PATHOGENESIS OF POSTPARTUM METRITIS IN BUFFALOES: A REVIEW

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ABSTRACT

Uterine function is often compromised in buffaloes by bacterial contamination of the uterine lumen after parturition, wallowing and insemination. Pathogenic bacteria frequently persist, causing uterine disease resulting in infertility. The presence of pathogenic bacteria in the uterus causes inflammation, histological lesions of the endometrium, delays uterine involution and perturbs embryo survival. Numerous bacteria in a variety of combinations have been isolated from infected uteri. *Arcanobacterium 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 in buffaloes. The incidence rate of uterine infection in buffaloes was much higher than in cattle. The earlier appearance and colonization of *E. coli* and lipopolysaccharide endotoxins in the uterus by affecting the phenotype and function of polymorphonuclear cells, and this might support the co-infection on by *A. pyogenes* and gram-negative anaerobes such as *Fusobacterium necrophorum* at later time.

Keywords: buffalo cow, pathogenesis, bacterial infection, metritis, endometritis, postpartum

INTRODUCTION

Water buffaloes are classified in to two main 'types': the river type located in South Asia and the swamp type spread across the South-East Asian region. The Mediterranean buffalo, which some consider to be a third type, is derived from the river type. Postpartum metritis and endometritis are the most important disorders in buffaloes (Azawi, 2006), causing high economic losses due to prolonged days open and prolonged intercalving intervals, resulting in involuntary culling (Taha and Azawi, 2003; Singh *et al*., 2000). Uterine function is often compromised in buffaloes by bacterial contamination of the uterine lumen after parturition, insemination and wallowing; pathogenic bacteria frequently persist, causing genital diseases, a key cause of infertility (Azawi *et al*., 2008a). The major problems faced by buffalo breeders and farmers include poor reproductive efficiency and prolonged intercalving intervals (Samad *et al*., 1984: Oswin-Perera, 1999: Barile, 2005: Perera, 1999: Sah and Nakao, 2006). This can be attributed to factors such as harsh environments (Abdalla, 2003), lack of year-round feed supply and minimal managerial inputs (Perera, 2008), in the majority of farming systems under which buffalo are raised (Sah and Nakao, 2006). In every survey of the factors causing endometritis, metritis and toxic puerperal metritis, dystocia and retained fetal membranes

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are identified as of major importance in buffaloes (Singh *et al*., 2000: Ahmed *et al*., 2009: Azawi *et al*., 2007). The presence of pathogenic bacteria in the uterus causes inflammation, histological lesions of the endometrium, delays uterine involution and perturbs embryo survival (Azawi and Taha, 2002: Azawi *et al*., 2008b). In addition, uterine bacterial infection, bacterial products or the associated inflammation, suppress pituitary LH secretion and perturb postpartum ovarian follicular growth and function, which disrupt ovulation in buffaloes and cattle (Sheldon *et al*., 2002: Hanafi *et al*., 2008). The incidence rate of uterine infection in buffaloes was much higher than in cattle (Usmani *et al*., 2001; Sheldon *et al*., 2002: Roman-Ponce *et al*., 2006; Azawi *et al*., 2008c; Hanafi *et al*., 2008). The annual incidences of uterine infections in postpartum cows range from 10 to 50% of dairy cattle (Lewis, 1997), 20 to 75% of the buffaloes (Rao, 1982; Jainudeen, 1986; Usmani *et al*., 2001). Postpartum metritis is one of the most important disorders in buffaloes (Rao and Sreemannarayana, 1983; Reddy *et al*., 1986; Singla and Verma, 1994; Singh and Sahni, 1995; Tailor *et al*., 1997; El-Wishy, 2007). Toxic puerperal metritis (i.e. acute septic metritis) is characterized by increased rectal temperature, depression, anorexia, and a fetid watery vulvar discharge (Azawi *et al*., 2007). Toxic puerperal metritis can be a severe problem, and uterine infections that are life threatening (Tomnar *et al*., 1984; Singh *et al*., 1997; Azawi *et al*., 2008d). Metritis and endometritis are inflammation of the uterus. Metritis involves the endometrium, the underling glandular tissues and the muscular layer (McEntee, 1990; Lewis, 1997). Endometritis involves only the endometrium, which includes the superficial (luminal) epithelium, the underlying stratus compactum (stromal cells and gland necks) and the stratum spongiosum (gland bodies and stroma) (Azawi and Jajo Azar, 2002), and without systemic signs (Azawi *et al*., 2008b). These diseases share common etiological factors, predispose to one another and, largely, share common treatment (Azawi, 2006).

In this species, the related knowledge available in the literature is very limited and most studies concerning uterine infection are in cattle. The goal of this review is to present comprehensive current information on the pathogenesis, incidence, bacterial causes, and uterine defense mechanism in buffaloes.

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 adheres to definitions used by theriogenologists (Noakes *et al*., 2001). However, frequently the definition or characterization of the various manifestations of uterine disease either lack 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 (Lewis, 1997). During the 15th 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. Sheldon (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

in buffaloes: a fetid red-brown watery uterine discharge and usually, pyrexia, reduced milk yield, dullness, inappetance or anorexia, and elevated heart rate, and apparent dehydration may also be present (Azawi *et al*., 2008d). 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 (Azawi *et al*., 2008a). 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 [14]. A new technique for the diagnosis of endometritis that has been used recently in bovine gynecology is uterine cytology, mainly to detect subclinical endometritis in clinically healthy cows (Barlund *et al*., 2008). The proportion of polymorphonuclear neutrophils (PMN) in the total number of endometrial cells is indicative for subclinical endometritis (Westermanna *et al*., 2010). Different threshold values for the proportion of PMN have been suggested, varying from 5 to 18% (Dubuc *et al*., 2010). Reports on the use of endometrial cytology for the diagnosis of clinical endometritis, however, polymorphonuclear cells are limited to one recent study that described endometrial cytology as the most reliable method of diagnosing endometritis in cattle (Westermanna *et al*., 2010). Subclinical 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 subclinical endometritis is defined by > 18% in uterine cytology samples. Recently Dubuc *et al*. (2010) defined postpartum endometritis as by its negative effect on subsequent reproductive performance, cytological and clinical diagnostic criteria were taken together to determine the optimal definition of endometritis. They also suggested that clinical endometritis terminology may not be appropriate and that purulent vaginal discharge may be more descriptive. Buffaloes may be classified according to their uterine health status as purulent vaginal discharge only, cytological endometritis only, or both purulent vaginal discharge and cytological endometritis.

Pathogenesis

Following calving the uterus of buffaloes becomes contaminated with bacteria [Azawi, 2006; Azawi *et al*., 2008a; Azawi *et al*., 2008e). Some of these bacteria are harmful and others are not (Azawi *et al*., 2007; Azawi *et al*., 2008b). When harmful bacteria are present; the uterus may become infected (Azawi, 2009). One should differentiate between uterine contamination and uterine infection. The uterus of postpartum buffaloes is usually contaminated with a range of bacteria, but this is not consistently associated with clinical disease (Azawi *et al*., 2008g). 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 (Azawi *et al*., 2007). The development of uterine disease depends on the immune response of the buffalo, as well as the species and number (load or challenge) of bacteria (Azawi, 2006; Azawi *et al*., 2008e). 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 (Singh *et al*., 1986). The postpartum uterus has a disrupted surface epithelium in contact with fluid and tissue debris that can support bacterial growth (Azawi *et al*., 2008e). The outcome of uterine contamination depends on the number and virulence of the organisms present (Azawi, 2006), as well as the condition

of the uterus and its inherent defense mechanism Azawi, 2008). A mild to severe endometritis occurs in 90% of postpartum buffaloes during the second through fourth postpartum weeks (Azawi, 2006). Resolution of the inflammation in cattle occurs with time, firstly being restored in the normal cow by 40 to 50 days postpartum (Jainudeen and Hafez, 1993). No information is available on the resolution of postpartum endometritis in buffaloes after normal parturition. The interval from calving to clinically completed involution of the uterus in buffaloes varied widely with a minimum of 25 days (Jainudeen and Hafez, 1993) and a maximum of 74 days (Devanathan *et al*., 1987; Qureshi *et al*., 1998). No study is available on the spontaneous clinical resolution of postpartum endometritis in buffaloes. In cattle, approximately three quarters of cows with postpartum endometritis had spontaneous clinical resolution (Gautam *et al*., 2010). The central question is why buffalo cows have persistent infection after the postpartum period without spontaneous clinical resolution of postpartum endometritis leading to prolonged days open and prolonged intercalving intervals. This could be due to the prolonged interval from calving to clinically completed involution of the uterus in dairy buffaloes (Qureshi *et al*., 1998) and to the period of postpartum anestrous or anestrous, which is usually longer in buffalo than in cattle (Dobson and Kamonpatana, 1986; Devanathan *et al*., 1987). Further studies in postpartum buffaloes concern the release of acute phase proteins after parturition that helps to promote tissue repair. Following the inflammatory process, hydroxyproline or prostaglandin (PG) F2α metabolites are released to enhance neutrophil chemotaxis and the ability of neutrophils to ingest bacteria and plasminogen activators. They are specific serine proteases that convert plasminogen to plasmin and are likely to play an important role during the inflammatory process of the uterus. Further study of them is needed to understand the impairment of spontaneous clinical resolution of postpartum endometritis.

A variety of species of bacteria, both gram-positive and gram-negative aerobes and anaerobes, can be isolated from the early postpartum uterus (Azawi, 2006; Azawi *et al*., 2007; Azawi *et al*., 2008a). Most of these are environmental contaminants. Buffaloes 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, predispose for postpartum metritis (Azawi *et al*., 2007). Metabolic disorders, some traditional practices by farmers and herdsmen in which the hand or implements in are inserted into the vagina of the 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, 2006). Lochia is then retained beyond the normal period, providing a medium for bacterial multiplication (Azawi *et al*., 2008e). Phagocytosis by uterine leukocytes is reduced in buffalo cow with dystocia, retained fetal membranes and metritis (Azawi *et al*., 2007). If the uterus is severely debilitated, any of a variety of contaminating organisms can cause a toxic puerperal metritis (Azawi *et al*., 2008d). In less severe cases, an endometritis is initiated that may become persistent and impair fertility (Usmani *et al*., 2001; Azawi and Taha, 2002; Roman-Ponce *et al*., 2006).

Bacterial causes of uterine infection

The most common cause of uterine

infection is the pathogenic microorganisms affecting productivity and fertility of buffaloes (Azawi, 2006). Pathogenic organisms isolated from an infected uterus are found generally in livestock environments and are capable of infecting other tissues and organs (Azawi *et al*., 2008a). Thus, uterine infections are classified as non-specific infections (Sheldon *et al*., 2004). They are called non-specific infection 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 uteri. *Arcanobacterium pyogenes* and *E. coli* are usually associated with uterine infection in buffaloes and cattle (Azawi, 2006; Azawi *et al*., 2007). The composition of the uterine flora changes somewhat at each recontamination, and no specific combination of organisms is associated consistently with postpartum infections (Azawi *et al*., 2008e and 2008f). Nevertheless, *Arcanobacterium pyogenes*, either alone or in combination with other bacteria such as the anaerobic *Fusobacterium necrophorum* and *Bacteroides spp* (Azawi *et al*., 2007), often is associated with uterine infections (Azawi, 2006; Azawi *et al*., 2007; Azawi *et al*., 2008f). Intrauterine oxygen reductase potential fell in the presence of infection (El-Azab *et al*., 1988) and mostly the aerobic bacteria, 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 microorganisms cultured from cases of uterine infection, *Fusobacterium necrophorum* and *Bacteroides spp*. have been identified (Azawi, 2006; Azawi *et al*., 2007). When *A. pyogenes* was isolated from uterine fluids, buffaloes developed severe endometritis and usually were infertile at first service (Usmani *et al*., 2001; Roman-Ponce *et al*., 2006). 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. Indeed, *Fusobacterium necrophorum* produce leukotoxin (Baron, 2004; Carter, 2004), while *Bacteroides* produce substances that prevent bacterial phagocytosis and *A. pyogenes* produce a growth factor for *Fusobacterium necrophorum* (Azawi, 2008). *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 (Azawi, 2008). 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, 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 mechanisms

Anatomical and functional barriers mediate effective defense against reproductive tract invasion by environmental organisms as well as nonspecific and specific immune responses

(Azawi, 2008). Dhaliwal *et al*. (2001) stated 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. Disruptions of these mechanisms allow opportunist pathogens, mostly microorganisms found in the posterior gastro-intestinal tract and around the perineal area (Azawi *et al*., 2008e), to colonize the endometrium and cause an endometritis (Azawi, 2008; Sheldon *et al*., 2008). A degree of bacterial contamination of the uterus usually occurs during, or immediately after, parturition (Azawi, 2006; Azawi *et al*., 2007; Azawi, 2008). Bacterial contamination of the uterus may also occur during coitus or insemination (Taha and Azawi, 2003; Azawi, 2008). Also in buffaloes, bacterial contamination of the vagina and other external reproductive organs might occur during wallowing (Jainudeen, 1986; Azawi, 2006). 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 (Azawi *et al*., 2007; Azawi *et al*., 2008a).

Under normal circumstances, there are several mechanisms, which prevent opportunist pathogens from colonizing the genital tract. 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 (Sheldon *et al*., 2008, 2009) as in cattle, while in buffaloes the larger soft loose vulval tissue might reduce its efficacy as a barrier (Azawi, 2006). In cattle and buffaloes, the cervix is formidable barrier composed of series of mucosal lined collagenous rings (Dhaliwal *et al*., 2001). 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 *et al*., 2009). The circular and longitudinal layers of the uterine musculature provide physical propulsion of particular material, including microbes.

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 the turnover of the extracellular matrix (Wira *et al*., 2005). Conversely, epithelial cells affect stromal cells function through the release of soluble factors and cell-to-cell contact. Pierro *et al*. (2001) suggested that PGE2 could regulate epithelial cells proliferation and may be 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 (Sheldon *et al*., 2009). Progesterone aids in endometrial gland differentiation and enhances uterine gland secretions, reducing cervical mucus production, prevents uterine contractility (Azawi *et al*., 2008f), and acts as a counter influence to estradiol in immune protective responses of the

reproductive tract (Wira *et al*., 2005). Cattle are resistant to uterine infections when progesterone concentrations are basal and they are susceptible when progesterone concentrations are increased (Lewis *et al*., 1997). 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 *et al*., 1997; Sheldon *et al*., 2009). Postpartum cows that received intrauterine infusions of *Arcanobacterium 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 (DelVecchio *et al*., 1994). In addition, none of the animals that received intrauterine infusions of *Arcanobacterium pyogenes and E. coli* during the estrus phase developed uterine infection, but all of those that received *Arcanobacterium pyogenes* and *E. coli* infusions during luteal phase of the estrus cycle developed uterine infections (Dhaliwal *et al*., 2001; Sheldon *et al*., 2009). 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 the cycling buffalo cow, the uterus is usually under progesterone influences. That is, the non-pregnant 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) (Perera, 1999; El-Wishy, 2007). 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 buffalo have higher overall plasma estradiol concentration than do swamp buffalo and cows. Values at estrus of 31±1.70 pq/Ml (Devanathan *et al*., 1987) compare with the lower values of 12.9 pq/Ml and 13.0 pq/Ml,, for swamp and cows (Glencross and Pope, 1981; Kani *et al*., 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" (Azawi, 2008). 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 (Sheldon *et al*., 2009). 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 (Sheldon *et al*., 2009). The immune functions of the uterus were found to be up regulated when estrogens were increased (Dhaliwal *et al*., 2001). It is difficult to determine whether increased estrogens during follicular phase induced the up-regulation or whether up-regulation was due to the removal of the suppressive effects of progesterone (Dhaliwal *et al*., 2001). Wira *et al*. (2005) demonstrated that changes in ovarian estrogens and progesterone regulate uterine immune function. The effect of estrogens and progesterone may seem antagonistic at first, but the two hormones seem to orchestrate uterine immune function in 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 (Lewis, 2003). The most critical factor in uterine defense against infection is rapid, physical clearance of inflammatory debris from the uterus after insemination or calving (Azawi, 2006). Compared to cattle, buffaloes have difficulty in clearing this debris from uterine cavity because they have lower estradiol secretion than cattle during estrous phase that decreases the uterine drainage (Kani *et al*., 1984; Perera, 2011).

CONCLUSION

The incidence rate of uterine infection in buffaloes was much higher than in cattle. The earlier appearance and colonization of *E. coli* and lipopolysaccharide endotoxins in the uterus by affecting the phenotype and function of polymorphonuclear cells, and this might support the co-infection on by *A. pyogenes* and gram-negative anaerobes such as *Fusobacterium necrophorum* at a later time. Serum complement proteins and immunoglobulins in the buffalo genital tract and secretions in the endometrium or other parts of the reproductive tract of the buffalo have not yet been studied as extensively as in cattle. Further studies are needed to understand the uterine defense mechanism in buffaloes and to compare them with those of cattle as most studies concerning uterine defense mechanism have been undertaken in cattle.

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