Immunology of the bovine uterus and immunological strategies to resolve endometritis*

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ABSTRACT

Endometritis in breeding cattle occurs during the postpartum period, and is mostly associated with ascending infection involving Arcanobacter pyogenes together with Gram negative anaerobes. Part of the uterine defence mechanisms against bacterial invasion is provided by uterine polymorphonuclear leukocytes (PMNs), whose phagocytic activity depends on bacterial opsonisation by humoral antibodies. The leukocyte count in the uterine lumen is relatively high during the met-oestrus period compared to other phases of the oestrus cycle. Humoral immune response in the bovine reproductive tract is stimulated following local antigen exposure, and the response is site dependent; IgG predominates in the uterus while IgA in the vagina. A part of IgG1 is synthesised locally in the bovine endometrium while the remaining IgG1 and whole of the IgG2 in the uterine lumen is derived from the uterine blood circulation. Intrauterine infusion of immunomodulators, such as *E. coli* lipopolysaccharides (LPS) or oyster glycogen, in healthy cows and those with endometritis, induces leukocytosis within the uterine lumen; leukocyte count is relatively high during dioestrus but their functional activity is not affected by the stage of the oestrous cycle. Uterine luminal immunosuppressant proteins elevate under progesterone dominance, which inhibit lymphocyte proliferation, thereby making the uterus more susceptible to infections. Exact mechanisms involved in the increased susceptibility of the uterus to infections during the luteal phase of the oestrous cycle are still not clear. *E. coli* lipopolysaccharides (LPS) when administered intrauterine at a dosage of 100 mg, are not absorbed by the healthy endometrium, and do not alter the oestrous cycle length. It is not known whether this dose of LPS will be absorbed from the inflamed uterus of naturally occurring cases of endometritis, and will cause systemic illness.

Key words : Endometritis, bovine, immunomodulators, uterine defence

The uterine defence mechanisms against contaminant microorganisms are 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 (PMNs) and humoral antibodies, however, the degree of interaction is not clear (Liu and Cheung, 1986; Yadav and Agarwal, 2002). Disruption of these mechanisms allows opportunist pathogens, mostly microorganisms found in the posterior gastro-intestinal tract and around the perineal area (Paisley *et al.*, 1986; Hussain *et al.*, 1990), to colonise the endometrium and cause endometritis (Vandeplassche and Bouters, 1976). In the present paper immunology of bovine uterus and immunological strategies to resolve endometritis have been discussed.

Inflammation of the endometrium may occur following coitus, artificial insemination (AI), or more commonly after parturition. In the majority of cattle, 1-4 weeks after calving, microorganisms contaminate the uterine lumen but self-cure usually occurs within 6 weeks post-partum. Cows, unable to eliminate infection endometritis may develop subsequently. A diagnosis is usually made either at routine examination after calving

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or when the cow is bred. Endometritis causes significant delay in calving to conception intervals.

A wide variety of bacteria, both Gram-positive and Gram-negative aerobes and anaerobes have been isolated from the bovine uterus post-partum (Olson *et al.*, 1984). The bacterium most frequently isolated is *Arcanobacter pyogenes* (formerly *Actinomyces pyogenes*. formerly *Corynebacterium pyogenes*) (Dohmen *et al.*, 1995). Other bacteria, such as *Streptococci. Staphylococci* and *Escherchia coli* have also been cultured and identified with endometritis of varying severity (Zemjanis, 1980). It has been difficult to identify the most significant microbes associated with endometritis because both pathogenic and non-pathogenic organisms colonise the bovine endometrium, and many of them are fastidious (Studer, 1981). Of the anaerobes cultured from cases of endometritis, *Fusobacterium necrophorum* and *Bacteroides melaninogenicus* have been identified (Olson *et al.*, 1984: Noakes *et al.*, 1989); it is likely that they act synergistically with *A. pyogenes* in severe endometritis (El Azab *et al.*, 1988).

Diagnosis of endometritis is easily made from endometrial biopsy or culture of uterine discharges (Noakes *et al.*, 1989; Dohmen *et al.*, 1995), but these techniques cannot be used in the field as a routine screening method to identify all cows that require treatment. Intrauterine oxygen reductase potential (Eh) and pH may be used in assessing the degree of bacterial contamination of the uterine lumen. Eh values fell (El-Azab *et al.*, 1988), thereby creating an anaerobic environment; this drop may be associated with either microorganism metabolism or increased oxygen consumption by polymorphonuclear inflammatory cells. May (1996) found that the pH of discharge collected from field cases of endometritis varied from 6.9 -7.3, which favoured the growth of *A. pyogenes*.

Uterine cellular immunity : Polymorphonuclear inflammatory cells (PMNs), blood monocytes and tissue macrophages are regarded as the 'professional phagocytes' in the cellular defences against pathogenic microorganisms. Phagocytosis involves chemotaxis, adherence and attachment of PMNs to cell surface antigens presented by the organism before it is ingested by the phagocyte and finally digested. In the uterus, the cellular defence against bacterial contaminants is provided by uterine leukocytes (Vandeplassche and Bouters, 1976; Frank *et al.*, 1983: Romaniukowa, 1984; Vandeplassche, 1984). After experimental intrauterine infection, the PMN population within the uterine lumen usually increases (Watson *et al.*, 1990; Butt *et al.*, 1991).

The cellular immune response in the uterus may be adversely affected by several therapeutic strategies commonly used to treat post partum disorders in cattle (Bouters and Vandeplassche, 1977). For example, manual removal of fetal membranes may inhibit uterine leukocyte phagocytic activity for several days, as does intrauterine administration of most antiseptics and disinfectants (Vandeplassche, 1984). Intrauterine administration of antibiotics also suppresses uterine leukocytic activity; this could have serious implications in the treatment of metritis should the bacteria involved be resistant to the particular antibiotic. Conversely, several intrauterine chemotherapeutic agents may stimulate the uterine defence mechanisms; for example, Lugol's iodine and polyvinyl-pyrrolidone-iodine both cause necrosis of endometrial epithelium (Gustafsson, 1984) with subsequent release of prostaglandin $F_2\alpha$. However, their use should be discouraged since they cause endometrial fibrosis.

Uterine humoral immunity : Immunoglobulin concentrations in uterine secretions reflect both the extent of the endometrial inflammatory process in the face of microbial challenge and its chances of clinical recovery (Aknarzov, 1988). The synthesis of immunoglobulins, classes IgA, IgG and IgM, in the reproductive tract of domestic animals and their significance in its local defense mechanisms has not been studied extensively. Immunoglobulins have been found in bovine uterine secretions and their protective role against pathogens widely reported (Whitmore and Archbald, 1977; Dhaliwal *et al.*, 1996a, 1996b). Following experimental 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 (Corbeil *et al.*, 1974). Their concentrations differ depending upon the site of sampling; IgG predominates in the uterine lumen and IgA in the vagina (Corbeil *et al.*, 1974, 1976; Watson *et al*, 1990). Following systemic immunisation, these immunoglobulins are undetectable in the reproductive tract (Butt *et al.*, 1993). Within the same species, differences recorded in immunoglobulin concentrations of uterine secretions have been attributed to sampling at different stages of the oestrous cycle. Results so far suggest that IgG concentrations are highest at oestrus (Whitmore and Archbald, 1977), but few studies have investigated the specific influence of steroid hormones on immunoglobulin concentrations in genital secretions.

In cows with abnormal puerperium, both IgA and IgG concentrations in uterine fluids rise rapidly as endometritis develops; the concentration of IgA may end up being 2-3 times greater than in healthy cows but IgM remains low (Aknazarov, 1988). IgA is synthesised locally in the bovine uterus at the mucosal surface. IgG is derived from two sites: part of the IgG1 fraction is synthesised locally in the endometrium, while the remaining IgG1 and all the IgG2 are derived from the peripheral circulation (Butt *et al.*, 1993).

Influence of steroid hormones on uterine immune responses : It is generally accepted that the cyclical pattern of steroid hormone concentration, characteristic for different stages of the oestrous cycle, regulates the potential pathogenicity of microorganisms that contaminate the uterus post partum. For example, the endometrium is more susceptible to infection under progesterone than oestrogen dominance. The precise mechanisms responsible for this are unclear, but several hypotheses have been propounded. During the oestrogen phase of the ovarian cycle there is increased blood flow to the uterus, increased mucus production, and intensified PMN activity (Hawk *et al.*, 1964). In the luteal phase, there is reduced endometrial epithelial permeability to bacteria that delays leukocyte stimulation, and an absence of detoxifying agents in uterine secretions. Ovarioectomized cows, treated with progesterone, develop infection more frequently after experimental infection with A. pyogenes than those treated with oestradiol (Carson *et al.*, 1988).

Dynamics of uterine PMN count and their functional activity during different phases of the oestrous cycle in healthy cows remains largely undefined. There are wide variations in the leukocyte population, especially PMNs, in histological sections of endometrium and in uterine flushings across different stages of the oestrous cycle (Priedkains, 1987; Klucinski *et al.*, 1990; Ohtani *et al.*, 1993; Faundez *et al.*, 1994). In general, the percentage of PMNs is higher during the met-oestrus period compared to other stages of the oestrus cycle (Klucinski *et al.*, 1990; Faundez *et al.*, 1994). Hawk *et al.* (1964) showed a delayed leukocyte infiltration response in endometrium of heifers following intrauterine bacterial challenge during the luteal phase compared to the oestrus phase. The uterine PMN count following intrauterine inoculation of chemoattractants, such as oyster glycogen, was significantly higher (Subandrio and Noakes, 1992) or tended to be higher (Chacin *et al.*, 1990) during the luteal phase than the follicular phase.

Functional activity of PMNs migrating into the uterus could also be affected by the influence of steroid hormones. Treatment of ovariectomized cows with oestrogen increased phagocytic activity of PMNs derived from the uterine lumen (Kerns *et al.*, 1985). However, Anderson *et al.* (1985) found that stage of the oestrous cycle had no effect on phagocytic activity of uterine-derived PMNs, collected after intrauterine infusion of oyster glycogen. Chacin *et al.* (1990) also found no evidence that migration or activity of uterine PMNs is greately reduced during dioestrus or in progesterone treated ovariectomized cows, though there was a tendency for greater PMN activity in animals under oestrogen influence. Concentrations of uterine luminal immunosuppresant proteins capable of inhibiting lymphocyte proliferation have been reported to elevate during the luteal phase (Segerson *et al.*, 1984). In ovariectomized heifers, the immunosuppressive activity of uterine secretions was enhanced following treatment with progesterone alone (Chacin *et al.*, 1990) or a combination of oestrogen and progesterone (Segerson *et al.*, 1986). Alterations in the migration of PMNs are unlikely to be the cause of decreased resistance of the uterus to infections during dioestrus, but progesterone may induce an immunosuppresant molecule in the uterine lumen which inhibits lymphocyte proliferation rather than acting itself as an immunosuppresant (Chacin *et al.*, 1990).

Role of immunomodulators in the treatment of endometritis : Much of the original research associated with immunomodulators of the uterine defense mechanism and their application as treatments for metritis or endometritis has been carried out in mares; more recently, Anderson *et al.* (1985), Hussain and Daniel (1992), Subandrio and Noakes (1992) reported similar work in cattle.

***E. coli* lipopolysaccharides** : When placed in the uterine lumen by infusion, *E. coli* lipopolysaccharides (LPS) are thought to act as a chemoattractant to PMNs. This increase in numbers of PMNs in the endometrium may help to resolve endometritis in both cows and mares (Asbury and Hansen, 1987; William *et al.*, 1987). A preliminary study by Targowski (1984) showed that intrauterine infusion of 100mg *E. coli* LPS in healthy cows increased the PMN number found in uterine secretions 6 and 24 hours after administration. Similar results were obtained after LPS treatment of both healthy cows and those where endometritis was experimentally induced (Hussain and Daniel, 1992) which suggested that this may offer an alternative therapy to the traditional use of intrauterine antibiotics, or parenteral prostaglandin. This treatment did not alter peripheral white blood cell counts or cause systemic illness in any cow. Corresponding results have been described following intrauterine administration of LPS in ewes and mares. When *E. coli* endotoxin has been similarly administered to cows 5 days after calving at rates of 5mg per kg bodyweight, the toxin was absorbed with associated transient clinical signs; however, when given 15 days later, there was no such effect (Peter *et al.*, 1990).

Oyster glycogen : PMN migration into the uterine lumen of healthy cows is stimulated after intrauterine administration of oyster glycogen (OG), up to 90% of all cells identified in uterine secretions being neutrophils (Anderson et al, 1985; Chacin *et al.*, 1990; Subandrio and Noakes. 1992). Variable concentrations of OG between 0.1 - 10%, all in 60ml of vehicle, produced identical responses with a peak in PMN concentration 12 hours after administration (Anderson *et al.*, 1985). Measurable IgG concentrations were found in uterine secretions following treatment, but no IgA; similar results were obtained in ovariectomized cows following both exogenous progesterone and oestrogen administration (Chacin *et al.*, 1990).

Leukotriene B4 : Leukotriene B4 (LT B4) is an effective chemoattractant, stimulating preferential migration of PMNs into the lumen of the bovine uterus (Zerbe *et al.*, 1996). A single intrauterine treatment of a 30-nmol/L solution increased the intrauterine leukocyte count 5-10 times within 24 hours. Before stimulation, PMNs comprised 25-30% of the total leukocyte count, after treatment it increased to 85%. Polymorphonuclear extracts and granulocyte-macrophage colony stimulating factor (GM-CSF; lymphokines) are highly effective chemoattractants in mares, but their activity in cattle has not been studied.

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