

## UTERINE INFECTION IN BUFFALO COWS: A REVIEW

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## ABSTRACT

Postpartum uterine infections result from uterine contamination with bacteria during parturition. The prevalence of uterine infections varies considerably among studies. Uterine infection implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium, and/or release of bacterial toxins that lead to establishment of uterine disease. The development of uterine disease depends on the immune response of the cow as well as the species and number (load or challenge) of bacteria. Postpartum metritis is one of the most important disorders in buffaloes. The incidence rate of uterine infection in buffalo cows has been found to be much higher than in cows. A variety of species of bacteria, both Gram-positive and Gram-negative aerobes and anaerobes, can be isolated from the early postpartum uterus. Most of these are environmental contaminants that are gradually eliminated during the first 6 weeks postpartum. Bacterial contamination of the vagina and other external reproductive organs might occur during wallowing.

**Keywords:** buffaloes, cows, uterine infections

## INTRODUCTION

The postpartum period is defined as the interval from parturition to complete uterine

involution (Olson *et al.*, 1986). Postpartum metritis is one of the most important disorders in buffaloes (Azawi *et al.*, 2008a), causing high economic losses due to prolonged days open and prolonged intercalving intervals, resulting in involuntary culling (Esslemont and Peeler, 1993). The incidence rate of uterine infection in buffalo cows has been found to be much higher than in cows (Jainudeen, 1986; Azawi, 2006). Uterine function is often compromised in cattle by bacterial contamination of the uterine lumen after parturition; pathogenic bacteria frequently persist, causing uterine disease, a key cause of infertility (Sheldon and Dobson, 2004). The presence of pathogenic bacteria in the uterus causes inflammation, histological lesions of the endometrium, delays uterine involution and perturbs embryo survival (Sheldon *et al.*, 2006). Postpartum metritis is one of the most important disorders in buffaloes (Rao, 1982; Rao and Sreemannarayana, 1983; Azawi *et al.*, 2007), causing high economic losses due to prolonged days open and prolonged intercalving intervals, resulting in involuntary culling (Esslemont and Peeler, 1993). Toxic puerperal metritis (i.e. acute septic metritis) is characterized by increased rectal temperature, depression, anorexia, and a fetid watery vulvar discharge (Paisley *et al.*, 1986; Smith *et al.*, 1998; Azawi *et al.*, 2007). Toxic puerperal metritis can be a severe problem, and uterine infections can be life threatening (Montes and Pugh, 1993). Metritis and endometritis are

inflammation of the uterus. Metritis involves the endometrium, the underlying glandular tissues and the muscular layer (Bartlett *et al.*, 1986; Lewis, 1997; Azawi *et al.*, 2008b). Endometritis, involves only the endometrium and the underlying glandular tissues (Bretzlaff, 1987; Bonnett *et al.*, 1993; Correa *et al.*, 1993), and without systemic signs (Bondurant, 1999). These diseases share common etiological factors, predispose to one another, and largely share common treatment (Roberts, 1986; Bretzlaff, 1987; Noakes *et al.*, 2002).

## CLASSIFICATION OF UTERINE INFECTION

Several systems have been described in attempt to classify and define uterine infection. Uterine infections are generally classified according to clinical signs and degree of severity, which is in adhere to definitions used by theriogenologists (Olson *et al.*, 1986; Roberts, 1986; Youngquist and Shore, 1997; Noakes *et al.*, 2002). However, frequently the definition or characterization of the various manifestations of uterine disease either lacks precision, or definitions vary among research groups and/ or were not validated as to their effect on reproductive performance, making assessing the effects of treatment difficult. Often the term endometritis incorrectly includes metritis and endometritis or is determined solely based on transrectal palpation of an enlarged uterus (Correa *et al.*, 1993). During the 15<sup>th</sup> International Congress on Animal Reproduction (Gilbert, 2004), it was suggested that the research field would be aided by clear definitions of uterine disease that researchers could adopt. Whilst it may not be possible to categorize every animal, it is important

to have practical definitions, particularly for research. Dohoo *et al.* (1984) classified uterine infection into three categories: primary metritis occurring within the first 21 days of calving, secondary metritis between 21 and 60 days, and tertiary metritis after 60 days postpartum. Mean while, Olson *et al.* (1986) have classified uterine infections slightly differently. Puerperal metritis occurs between the time of calving and recovery of the sensitivity of the pituitary gland to GnRH at 10-12 days postpartum. Metritis and endometritis usually occur between resolution of pituitary sensitivity to GnRH and the first postpartum ovulation. Postovulatory infections arise during the time between the first ovulation and complete uterine involution. Diseases of the postovulatory period include chronic metritis, endometritis, and pyometra. Roberts (1986) classified endometritis into mild endometritis that is characterized by little infiltration of lymphocytes and plasma cells with few fibrosis and cystic glandular degeneration. The second type is severe endometritis, characterized by scar tissue formation in the endometrium, the thickness of uterine tissue due to high infiltration of leukocytes associated with severe changes in uterine glands including atrophy, cystic degeneration and necrosis. Dohmen *et al.* (1995) classified uterine infection according to clinical symptoms and time of occurrence. Acute endometritis occurs within 14 days of parturition with two types: first, acute endometritis characterized by a large amount of uterine exudates and a thin uterine wall, and secondly, acute endometritis with a limited amount of uterine exudates and a thick uterine wall. Sub-acute or chronic endometritis occurs from 14 days after parturition. Pyometra occurs from 3-4 weeks after parturition. Recently, Sheldon *et al.* (2006) provided a clear clinical definition of uterine diseases: toxic puerperal metritis

is an acute systemic illness due to infection of the uterus with bacteria, usually within 10 days after parturition. The following clinical signs characterize toxic puerperal metritis: a fetid red-brown watery uterine discharge and usually, pyrexia, reduced milk yield, dullness, inappetance or anorexia, elevated heart rate and apparent dehydration may also be present (Azawi *et al.*, 2007). The term metritis is used for animals that are not systemically ill but have an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina. Clinical endometritis is characterized by the presence of a purulent (>50% pus) or mucopurulent (approximately 50% pus, 50% mucus) discharge detectable in the vagina after 26 days postpartum. While sub clinical endometritis can be defined as endometrial inflammation of the uterus usually determined by cytology in the absence of purulent material in the vagina. A cow with sub clinical endometritis is defined by >18% polymorphonuclear cells in uterine cytology samples. Pathologists Jubb *et al.* (1985) and McEntee (1990) classified uterine infection depending on histopathological changes of uterine tissues. The histopathological changes in mild endometritis are not striking and consist for the most part of a diffuse, light infiltration of inflammatory cells with slight desquamation of the superficial epithelium and no significant vascular changes. While in acute endometritis, there is a prominent leukocyte infiltration involving all mucosal elements including the glands, and massing at the surface, with suppurative and superficial necrosis. In chronic endometritis, there is a prominent leukocyte infiltration involving all mucosal elements, the uterine wall is thickened with suffused blood and edema, serosa is dull and finely granular with "paint brush" hemorrhages

and thin deposition of fibrin or subserosal vessels may be darkly congested (Dzhuroua and Gulubinov, 1981). In puerperal metritis, the subserosal connective tissue is edematous and infiltrated with leukocyte, and the same process surrounds the blood vessels of the myometrium and permeates between bundles of and individual muscle fibers, which themselves undergo granular degeneration (Bonnett *et al.*, 1991). However, when metritis of the acute type, the leukocytes masses on the mucosal surface are associated with extensive hemorrhage, necrosis and sloughing, and invasion of blood vessels, both arterioles and venules, aggravates the lesion. Thrombosis may extend to the vessels of mesometrium with the usual squeal. Recovery from the acute phase of metritis often results in chronic metritis, with greater or lesser degree of endometrial destruction and replacement by granular scar tissue, and the uterus takes on the nature of a fistulous tract. With thickening of endometrium by inflammatory tissue, glands are depleted, atrophied, flattened and attenuated or cystic due to periglandular fibrosis (Hussain and Daniel, 1991; Dogan *et al.*, 2002). The lining mucosa may be intact, denuded in places, or show foci of polyploidy hyperplasia or squamous metaplasia. Endometrial stroma, especially in caruncles, may be replaced by scar tissue and dystrophic calcification of necrotic portions of the endometrium may sometimes be extensive enough that the lining of uterus feels gravelly. To the pathologists, the general definition of inflammation of the genital tract is simple. Inflammation limited to the endometrium is termed endometritis, involvement of the entire thickness of uterine wall is metritis; of serosa, perimetritis, and of the suspensory ligaments, parametritis (McEntee, 1990).

## PREVALENCE OF UTERINE INFECTIONS

The incidence rate of uterine infection in buffalo cows (24.7%) was much higher than in cows, in India (Gupta *et al.*, 1978; Rao and Sreemannarayana, 1983). Raman and Bawa, (1977) found a high prevalence of postpartum infections (38.54%) in buffalo cows. While metritis was recorded as incidence rate of 25% (Sar *et al.*, 1996) and endometritis 20.68% (Narasimha Rao and Sreemannarayana, 1983), while Rao (1982) recorded 30% incidence of endometritis among Indian buffaloes. In Pakistan Usmani *et al.* (2001) recorded an incidence of 24% of uterine infection among buffalo cows. In Malaysia, the incidence rate was the same as in India, with a high incidence of ovaro-bursal adhesions (Jainudeen, 1986). In Egypt, Serur *et al.* (1982) recorded an incidence of 38.9% and recently, Ghanem *et al.* (2002) recorded an incidence of endometritis of 22.4% in Egyptian buffalo cows. In Iran, Moghami *et al.* (1996) recorded an incidence of endometritis of 33.2% in buffalo cows, and recently, Moghaddam and Mamoei (2004) recorded an incidence of 29.4% of infertility problems including endometritis and metritis in Iranian local breed buffalo cows. In Iraq, the incidence of uterine infections was recorded as 45.3% (El-Dosseky and Juma, 1973). Recently Azawi *et al.* (2008c) recorded an incidence of uterine infections of 17% in Mosul buffalo cows. Ptaszynska (2003) revealed that the higher incidence of uterine infections in buffaloes than in cows might be due to poor hygiene, vaginal stimulation for milk let down and possibly, wallowing.

## PATHOGENESIS

Following calving, the uterus of over 90% off all cows becomes contaminated with bacteria (Paisley *et al.*, 1986; Azawi *et al.*, 2008b; Azawi *et al.*, 2008f). Some of these bacteria are harmful and others are not (Studer and Morrow 1978). When harmful bacteria are present; the uterus may become infected (Bondurant, 1999; Sheldon *et al.*, 2004; Azawi *et al.*, 2008f). One should differentiate between uterine contamination and uterine infection. The uterus of postpartum cows is usually contaminated with a range of bacteria, but this is not consistently associated with clinical disease. Infection implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium, and/or release of bacterial toxins that leads to establishment of uterine disease (Sheldon *et al.*, 2006; Azawi *et al.*, 2008c). The development of uterine disease depends on the immune response of the cow, as well as the species and number (load or challenge) of bacteria (Sheldon *et al.*, 2006; Azawi *et al.*, 2008f). The number of pathogenic bacteria in the uterus of postpartum cows may be great enough to overwhelm uterine defense mechanisms and cause life-threatening infection (Sheldon and Dobson, 2004; Azawi *et al.*, 2008d). The postpartum uterus has a disrupted surface epithelium in contact with fluid and tissue debris that can support bacterial growth (Etherington *et al.*, 1985; Konigsson *et al.*, 2002; Azawi *et al.*, 2008f). The outcome of uterine contamination depends on the number and virulence of the organisms present (Cohen *et al.*, 1995; Azawi, 2008), as well as the condition of the uterus and its inherent defense mechanism (Hussain, 1989). A mild to severe endometritis occurs in 90% of postpartum cows during the second through fourth postpartum weeks (Griffin *et al.*, 1974; LeBlanc *et al.*, 2002b). Resolution of

the inflammation occurs with time, in the normal cow, by 40 to 50 days postpartum (Bretzlaff, 1987; Azawi, 2008).

A variety of species of bacteria, both gram-positive and gram-negative aerobes and anaerobes, can be isolated from the early postpartum uterus (Noakes *et al.*, 1989; Bekana *et al.*, 1997; Sheldon *et al.*, 2004; Azawi, 2006; Azawi *et al.*, 2007; Azawi *et al.*, 2008a, Azawi, 2009). Most of these are environmental contaminants that are gradually eliminated during the first 6 weeks postpartum (Roberts, 1986; Arthur *et al.*, 1989; Noakes *et al.*, 2002). A normal postpartum cow resolves uterine infection by rapid involution of the uterus and cervix, discharge of uterine content, and mobilization of natural host defenses, including mucus, antibodies and phagocytic cells (Hussain and Daniel, 1992; Azawi, 2006; Azawi *et al.*, 2008a). Cows with certain periparturent problems have a reduced ability to control uterine infections. Excess stretching of the uterus, as with hydrops allantois, traumatization of genital tissues during dystocia or obstetric manipulation (Azawi *et al.*, 2008c) predispose for postpartum metritis. Metabolic disorders, traditional practices of farmers and herdsmen such as inserting the hand or implements in the vagina of buffalo cow to stimulate milk letdown, as well as, unhygienic conditions under which animals are allowed to calve, can diminish uterine tonus. In addition, some farmers suture the buffalo cow's vulva to prevent uterine prolapse immediately after postpartum (Azawi *et al.*, 2006). Lochia is then retained beyond the normal period, providing a medium for bacterial multiplication (Roberts, 1986; Noakes *et al.*, 2002). Phagocytosis by uterine leukocytes is reduced in cow with dystocia, retained fetal membranes and metritis (Paisley *et al.*, 1986; Azawi *et al.*, 2008f). If the uterus is severely debilitated,

any of a variety of contaminating organisms can cause a toxic puerperal metritis (Smith *et al.*, 1998; Drillich *et al.*, 2001; Melendez *et al.*, 2004; Azawi *et al.*, 2007). In less severe cases, an endometritis is initiated that may become persistent and impair fertility (El-Azab *et al.*, 1988; Slama *et al.*, 1991; Bateman *et al.*, 2002; LeBlanc *et al.*, 2002a; Seals *et al.*, 2002).

### NON-SPECIFIC BACTERIA CAUSING UTERINE INFECTION

The most common cause of uterine infection is the pathogenic microorganisms affecting productivity and fertility of cows (Lewis, 1997; Bondurant, 1999). Pathogenic organisms isolated from an infected uterus are found generally in livestock environments and are capable of infecting other tissues and organs (Griffin *et al.*, 1974; Roberts, 1986; Azawi, 2006; Azawi *et al.*, 2008a). Thus, uterine infections are classified as non-specific infections (Dawson, 1959; Griffin *et al.*, 1974; Bekana *et al.*, 1994; Bonnett and Martin, 1995; LeBlanc *et al.*, 2002b; Sheldon *et al.*, 2004; Azawi *et al.*, 2008f). They are called nonspecific infections because the initial colonizing bacterium is not known and the specific bacteria causing the signs of infection are not known (Lewis, 1997). Numerous bacteria in a variety of combinations have been isolated from infected uterus. *Archaeobacterium pyogenes* and *E. coli* are usually associated with uterine infection in cattle (Usmani *et al.*, 2001; Seals *et al.*, 2002). The composition of the uterine flora changes somewhat at each recontamination, and no specific combination of organisms is associated consistently with postpartum infections (Griffin *et al.*, 1974; Ruder



*et al.*, 1981). Nevertheless, *Archaeobacterium pyogenes* either alone or in combination with other bacteria such as the anaerobic *Fusobacterium necrophorum* and *Bacteroides spp* (Bretzlaff, 1987; Bondurant, 1999; Azawi *et al.*, 2007; Azawi *et al.*, 2008c) often is associated with uterine infections (Lewis, 1997; Sheldon *et al.*, 2004). Intra- uterine oxygen reductase potential fell in the presence of infection (El-Azab *et al.*, 1988), thereby creating an anaerobic environment. This drop in intrauterine oxygen reductase potential may be associated with either microorganism metabolism or increased oxygen consumption by polymorphonuclear inflammatory cells. Of the anaerobic organisms cultured from cases of uterine infection, *Fusobacterium necrophorum* and *Bacteroides spp.* have been identified (Azawi *et al.*, 2007; Azawi *et al.*, 2008c; Azawi, 2008; Azawi *et al.*, 2009). When *A. pyogenes* was isolated from uterine fluids approximately 21 days postpartum, cows developed severe endometritis and usually were infertile at first service (Ball *et al.*, 1984; Dohmen *et al.*, 1995; Noakes *et al.*, 2002). Azawi *et al.* (2007) suggested that organisms other than *A. pyogenes* and gram-negative anaerobes such as *Fusobacterium necrophorum*, as well as, *E. coli*, *Streptococcus spp.*, *Staphylococcus spp.*, and *Pseudomonas spp.* are responsible for toxic puerperal metritis. The growth of anaerobic bacteria may enhance the establishment of *A. pyogenes* and lead to the development of severe uterine infections (El-Azab *et al.*, 1988). Indeed, *Fusobacterium necrophorum* produce leukotoxin (Bretzlaff, 1987; Carter and Wise, 2004), while *Bacteroides* produce substances that prevents bacterial phagocytosis and *A. pyogenes* produce a growth factor for *Fusobacterium necrophorum* (Bekana *et al.*, 1994; Kamiyama *et al.*, 2004). *Bacteroides* and *Fusobacterium* species are

prevalent in the indigenous flora on all mucosal surfaces. Tissue necrosis and poor blood supply lower the oxidation-reduction potential, thus favoring the growth of anaerobes (Baron, 2004). In addition, *Fusobacterium necrophorum* is frequently a secondary invader, and mixed infection with *A. pyogenes* is not common (Zerbe *et al.*, 2001). In addition *F. necrophorum* produces a variety of extra-cellular products including hemolysin, hemagglutinin, adhesions, platelet aggregation factor, proteases and DNase. The significance of these products relative to virulence is not clear (Carter and Wise, 2004). Azawi *et al.* (2007) suggested that the earlier appearance of *E. coli* in the uterus affected the phenotype and function of polymorphonuclear cells, and this might support the co-infection on by *A. pyogenes* at a later time.

## UTERINE DEFENSE MECHANISM

Anatomical and functional barriers mediate effective defense against reproductive tract invasion by environmental organisms as well as, nonspecific and specific immune responses (Bondurant, 1999; Azawi, 2008). While Dhaliwal *et al.* (2001) reviewed that the uterine defense mechanisms against contaminant microorganisms were maintained in several ways: anatomically, by the simple or pseudostratified columnar epithelium covering the endometrium; chemically by mucus secretions from the endometrial glands; immunologically, through the action of polymorphonuclear inflammatory cells and humoral antibodies, but the degree of interaction is not clear. Disruption of these mechanisms allows opportunist pathogens, mostly micro-organisms found in the posterior gastro-intestinal tract and

around the perineal area (Paisley *et al.*, 1986; Azawi *et al.*, 2008f), to colonize the endometrium and cause an endometritis (Dhaliwal *et al.*, 2001). A degree of bacterial contamination of the uterus usually occurs during, or immediately after, parturition (Azawi *et al.*, 2008c). Bacterial contamination of the uterus may also occur during coitus or insemination (Griffin *et al.*, 1974; Lewis, 1997). Also in buffaloes, bacterial contamination of the vagina and other external reproductive organs might occur during wallowing (Ali *et al.*, 2000; Ghanem *et al.*, 2002). Whether or not a persistent infection of the uterus becomes established depends upon the level of contamination, the animal's uterine defense mechanism and the presence of substrates (such as devitalized tissue) for the growth of bacteria (Roberts, 1986; Noakes *et al.*, 2002).

Under normal circumstances, there are several mechanisms, which prevent opportunist pathogens from colonizing the genital tract (Bondurant, 1999; Noakes *et al.*, 2002). The major anatomical barriers between the contaminated world and the relatively sterile environment of the uterus, include the vulva, the vestibule (guarded by a muscular sphincter), and the cervix. It should be noted that, although the vulva may appear of little consequences as a barrier, it is, in fact, remarkably efficient at preventing faecal contamination of the tubular genitalia (Hussain, 1989; Hussain and Daniel, 1992). In cattle, the cervix is formidable barrier composed of series of mucosal lined collagenous rings (Arthur *et al.*, 1989; Azawi, 2006; Azawi, 2008). In addition, the cervical-vaginal mucus (especially the scant, tenacious mucus of the luteal phase) can function as a physical barrier for organisms that would otherwise ascend the reproductive tract (Sheldon and Dobson, 2004). The circular and longitudinal layers of the uterine

musculature provide physical propulsion of particular material, including microbes (Herath *et al.*, 2006a). Epithelial cells are the first to make contact with potential pathogens that enter the uterus (Wira *et al.*, 2005). Epithelial and stromal cell interactions are critically important for endometrial function, with stromal cells affecting epithelial cells through both the release of soluble factors and turn over of extra cellular matrix (Wira *et al.*, 2005). Conversely, epithelial cells affect stromal cell function through the release of soluble factors and cell-to-cell contact (Wegner and Carson, 1992). Pierro *et al.* (2001) suggest that PGE2 regulates epithelial cells proliferation and is mediated indirectly by uterine stroma.

Estradiol and progesterone have both opposing and complementary effects on the female genital tract with estradiol stimulating epithelization (especially of the vaginal lining and endometrial gland), and vascularization of the endometrium (Liu and Hansen, 1993; Noakes *et al.*, 2002). Progesterone aids in endometrial gland differentiation and enhances uterine gland secretions, reducing cervical mucus production, prevents uterine contractility (Rodriguez-Martinez *et al.*, 1987), and acts as a counter influence to estradiol in immune protective responses of the reproductive tract (Wira and Rossoll, 1995). Cattle are resistant to uterine infections when progesterone concentrations are basal, and they are susceptible when progesterone concentrations are increased (Lewis, 2003). For example, spontaneous uterine infection in cattle do not usually develop until after formation of the first postpartum corpus luteum, although bacterial contamination can be sufficient to induce the onset of puerperal metritis very soon after calving when progesterone concentrations are basal (Lewis, 1997; Seals *et al.*, 2002). Postpartum cows that received intrauterine infusions of

*Archaeobacterium pyogenes* and *E. coli* when progesterone concentrations were basal did not develop uterine infections, whereas all cows developed uterine infections when the bacteria were infused after the onset of luteal function and progesterone concentrations had begun to increase (Del Vecchio *et al.*, 1994). In addition, none of the animals, that received intrauterine infusions of *Archaeobacterium pyogenes* and *E. coli* during estrus phase developed uterine infection, but all of them that received *Archaeobacterium pyogenes* and *E. coli* infusions during luteal phase of the estrus cycle developed uterine infections (Ramadan *et al.*, 1997). The previous examples clearly support the idea that progesterone converts the uterus from an organ that is resistant to one that is susceptible to infection.

In a cycling buffalo cow, the uterus is usually under progesterone influences. That is, the nonpregnant uterus is in the luteal phase (under the influence of progesterone) for about 14 to 15 days of its 21-day cycle (i.e. from about day 3 to 17 after estrus and ovulation) (Stevenson, 1997). It is under its most significant estradiol influence, with no progesterone to counter its effect, for about 1 day (immediately preceding standing estrus). It has been reported that Murrah buffaloes have higher overall plasma estradiol concentration than do swamp buffalo and cows. Values at estrus of  $31 \pm 1.70$  pg/ml (Batra and Pandey, 1982) compare with the lower values 12.9 pg/ml, and 13.0 pg/ml, respectively, for swamp buffaloes and cows (Glencross and Pope, 1981; Kani and Shimizu, 1984; Avenell *et al.*, 1985). The high estradiol concentrations that occur at estrus and parturition cause changes in the number and proportions of circulating white blood cells, with a relative neutrophilia and a "shift to the left" (Noakes *et al.*, 2002). Moreover, at estrus, the blood supply to the uterus is increased under the

influence of estradiol, whilst at parturition, there is a massive blood supply to the gravid uterus. This increased blood supply, coupled with the migration of white cells from the circulation to the uterine lumen, enables vigorous and active phagocytosis of bacteria to occur (Hussain and Daniel, 1991). Estradiol also causes an increase in the quantity and nature of vaginal mucus, which also plays an important role in defense of the uterus against bacteria by providing a protective physical barrier and by flushing and diluting the bacterial contaminants (Youngquist and Shore, 1997). The immune functions of the uterus have been found to be up regulated when estrogens were increased (Ramadan *et al.*, 1997). It is difficult to determine whether increased estrogens during the follicular phase induced the up-regulation or whether up-regulation was due to the removal of the suppressive effects of progesterone (Hussain, 1989; Seals *et al.*, 2002; Padua *et al.*, 2005). Recently Wira *et al.* (2005) demonstrated that changes in ovarian estrogens and progesterone regulate the uterine immune function. During estrus, when progesterone concentrations are decreased and estradiol concentrations are increased, uterine production of  $\text{PGF}_{2\alpha}$  is increased, endometrial leukotriene B4 production is increased, and the uterus is normally able to prevent infections from developing (Lewis, 2003). Uterine  $\text{PGF}_{2\alpha}$  and leukotriene B4 production decrease to basal within a few days after estrus, when progesterone concentrations begin to increase, and the uterus again becomes susceptible to infections. Prostaglandin F enhances neutrophil chemotaxis and the ability of neutrophils to ingest bacteria and leukotriene B4 enhanced chemotaxis, random migration and antibody independent cell mediated cytotoxicity (Hoedemaker *et al.*, 1992). Prostaglandin  $\text{F}_{2\alpha}$ , which is considered a proinflammatory molecule, may stimulate the



production of proinflammatory cytokines that enhance phagocytosis and lymphocytes function (Kelly *et al.*, 2001). When exogenous  $\text{PGF}_{2\alpha}$  is administered, it is followed by an increase in uterine  $\text{PGF}_{2\alpha}$  production, and this probably increases phospholipase A2, which would increase the amount of free arachidonic acid that could be used to produce cyclooxygenase (e.g.  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$ ) and lipoxygenase (e.g. leukotriene B4) products. The effect of estrogens and progesterone may seem antagonistic at first, but the two hormones seem to orchestrate uterine immune function in the favor of the animal. Indeed, uterine immune function is up regulated at estrus when there are many opportunities for the introduction of pathogens and down regulated during the luteal phase when the uterus is capable of supporting a conceptus, and this down-regulation during the luteal phase seems to allow the uterus to tolerate a fetal allograft (Liu and Hansen, 1993).

Intra- uterine inoculation with bacteria increases  $\text{PGF}_{2\alpha}$  concentrations, regardless of the stage of the cycle when the bacteria were introduced (Ramadan *et al.*, 1997), as well as, in postparturent cows (DeVecchio *et al.*, 1994). Ramadan *et al.* (1997) assumed that the increase in  $\text{PGF}_{2\alpha}$  in uterine infections was not known. Nevertheless, *in vitro* experiments indicate that  $\text{PGF}_{2\alpha}$  and other arachidonic acid cascade products such as leukotrienes are chemo-attractive to neutrophils, and this may indicate that the bacteria induced increase in  $\text{PGF}_{2\alpha}$  is an important signal for the immune defense of the uterus (Hoedemaker *et al.*, 1992). Free arachidonic acid can be converted through the cyclo- oxygenase pathway to leukotrienes. In addition to the chemo-tactic effects of leukotrienes and  $\text{PGF}_{2\alpha}$ , leukotrienes are a potent activator of neutrophils function,  $\text{PGF}_{2\alpha}$  reduces intracellular cAMP concentrations, and

reduced cAMP is associated with active immune cells (Hoedemaker *et al.*, 1992). The increased uterine secretions of  $\text{PGF}_{2\alpha}$  may be an important component of the uterine defense mechanism. During the periparturent period, physiological changes occur that depress the defense mechanism of the uterus and render it more prone to uterine puerperal infections (Cai *et al.*, 1994). The polymorphonuclear phagocytes activity remains high through out the peripartum period (Cai *et al.*, 1994), but the polymorphonuclear killing capacity is impaired (Zerbe *et al.*, 1996). The increase in uterine polymorphonuclear cells might favor the spontaneous resolution of endometritis (Mateus *et al.*, 2002a). Tizard (1996) reported a decreases level of polymorphonuclear cells in blood circulation when there is a uterine infection. Wade and Lewis (1996) suggested that the reduction in polymorphonuclear cells in blood circulation might be because they leave the circulation and move to the site of infection in the uterus. Klucinski *et al.* (1990) demonstrated that intrauterine instillation of specific and non-specific antigens in cows caused a significant rise in polymorphonuclear cells percents. The induction of cell-mediated immune reaction in the uterus significantly boots the intracellular capability of uterine cells to kill bacteria through the oxidative system (Klucinski *et al.*, 1995; Tekin and Hansen, 2002). The uterus is part of the common mucosal immune system, sharing structural and functional similarities and common lymphocyte trafficking network (Robertson, 2000). The uterus is exceptional among mucosal tissues, in that, ovarian steroid hormones also have considerable effect on both humeral and cell mediated immunity (Wira *et al.*, 1999). Recently Herath *et al.* (2006b) concluded that epithelial and stromal cells detect and respond to bacteria, which modulate their endocrine function.

Thus, the outcome of an immune response and the elicitation of protective immunity to pathogens can be markedly influenced by the stage of the estrus cycle at which priming or infection takes place (Kaushic *et al.*, 1998; Parr and Parr, 1999). The uterus is supplied with ample lymphocytic drainage and contains the full range of lymphohemopoietic cells and molecular regulators required to generate and elicit adaptive antigen specific immunity (Hunt *et al.*, 1997). Antibody mediated (humeral) and cell mediated immunity can be induced in the estrus after infection, or immunization by antigen delivery to the reproductive tract or other mucosal surfaces (Cai *et al.*, 1994; Zerbe *et al.*, 2001). It is clear that the nature of the antigen, the route of sensitization and the use of adjuvant all have major influence on the magnitude or quality of uterine response to exogenous antigen (Robertson, 2000).

Polymorphonuclear cells, blood monocytes and tissue macrophages are regarded as the 'professional phagocytes' in the cellular defense against pathogenic microorganisms. The main function of neutrophils is phagocytosis and killing of invading bacteria (Dhaliwal *et al.*, 2001). These include many stages, chemotaxis, opsonization, adherence and attachment, ingestion and digestion. Chemotaxis is the directed movement of the neutrophils toward the invading pathogen under the effect of different substances such as bacterial products and substances released by destroyed cells, by components of the complement system (Shaw and Griffin, 1981; Kimura *et al.*, 2002). Around 48 h after normal unassisted calving, leukocytes accumulated in the uterine lumen as well as contaminant microorganisms; this marks the beginning of a normal cleansing and involution process in the uterus (Dhaliwal *et al.*, 2001). The cellular immune response in the uterus may be adversely affected by several therapeutic strategies

commonly used to treat postpartum disorders in cattle (Bretzlaff, 1987). For example, manual removal of fetal membranes may inhibit uterine leukocyte phagocytic activity for several days as may intrauterine administration of most antiseptics and disinfectants (Noakes *et al.*, 2002).

Luminal and glandular epithelial cells of the endometrium have been shown to produce numerous cytokines (Wira *et al.*, 2005). Cytokines are now recognized as principal components of the complex intracellular communication among cells in the uterus, and temporal release patterns during estrus cycle show they may be regulated by sex hormones (Robertson *et al.*, 1997; Hunt *et al.*, 1997; Kaushic *et al.*, 1999). Jacobs and Carson (1993) found that the release of interleukin by uterus epithelium and stromal cells result in increased secretions of PGE<sub>2</sub> and PGF<sub>2α</sub>. Also uterine epithelial cells produce a spectrum of antimicrobials, including lactoferrin, isozymes, and complements, which enter the uterine lumen along with immunoglobulin A (IgA) and IgG that confer protection against potential pathogens (Buchanan *et al.*, 1999). Immunoglobulin concentration in uterine secretions reflects both the extent of the endometrial inflammatory process in the face of microbial challenge and its chances of clinical recovery (Parr and Parr, 1994). Following intrauterine inoculation of known pathogenic microorganisms, immunoglobulins in cervical and vaginal secretions appear in the order IgM, IgA and IgG and disappear in the order IgM, IgG and IgA (Dhaliwal *et al.*, 2001; Azawi, 2008). Their concentrations differ depending upon the site of sampling; IgG predominates in the uterine lumen and IgA in the vagina (Watson *et al.*, 1990). In cows with abnormal puerperium concentrations of IgA and IgG in the uterine fluid rise rapidly as endometritis develops (Dhaliwal *et al.*, 2001).

IgA is synthesized locally in the bovine uterus at the mucosal surface, while IgG is derived from two sites, part of the IgG being synthesized in the endometrium and the remainder derived from peripheral circulation (Butt *et al.*, 1991).

Cytokines are proteins that are involved in the regulation of the immune response. When these proteins are produced by lymphocytes, they are referred to generically as lymphokines (Blecha, 1991), and when they are produced by monocytes or macrophages, they may be called monokines. The term interleukin is applied to protein that functions as communicator between leukocytes (Blecha, 1988). Cytokines induced interferons ( $\alpha$ ,  $\beta$  and  $\gamma$ ), tumor necrosis factor, lymphotoxin, interleukin (IL-1 to IL-10), colony stimulating factor and transforming growth factor. Interleukin 1 is a predominantly macrophage derived protein that modulates many of the responses involved in the process of host defense to uterine infection (Robertson, 2000). Interleukin 2 is secreted by a subset of T-cells and large granular lymphocytes after stimulation with antigen (Miller *et al.*, 1980). These lymphokines induce the clonal expansion of activated T-cells and B-cells and activate natural killer cells (Lanier *et al.*, 1985). The most important T-lymphocytes in uterine immunity is type 2, which stimulate a humeral response by releasing cytokines that induce B-lymphocytes to proliferate, differentiate, and secrete antigen reaction antibodies (Hansen, 1998). These cells are fewer in number than non-specific populations of other lymphocytes, but comprise a significant action and are classified as natural uterine killer cells (Hansen, 1998). B-lymphocytes and plasma cells are also present in uterine tissues, demonstrating active humeral immunity (Parr and Parr, 1994).

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