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Infections distant to the ovary impact ovarian function mediated by innate immune mechanisms

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ABSTRACT

After parturition many dairy cattle do not conceive within the desired time period whilst others are infertile. Bacterial infections that cause uterine disease or mastitis are common during this period and these animals also have ovarian dysfunction, with perturbed follicle growth and steroidogenesis. The pathogen-associated molecular pattern (PAMP), lipopolysaccharide, from Gram-negative bacteria is present in the ovarian follicular fluid of animals with uterine disease. However, the mechanisms linking ovarian dysfunction to bacterial infections at sites distant to the ovary are intriguing because healthy ovarian follicles are devoid of immune cells. Granulosa cells from ovarian follicles expressed most Toll-like receptors (TLRs) that detect PAMPs, and secreted inflammatory mediators IL-6 and IL-8 in response to bacterial PAMPs via TLR-dependent pathways. Throughout follicle development, PAMPs perturbed granulosa cell steroidogenesis and there was cross-talk between endocrine and immune pathways. Cumulus-oocyte complexes mounted inflammatory responses and the meiotic spindle of oocytes was disrupted. In conclusion, PAMPs impact granulosa cell and oocyte health via innate immune mechanisms, which may explain how infections distant to the ovary cause infertility.

KEY WORDS: Cow, ovary, oocyte, bacteria, innate immunity.

INTRODUCTION

After parturition many dairy cows do not conceive within the desired time period whilst others are infertile. Bacterial infections that cause uterine disease or mastitis are common during this period and these animals also have ovarian dysfunction, with perturbed follicle growth and steroidogenesis (Lavon and others 2011, Sheldon and others 2002). Uterine infection after parturition causes metritis in 40% of animals, and the resulting decreased milk yields, delayed ovulation, reduced fecundity, and culling for failure to conceive cost the European Union dairy industry €1.4 billion/year (Sheldon and others 2006, Sheldon and others 2009). Infection of the mammary gland causes mastitis in a comparable proportion of animals, and these infections also reduce conception rates (Huszenicza and others 2004, Perrin and others 2007). Metritis or mastitis also retard follicular growth, reduce circulating and intrafollicular estradiol concentrations, extend luteal phases, and disrupt ovarian cyclic activity. Gram-negative *Escherichia coli* often cause metritis and mastitis, and these infected animals have reduced fecundity even after resolution of clinical disease (Borsberry and Dobson 1989, Hertl and others 2010). Accumulation of lipopolysaccharide (LPS) from Gram-negative bacteria in follicular fluid of animals with metritis may link infection and ovarian dysfunction (Herath and others 2007). The present review will consider mechanisms linking bacterial infection distant to the ovary and perturbation of ovarian function and oocyte quality.

IMMUNITY AND INFLAMMATION

Innate and adaptive immunity are the two main arms of immunity. Innate immunity provides non-specific, immediate alarm-type responses to microbes, initiates inflammation and directs the adaptive immune response. The components of the innate immune system include pattern recognition receptors, antimicrobial peptides, physical tissue barriers, and the complement cascade.The most important are the cellular pattern recognition receptors that detect pathogen-associated molecular patterns (PAMPs) commonly associated with microbes (Ronald and Beutler 2010, Ferrandon and others 2007). This concept was founded on the discovery in the 1990's that

53 "Toll" protein was required for an effective immune response to the fungus *Aspergillus* in *Drosophila*
54 *melanogaster* as well as the previously established role for Toll in embryonic development of the fly
55 (Ronald and Beutler 2010, Lemaitre and others 1996). The next advance was the discovery in mice
56 that Toll-like receptor 4 (TLR4) was necessary for the inflammatory response to the
57 lipopolysaccharide (LPS) cell wall component of bacteria, which acts as a PAMP (Ferrandon and
58 others 2007, Poltorak and others 1998). Subsequently, multiple TLRs were identified that bind
59 PAMPs from viruses, fungi and bacteria. Whilst Gram-negative bacteria are mainly recognised by
60 TLR4 on host cells binding LPS, Gram-positive bacteria are often identified by TLR1, TLR2 and TLR6
61 binding bacterial lipoproteins (Oliveira-Nascimento and others 2012). The TLRs are primarily
62 associated with immune cells, such as macrophages and neutrophils, and generate the initial
63 inflammatory response including production of pro-inflammatory cytokines, such as IL-1β, IL-6, TNFα,
64 and chemokines, such as IL-8 (Takeuchi and Akira 2010).
65
66 The pattern recognition receptors of the innate immune system are important for the detection of
67 microbial infection and PAMPs in the uterus of cattle, which we have reported previously (Cronin and
68 others 2012, Herath and others 2009b, Herath and others 2006b). In brief, the TLRs were expressed
69 by purified populations of epithelial and stromal cells isolated from the endometrium of dairy cattle
70 (Herath and others 2009b, Davies and others 2008). Furthermore, TLR4 was functionally active in
71 endometrial epithelial and stromal cells from many species and was able to detect LPS from *E. coli*
72 leading to the production of prostaglandins, cytokines such as IL-6, and chemokines such as IL-8
73 (Sheldon and Bromfield 2011, Cronin and others 2012, Sheldon and Roberts 2010).
74
75 One aspect that may link uterine infection to ovarian function is the mechanism of luteolysis. Corpus
76 luteum lifespan is often prolonged in animals with bacterial infections, and this may be because the
77 TLR4-mediated response to LPS in the endometrium switches prostaglandin production by the
78 epithelial cells from the luteolytic F series to the luteotrophic E series (Herath and others 2009a).

Conversely, if animals ovulate but still have microbes in the uterus, then progesterone perturbs the TLR4 mediated response to LPS (Herath and others 2006a, Herath and others 2006b). Perhaps removing the negative impact of progesterone on immunity, when animals are treated with prostaglandin $F_{2\alpha}$, helps to resolve endometritis. However, the specific role of innate immunity in the ovary is less clear. Healthy ovarian follicles are devoid of immune cells within the basement membrane, at least in cattle (Spanel-Borowski and others 1997, Bromfield and Sheldon 2011). We therefore considered whether the granulosa cells lining ovarian follicles may play roles in the response to PAMPs.

IMMUNITY AND INFLAMMATION IN THE OVARY

We discovered that bacterial infection of the uterus perturb postpartum ovarian function. Specifically, the presence of bacterial infection in the uterine lumen reduced the rate of ovarian follicle growth and perturbed follicle function as determined by reduced oestradiol secretion (Sheldon and others 2002). Furthermore, the peripheral plasma concentrations of progesterone are lower in cows with uterine disease than those in normal fertile animals (Williams and others 2007). The mechanisms linking uterine disease to ovarian function were not obvious at the time because bacteria are rarely found in the ovary and ovarian follicles are devoid of immune cells such as macrophages. However, LPS was found in the follicular fluid of diseased cows, perhaps reaching the ovary from the uterus by the same vascular mechanisms used by prostaglandin. Furthermore, the granulosa cells that line ovarian follicles expressed TLRs, and PAMPs perturbed their endocrine function (Herath and others 2007, Bromfield and Sheldon 2011). Granulosa cells also have increased abundance of mRNAs that encode cytokines and chemokines in response to PAMPs and this mechanism applies across species (Bromfield and Sheldon 2011, Sheldon and Bromfield 2011, Price and others 2012). The essential role of granulosa cells in innate immunity was confirmed by targeting TLR4 using siRNA, which reduced TLR4 expression and these cells secreted less IL-6 protein in response to LPS (Bromfield and Sheldon 2011).

IMMUNITY AND THE OOCYTE

A further question was whether perturbations of the ovarian antral follicle were the only mechanism linking uterine disease to lower ovarian fertility, or whether there may also be a direct impact on the egg. Mammalian oocyte growth and maturation from the primordial follicle until ovulation is dictated by a highly ordered cascade of hormones, growth factors, nutrients, and signalling molecules from the surrounding environment (Matzuk and others 2002, Albertini and others 2001). *Ex vivo*, LPS increases primordial follicle activation in the ovarian cortex, which reduces the primordial follicle pool (Bromfield and Sheldon 2013). In addition, ovarian cortex cultures produce the inflammatory mediators IL-1β, IL-6 and IL-8 in a LPS concentration-dependent manner and modulated typical intracellular regulators of follicle activation (Bromfield and Sheldon 2013). The reduction in the primordial ovarian follicle pool in the bovine ovarian cortex could help explain why infections around calving have longer term effects on fertility.

Oocytes must undergo nuclear and cytoplasmic maturation for successful fertilization and embryonic development, and oocytes progress from the germinal vesicle stage until pausing at the M-phase of meiosis II. The oocyte is nurtured by the surrounding cumulus granulosa cells via trans-zona projections from the cumulus cells which cross the zona pellucida and synapse on the oolema, allowing bidirectional communication between the granulosa and oocyte (Albertini and others 2001, Matzuk and others 2002). These intimate interactions expose mammalian oocytes to exogenous factors more than eggs enclosed in an impermeable shell. We therefore considered whether in the absence of immune cells, cumulus granulosa cells may play a role in protecting mammalian oocytes against PAMPs. Indeed, the cumulus granulosa cells mount inflammatory cytokine responses to PAMPs (Bromfield and Sheldon 2011). Furthermore, we were the first to show in any species that the meiotic spindle, containing the chromosomes that should form the embryo, was disrupted in oocytes

treated with LPS (Bromfield and Sheldon 2011). This damage of oocytes by PAMPs may help explain how infertility persists beyond the duration of clinical uterine disease in dairy cows.

CONCLUSIONS

Bacterial diseases are common and an important cause of infertility in cattle, which includes perturbation of ovarian function. The granulosa cells lining ovarian follicles and nurturing the oocyte have roles in innate immunity and respond to PAMPs via TLR pathways. Furthermore, even the oocyte is susceptible to damage by PAMPs. These findings provide mechanisms linking bacterial infections distant to the ovary with infertility.

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