Perspectives: Possible roles of polymorphonuclear neutrophils in angiogenesis and lymphangiogenesis in the corpus luteum during development and early pregnancy in ruminants1

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ABSTRACT: The establishment of pregnancy requires well-balanced regulation of the endocrine and immune systems and involves interactions among the conceptus, oviduct–uterus, and corpus luteum (CL). In particular, a rapid increase in plasma progesterone during the first week after ovulation is critical for the growth of the conceptus and successful pregnancy in cattle. Events involved in maternal recognition of pregnancy (MRP) may commence within 1 wk from AI, when interferon-stimulated gene expression in circulating polymorphonuclear neutrophils (PMN) increases in pregnant cows. To regulate optimal endocrine conditions within this time, the CL must develop rapidly, with active angiogenesis and lymphangiogenesis. The major angiogenic factors, vascular endothelial growth factor and fibroblast growth factor 2, contribute to the development of the CL but may also act as chemoattractants for PMN. Indeed, the number of PMN is greatest in the new CL, where PMN together with IL-8 induce active angiogenesis and lymphangiogenesis. During MRP, the conceptus secretes interferon tau (IFNT), which prevents CL regression by inhibiting luteolytic release of PGF_2α from uterine endometrium. In addition, IFNT and PGE_2 reach the CL and may contribute to desensitizing the CL to the luteolytic effects of PGF_2α. In the bovine CL, lymphangiogenesis, stimulated by IFNT, may occur during MRP, and thus a shift of local immunity might occur at this timing. The aforementioned evidence supports the possible involvement of PMN in the establishment of pregnancy via CL regulation. Further investigation could expand our understanding of the communication between zygotes, PMN, and reproductive organs during early pregnancy. This should provide new insight into the contribution of neutrophils to CL function and immune tolerance during early pregnancy in ruminants.

Key words: angiogenesis, corpus luteum, early pregnancy, lymphangiogenesis, neutrophils, ruminants

INTRODUCTION

The establishment of pregnancy, regulated by well-balanced endocrine and immune systems, involves interactions among the conceptus, oviduct–uterus, and corpus luteum (CL). In particular, a rapid increase in the plasma progesterone level during the first week after ovulation is critical for growth of the conceptus and successful pregnancy in cattle (Mann et al., 1999). The CL forms rapidly from the ovulated follicle and secretes progesterone, thereby supporting implantation and the maintenance of pregnancy. Angiogenesis and lymphangiogenesis are essential for the formation of the CL structure during development and, thus, its ability to generate active steroidogenesis and secrete high levels of progesterone into the circulation (Reynolds and Redmer, 1999).

Recently, the critical importance of ovarian angiogenesis, including its role in CL function, was intensively discussed in a review series published elsewhere (Duncan and Nio-Kobayashi, 2013; McFee and Cupp, 2013; Meidan et al., 2013; Robinson, 2013; Shirasuna et al., 2013). Several major components that regulate angiogenesis in cattle (Mann et al., 1999). The CL forms rapidly from the ovulated follicle and secretes progesterone, thereby supporting implantation and the maintenance of pregnancy. Angiogenesis and lymphangiogenesis are essential for the formation of the CL structure during development and, thus, its ability to generate active steroidogenesis and secrete high levels of progesterone into the circulation (Reynolds and Redmer, 1999).
CL development and maintenance during early pregnancy overlap in the regulation of steroidogenesis, angiogenesis, vascular function, and the innate and adaptive immune systems (Pate and Keyes, 2001; Schams and Berisha, 2004; Miyamoto et al., 2010, 2013; Pate et al., 2010; Shirasuna et al., 2012b; Walusimbi and Pate, 2013). However, the local immune events that occur in the CL during early pregnancy remain unclear. It has been proposed that innate immunity orchestrates follicular atresia, follicle rupture, and follicle transformation into the CL as well as CL regression through sterile inflammation and tissue repair (Spanel-Borowski, 2011).

Of note, the major innate immune cells, polymorphonuclear neutrophils (PMN), might be involved in maternal recognition of pregnancy (MRP) in cows (Shirasuna et al., 2012a; Kizaki et al., 2013). Because investigation of PMN and the innate immune system in reproductive phenomena related to the regulation of CL function and early pregnancy has just begun, findings are limited. However, these findings may provide a broader view of the reproductive biology associated with the establishment of pregnancy. Therefore, we provide a brief perspective on the possible role of neutrophils in angiogenesis and lymphangiogenesis in the CL during the establishment of pregnancy in ruminants.

**ROLE OF THE INNATE IMMUNE SYSTEM IN ANGIOGENESIS DURING CORPUS LUTEUM FORMATION**

*Angiogenesis and Lymphangiogenesis in Corpus Luteum Formation*

The LH surge is the main trigger of ovulation and luteinization. Both LH and GH are necessary for normal luteal development ad function (Niswender et al., 2000; Schams and Berisha, 2004; Kobayashi et al., 2001). Following the preovulatory LH surge, follicular cells undergo morphological, endocrinological, and biochemical changes associated with luteinization. The LH surge sharply increases the local production of angiogenesis and lymphangiogenesis in the CL during the establishment of pregnancy in cows (Schams et al., 2004; Miyamoto et al., 2010, 2013; Pate et al., 2010; Shirasuna et al., 2012b; Walusimbi and Pate, 2013). However, the local immune events that occur in the CL during early pregnancy remain unclear. It has been proposed that innate immunity orchestrates follicular atresia, follicle rupture, and follicle transformation into the CL as well as CL regression through sterile inflammation and tissue repair (Spanel-Borowski, 2011).

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Analysis of the expression of VEGFA, its receptor (i.e., VEGFR-2), and FGF2 and its receptors showed that the VEGF and FGF systems peaked during the early luteal phase, decreased during the mid and late luteal phases, and further decreased during luteal regression in cows (Schams et al., 1994; Berisha et al., 2000). For the formation of luteal vascular networks, FGF2 is more crucial than VEGFA. It is likely that FGF2 plays a key role in the initiation of angiogenesis during the very early stages of luteal development in cows (Woad et al., 2009). These findings are consistent with the finding that antibody neutralization of VEGFA or FGF2 during the early luteal phase in cows and marmoset monkeys suppressed VEGFA and FGF2 expression, inhibited endothelial cell proliferation, and reduced the plasma progesterone concentration (Fraser et al., 2000; Yamashita et al., 2008).

In the CL, the VEGFC/VEGFD-VEGFR3 system regulates lymphangiogenesis and CL formation in primates (Xu and Stouffer, 2009) and in mice (Rutkowski et al., 2013). This lymphangiogenic system has also been identified in the CL of cattle (Nitta et al., 2011) and water buffalo (Ali et al., 2014). The system is most active in the mid-cycle CL. These investigations indicate that the bovine CL possesses lymphatic vessels as well as blood vessels, which appear to be a prerequisite for driving immune cells in the CL.

**The Innate Immune System in Developing Corpus Luteum**

Lobel and Levy (1968) first described the presence of white blood cells in the bovine CL. Later, experimentally induced lymphopenia was shown to cause luteal dysfunction in cattle (Alila and Hansel, 1984). While cluster of differentiation (CD) 4- and CD8-positive T cells, involved in adaptive immunity, localize to the CL throughout the estrous cycle, the number of innate immunity-related inflammatory cells, including neutrophils, macrophages, and eosinophils, increases sharply in the developing CL.

In the innate immune system, PMN primarily act as a cellular defense during acute inflammatory reactions. Large numbers of neutrophils have been detected in the human CL throughout the menstrual cycle (Brannstrom et al., 1994b) and in the rat CL during early pregnancy (Brannstrom et al., 1994a). In a recent study, we observed a large number of PMN, along with a high level of IL-8 (a potent neutrophil chemotactant), during the early luteal phase in cows (Jiemtaweeboon et al., 2011). The supernatant from cultures of early CL induced PMN migration in vitro and increased IL-8 production; IL-8 stimulated endothelial cells of the CL to form capillary-like structures, indicating that IL-8 acts as a major PMN chemoattractant and a strong stimulator of angiogenesis in the early CL (Jiemtaweeboon et al., 2011). Interestingly, the major angiogenic factors VEGFA and FGF2 also enhanced PMN migration in vitro (K. Shirasuna and A. Miyamoto, unpublished data). In fact, PMN and IL-8 can induce angiogenesis in vivo (Koch et al., 1992) and in vitro (Yasuda et al., 2000; Schruefer et al., 2005), indicat-
ing that the PMN and IL-8 duo generates angiogenesis in the developing CL together with VEGFA and FGF2. The concept of functional polarization of neutrophils (classic proinflammatory N1 vs. novel anti-inflammatory N2) has been proposed to explain their action in angiogenesis (Fridlender et al., 2009).

The number of macrophages in the CL increases during the early stages of development in cows (Penny et al., 1999) and humans (Gaytan et al., 1998). In response to local cytokines and other signals, macrophages differentiate to acquire a functional phenotype that is specific to the requirements of the tissue. Recently, it was reported that the conditional ablation of macrophages disrupts the ovarian vasculature in mice (Turner et al., 2011).

Polymorphonuclear eosinophils have been detected in the CL during the estrous cycle in several species (Murdoch, 1987; Aust et al., 2000). Eosinophils are involved in parasitic infections, asthma, and allergic conditions. The number of eosinophils in the CL increases rapidly during the early stages of development to comprise approximately 90% of the CD18+ leukocytes in cows (Reibiger and Spanel-Borowski, 2000). The expression of P-selectin on endothelial cells appears to recruit eosinophils into the developing CL (Aust et al., 2000). An in vivo bovine model using dexamethasone-induced eosinopenia showed lower plasma progesterone concentrations with reduction of VEGFA protein production in the CL (Kliem et al., 2013). This indicates that the migration of eosinophils into the early CL is an important, but not essential, stimulus for angiogenesis during the early stages of CL development.

**TRANSITION TO THE CORPUS LUTEUM OF EARLY PREGNANCY**

*Angiogenesis in the Corpus Luteum during Early Pregnancy*

When pregnancy is established, embryonic trophoblasts secrete interferon tau (IFNT), a well-known pregnancy recognition signal in ruminants (Imakawa et al., 1987). Interferon tau indirectly maintains the CL by inhibiting luteolytic pulses of uterine PGF$_{2\alpha}$ while not affecting basal secretion during diestrus in ewes (Zarco et al., 1988) but attenuating basal secretion as well as release of PGF$_{2\alpha}$ pulses in cows (Meyer et al., 1995) at approximately d 15 to 18 postinsemination. This period is, therefore, defined as the maternal recognition period for pregnancy (Spencer et al., 2004). Additionally, IFNT appears to act in an endocrine manner to make the CL refractory against the luteolytic action of PGF$_{2\alpha}$ in ewes (Antoniazzi et al., 2013). Thus, changes in CL function, including those involving the vasculature and endothelial cells, are critical during early pregnancy in ruminants. The same number of endothelial cells persisted from the late luteal phase until early pregnancy in cows (Beindorff et al., 2010). On the other hand, the number of pericytes and smooth muscle cells decreased sharply in the CL during early pregnancy in cows (Beindorff et al., 2010). In addition, the expression of VEGFA did not change, indicating that the vasculature does not grow but becomes thinner from the late luteal phase of the nonpregnant cycle to early pregnancy (Beindorff et al., 2010). In humans, VEGFA expression is greater in early pregnancy than in the mid-luteal phase (Sugino et al., 2005). However, an increase in VEGFA expression was not observed in the CL of marmosets (Rowe et al., 2002) and cattle (Berisha et al., 2000) during early pregnancy. Thus, it is likely that angiogenesis is not promoted in the CL of cows during early pregnancy.

**Lymphangiogenesis in the Corpus Luteum during Early Pregnancy**

The vascular system comprises blood and lymph vessels. The lymphatic vascular system is essential for maintaining interstitial fluid pressure equilibrium and transporting tissue fluid, proteins, and cells (Wang and Oliver, 2010). The lymphatic system is also crucial during the immune response to infectious agents, because lymphatic vessels are the route by which dendritic cells and macrophages migrate to the lymph nodes and lymphoid organs to present antigen to T cells. We recently found that the expression of a lymph vessel marker, lymphatic endothelial hyaluronan receptor 1 (LYVE1), was increased in the bovine CL at d 40 of pregnancy; furthermore, expression of the lymphangiogenic factors, VEGFC and LYVE1, in the CL increased on d 16 of pregnancy (Nitta et al., 2011). These results indicate that the lymphatic system, but not the blood vascular system, of the bovine CL is reconstituted during early pregnancy (Nitta et al., 2011). Interestingly, the progesterone concentration in ovarian lymph vessels was greater than that in uterine lymph or ovarian venous plasma during all stages of pregnancy in cows (Hein et al., 1988). An injection of soluble VEGFR3, which acts as an anti-VEGFR3 antibody, into the preovulatory follicle inhibited follicle rupture and ovulation and suppressed progesterone production in the CL of monkeys (Xu and Stouffer, 2009). Additionally, we found that IFNT stimulated lymphatic endothelial cells to proliferate and form capillary-like tubes in vitro (Nitta et al., 2011). Moreover, the number of PMN that migrated into the CL during early pregnancy was greater than the number in the cyclic mature CL, and IFNT
was able to stimulate bovine PMN to migrate in vitro (Shirasuna et al., 2011).

**SUMMARY AND CONCLUSIONS**

In ruminants, the maternal progesterone concentration has a marked influence on the development of the embryo and its ability to produce IFNT. Thus, progesterone plays a vital role in determining the fate of an embryo. Increases in CL size, plasma progesterone concentration, and luteal blood flow occur in parallel over the first 7 d after ovulation (Acosta et al., 2003). This underlines the impact of the rapid formation of CL structure and vasculature on CL function. It is clear that VEGFA and FGF2 play a central role in promoting angiogenesis during CL development. This local system is first triggered by the LH surge and then amplified by local regulators, including IL-8 and innate immune cells (i.e., neutrophils, macrophages, and eosinophils) recruited into the developing CL.

In the CL of early pregnancy, IFNT can induce lymphangiogenesis but not angiogenesis. This might relate to changes in the local immune environment in the CL at MRP. For example, the CL of early pregnancy expresses greater levels of forkhead box P3 and PGE₂, both of which generally relate to immune tolerance (Lee et al., 2012; Magata et al., 2012). In addition, in cows, circulating PMN are upregulated in interferon-stimulated gene (ISG) expression on d 5 to 14 of pregnancy compared with those of nonpregnancy (Shirasuna et al., 2012a; Kizaki et al., 2013). Neutrophils were more sensitive than lymphocytes to IFNT stimulation in vitro (Shirasuna et al., 2012a). Moreover, because PMN are present in the bovine oviduct fluid under physiological conditions around the time of ovulation (Marey et al., 2014), they might contact the sperm or embryo in the oviduct.

Clearly, maternal immunological adjustments to pregnancy are largely regulated by adaptive immunity (Hansen, 2013). The aforementioned evidence supports the possible involvement of PMN in these phenomena. Further investigations could expand our understanding of the communication between zygotes, PMN, and reproductive organs during early pregnancy. This should help in providing new insight into the contribution of neutrophils to CL function and immune tolerance during early pregnancy in ruminants.

**LITERATURE CITED**


