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Research paper

Leucocyte phagocytosis during the luteal phase in bitches

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ABSTRACT

Pyometra is a disease that affects a large proportion of intact bitches, and typically is seen during the latter half of dioestrus. Several factors contribute to the development of pyometra, including genetic factors, an infectious component (most often *Escherichia coli*), and hormonal factors. Hormones may act directly on the endometrium, and also affect the immune system. In dogs, the phagocytic ability has been shown to decrease with age, and ovarian hormones have also been shown to affect immune resistance. The aim of the present study was to examine whether phagocytosis by canine leucocytes varies significantly during the luteal phase. Eight bitches were followed by repeated blood sampling. Samples were taken at the calculated optimal day for mating (Day 1), and thereafter on days 8, 15 and 22 (early luteal phase) and 29, 43, 57 and 71 (late luteal phase). Blood was collected from the cephalic vein into EDTA tubes for leucocyte counts and heparinised tubes for testing of phagocytosis and oxidative burst using commercial kits and flow cytometry. The cell activity of the phagocyting leucocytes, expressed as mean fluorescence activity, MFI, was significantly lower during late luteal phase than during early luteal phase. The proportion of leucocytes that was induced to phagocyte did not differ significantly. The percentage of cells stimulated by *E. coli* to oxidative burst was significantly lower during late luteal phase. Their activity did not differ between the two periods. The number of cells stimulated to oxidative burst by a low stimulus was too low to evaluate, and leucocytes stimulated with the high stimulus did not vary in oxidative burst between the two periods. The changes in phagocytic activity and in the number of leucocytes that showed oxidative burst were not associated with any change in the proportion of different leucocytes. The decreased phagocytic capacity possibly contributes to the higher incidence of diseases such as pyometra during the latter part of the luteal phase.

1. Introduction

Uterine diseases are common in intact bitches, and pyometra affects nearly 25% of all bitches by the age of 10 years (Egenvall et al., 2001). Several factors contribute to the development of pyometra, including genetic, infectious and hormonal factors. The importance of genetics is demonstrated by the strong breed predilection for pyometra (Egenvall et al., 2001). Risk factors such as parity and oestrogen administration have been demonstrated (Niskanen and Thrusfield, 1998), but risk and protective factors may vary between breeds (Hagman et al., 2011). There is an infectious component in pyometra,

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and *Escherichia coli* is the bacterium most commonly isolated (Fransson et al., 1997; Hagman and Greko, 2005). Subclinical and clinical urinary tract infection is associated with pyometra (Hagman and Kuhn, 2002; Sandholm et al., 1975), and the gastrointestinal tract may also serve as a reservoir (Tsumagari et al., 2005). Pyometra typically occurs during dioestrus, and most cases are seen from 4 weeks to 4 months after oestrus (Dow, 1957; Smith, 2006). Bacteria may enter the uterus during oestrus (Watts et al., 1998), and the influence of progesterone on the endometrium following oestrogen stimulation is thought to predispose to pyometra (Dow, 1959). The simultaneous presence of corpora lutea and follicles has been described in bitches with pyometra (Strom Holst et al., 2001), presumably reflecting a hormonal imbalance. It has also been suggested that the down regulation of oestrogen receptors in the endometrium under the influence of progesterone is defective in bitches with cystic endometrial hyperplasia (De Cock et al., 1997), predisposing to pyometra.

Hormonal changes during the oestrous phase may also increase the risk of pyometra by immunological mechanisms, via changes in the innate immune system. The innate immune system is the first line of host defence against invading microorganisms, and includes, among other mechanisms, phagocytosis of pathogens and cells (Kobayashi and DeLeo, 2009). Neutrophils are the predominant phagocytic cells in peripheral blood and are the most important cellular component of innate immunity. The phagocytic process can be separated into several steps: chemotaxis, attachment of the particles to the surface of the phagocytic cell, phagocytosis (ingestion), and intracellular killing by oxygen-dependent (oxidative burst) and oxygen-independent mechanisms (Hostetter, 2012).

In septic dogs, phagocytic activity has been shown to increase whereas oxidative burst decreases (Webb et al., 2007). The innate immune response, including phagocytic ability, also changes during different physiological states. It has been shown to decline with age in beagle dogs (Hall et al., 2010). Innate immunity has been described to vary with reproductive state in humans. In pregnant women, among the most substantial immunologic deviations are an increase in circulating granulocytes and a decrease in lymphocytes, with additional activation of monocytes and granulocytes (Luppi et al., 2002). In bovines, progesterone reduces oxidative burst in vitro when added to leucocytes (Chaveiro and Moreira da Silva, 2010). Ovarian hormones have been shown also to affect immune resistance in dogs (Sugiura et al., 2004). The proliferative response of peripheral blood mononuclear cells (PBMNCs) to *E. coli* increased in proestrus/oestrus and decreased by day 10 of dioestrus (Sugiura et al., 2004). The proliferative response and expression of gamma interferon of cell cultures of PBMNCs collected in anoestrus was also enhanced upon addition of estradiol -17β, and suppressed by progesterone (Sugiura et al., 2004). A differential localisation and expression of toll-like receptor 4, also part of the innate immune system, has been described in the canine endometrium throughout the oestrous cycle and in pyometra (Chotimanukul and Sirivaidyapong, 2011).

The aim of the present study was to examine whether phagocytosis by leucocytes in peripheral blood varies significantly during the luteal phase in bitches.

2. Materials and methods

2.1. Animals and sampling procedure

Eight bitches were included in the study: five beagles, one German shepherd, one rottweiler and one cross bred bitch. Their mean age was 3.4 years (SD 1.2). The study period was from September 2009 to May 2010. Two mL whole blood samples were collected from the cephalic vein in EDTA Vacutainer tubes (K_3EDTA, Vacuette, Hettich Labinstrument AB, Sollentuna, Sweden) and in heparinised Vacutainer tubes (Lithium heparin, Vacuette, Hettich Labinstrument AB, Sollentuna, Sweden), which were properly filled and kept at room temperature until analysis. All samples were analysed within 2 h of collection. The study was approved by the Uppsala Ethical Committee of Animal Experimentation (C23/9) and the Swedish Board of Agriculture (31-1365/09).

2.2. Analysis of oestrous cycle stage

The bitches were followed with vaginal cytology and blood samples for progesterone assays during oestrus. Cytological staging was done according to Schutte (1967a,b). The day of ovulation was defined as the day when progesterone levels reached 15–24 nmol/L.

2.3. Experimental design

Based on progesterone levels, day 0 of the study was set at as the day optimal for mating, 2–5 days after ovulation, with progesterone levels higher than 30 nmol/L and cytological oestrus. Thereafter, blood samples were taken on days (±2) 1, 8, 15 and 22 (early luteal phase) and days 29, 43, 57 and 71 (late luteal phase). Progesterone values during the luteal phase are shown in Fig. 1.

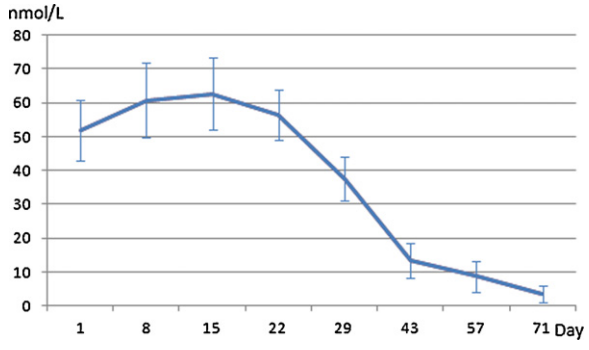

Fig. 1. Serum concentrations of progesterone (nmol/L) during the luteal phase. Mean values and 95% confidence intervals. Early luteal phase: Days 1–22; late luteal phase: Days 29–71.