Blood and lymphatic vasculature in the ovary: development, function and disease

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BACKGROUND: The remodelling of the blood vasculature has been the subject of much research while rapid progress in the understanding of the factors controlling lymphangiogenesis in the ovary has only been reported more recently. The ovary undergoes cyclic remodelling throughout each menstrual/estrous cycle. This process requires significant vascular remodelling to supply each new cohort of growing follicles.

METHODS: Literature searches were performed to review studies on the ovarian lymphatic vasculature that described spatial, temporal and functional data in human or animal species. The role of ovarian blood and lymphatic vasculature in the pathogenesis of ovarian disease and dysfunction was also explored.

RESULTS: Research in a number of species including zebrafish, rodents and primates has described the lymphatic vasculature within the remodelling ovary, while recent research in mouse has confirmed hormonal regulation of lymphangiogenic growth factors, their receptors and also a role for the protease, ADAMTS1, in the development of the lymphatic vasculature. With a critical role in the maintainence of fluid homeostasis, the ovarian lymphatic vasculature is important for normal ovarian function and has been linked to syndromes involving ovarian fluid imbalance, including ovarian hyperstimulation syndrome and massive ovarian edema. The lymphatic vasculature has also been heavily implicated in the metastatic cancer process.

CONCLUSION: The spatial and temporal regulation of the ovarian lymphatic vasculature has now been reported in a number of species and the data also implicate the ovarian lymphatic vasculature in ovarian pathologies, including cancer and those linked with use of artificial reproduction technologies.

Key words: blood / lymphatic / vessel / remodelling / ovary
Introduction

While the processes surrounding angiogenesis and the development and function of blood vascular networks in the ovary have received significant attention over the past few decades, our understanding of the development and function of the lymphatic vasculature has advanced only recently due to a combination of technologies including selective lymphatic markers, genetically modified mouse models and pharmaceutical development. The female reproductive organs, including the ovary and uterus, possess unique capacity to remodel tissue structure and vascular networks with each reproductive cycle. Such active remodelling is more often observed in pathological systems including wound healing and cancer development and metastasis. In the ovary these processes are continuous and under tight control. This review explores the development and remodelling processes of the blood and lymphatic vasculature in association with the cyclic remodelling events within the ovary. A rich and highly fenestrated blood vascular network supplies the ovary with hormones and the nutrients required for its high rate of metabolism, as well as matrix factors that are incorporated into the cumulus–oocyte complex prior to ovulation. Hundreds of growing follicles within the ovary at any one time actively recruit and remodel a vascular bed necessary to sustain their changing needs as they increase in size and complexity. After ovulation, the formation of a corpus luteum (CL) involves the most rapid angiogenic event known outside of aggressive pathological processes. The high levels of progesterone production by CL and its transport to the uterus to support endometrial preparation and maintenance of pregnancy depend on a robust vascular network (Reynolds et al., 1992; Augustin et al., 1995; Gordon et al., 1995). The reproductive organs are thought to be the only system that continually undergoes a remodelling process throughout adulthood, making it an excellent model in which to study controlled tissue morphogenesis, as well as angiogenesis. While research has predominantly focused on the ovarian blood vasculature to date, this review explores the presence, regulation and possible functional roles of the lymphatic vasculature in the ovary.

Methods

We searched Pubmed for items published until May 2013, including clinical and experimental, in vivo and in vitro studies in all species, using the following search terms: ovary AND lymphatics or ovary AND lymphangiogenesis: the search yielded 632 and 14 publications, respectively. Reference lists of the preselected articles, which included review articles, were also searched. After detailed screening of titles, abstracts and full texts, we selected the studies published in the English language, evaluating lymphatic vasculature within the ovary. Additional literature was sourced following a search of ovary AND vasculature (3487 publications) for comparison between the blood and lymphatic vasculature in the ovary. The data were then interpreted and summarized by all authors. No quantitative or statistical analysis was performed.

The lymphatic vasculature

The lymphatic vascular system has been recognized anatomically for centuries (Aselli, 1627; Nakamura and Rockson, 2007) but more recent identification of specific markers, gene ablation models and other molecular strategies led to the discovery of lymphatic-specific growth factors, receptors and the elucidation of the molecular mediators underlying lymphatic development (reviewed in Alitalo, 2011). The lymphatic vascular system plays three major physiological roles. First, it is responsible for mediating tissue fluid homeostasis by collecting extra-vascular fluid and proteins and transporting them back to the bloodstream, and therefore contributing to fluid reabsorption and tissue perfusion (Alitalo et al., 2005). Secondly, the lymphatic system is involved in absorption of lipids and the fat-soluble vitamins A, D, E and K within the gastrointestinal tract. Thirdly, in tight association with the blood vasculature, the lymphatic vasculature is integral for immune cell trafficking. More recently, pathophysiological roles, including cancer metastasis to lymph nodes and other organs, have been associated with the lymphatic vasculature, and has prompted a rejuvenated interest in research into the structure–function and regulation of the lymphatic system.

The lymphatic system is part of both the circulatory and immune systems, its functions stretching as widely as the gastrointestinal system and has only been reported to be absent from the brain and retina in addition to known avascular structures including epidermis, hair, nails, cartilage and the cornea (Hong and Detmar, 2003). The lymphatic vascular system is prevalent in the organs in direct contact with the external environment, including the skin, lungs and gastrointestinal tract and as such mediates the first recognition of foreign antigens by the immune system. The lymphatic vasculature comprises blind-ended capillaries, without a structural basement membrane, consisting of an overlapping layer of lymphatic vascular endothelial cells (LEC), lacking either pericyte or smooth muscle coverage. The endothelium is anchored to the underlying extracellular matrix by filamentous structures, allowing for large inter-endothelial openings and permitting passive transport of fluids and large protein moieties. Increased interstitial pressure transfers tension to the filaments and therefore the lymphatic endothelium, resulting in a widening of the vessel lumen and fenestrations, facilitating transport of fluid, macromolecules and cells. The remainder of the lymphatic vascular system comprises larger collecting vessels through which lymph fluid is returned to the venous circulation via the inferior vena cava (Witte et al., 2001). These larger collecting vessels are supported by a basement membrane and smooth muscle, which contributes to the propulsion of lymph. Without a direct pump (as found in the circulatory system) the pressure gradient of the lymphatic vascular system relies on one-way valves found within the vessels as well as changes in intrathoracic pressure generated by respiration and musculoskeletal contraction within the limbs in order to stimulate lymphatic flow (Oliver and Alitalo, 2005).
the formation of rudimentary lymph sacs (Wigle and Oliver, 1999). At E12.5, PROX1-positive, specified LECs sprout from the sac and spread throughout the embryo, whilst the Prox1-negative venous system switches off Vegfr3 and Lyve1. LECs then migrate peripherally, under the control of VEGFC, and undergo higher levels of differentiation, including the accumulation of a number of LEC-specific markers, to achieve lymphatic specification (Nakamura and Rockson, 2007). At E14.5, terminal differentiation is virtually complete. The established primary vessel network forms a hierarchical vascular tree, composed of lymphatic capillaries and collecting vessels. By E15, the lymphatic vasculature is present within distal organs, including the heart, diaphragm, lungs, gut and skin. However, certain organs, specifically the ovary, remain devoid of lymphatics until well after birth.

Gene ablation and overexpression studies have demonstrated roles for VEGFC in lymphatic development. Vegfc-null mice lack a lymphatic vascular network (Karkkainen et al., 2004), whilst ablation of the Vegfr3 gene results in defective blood vessel development in the early embryo, implicating the receptor in both angiogenesis and lymphangiogenesis (Dumont et al., 1998). Overexpression of Vegfd has been shown to promote formation of lymphatic vessels within tumours and promotion of metastasis of tumour cells (Stacker et al., 2001); in contrast, ablation of Vegfd results in only a subtle lymphatic phenotype involving a decrease in the abundance of lymphatic vessels in the lungs (Baldwin et al., 2005; Haiko et al., 2008).

Lymphatic vascular remodelling, or secondary lymphangiogenesis, is thought to occur in the adult during wound healing (Paavonen et al., 2000; Lohela et al., 2003) or in inflammation, and involves the sprouting of lymphatic vessels from already existing vessels (Paavonen et al., 2000; He et al., 2004). A number of animal post-surgical models have been described (Szuba et al., 2002; Daniels et al., 2003; Breier, 2005; Blacker et al., 2007); however, the mechanism by which lymphangiogenesis is achieved and regulated is poorly understood and likely to involve multiple factors, such as inflammation, physical changes to interstitial fluid flow, matrix metalloproteinase activity (extracellular matrix remodelling) and VEGFC/VEGFR3 signalling (Ristimaki et al., 1998; Boardman and Swartz, 2003; Mouta and Heroult, 2003; Rutkowski, Boardman et al., 2002; He et al., 2004). A number of animal post-surgical models have been described (Szuba et al., 2002; Daniels et al., 2003; Breier, 2005; Blacker et al., 2007); however, the mechanism by which lymphangiogenesis is achieved and regulated is poorly understood and likely to involve multiple factors, such as inflammation, physical changes to interstitial fluid flow, matrix metalloproteinase activity (extracellular matrix remodelling) and VEGFC/VEGFR3 signalling (Ristimaki et al., 1998; Boardman and Swartz, 2003; Mouta and Heroult, 2003; Rutkowski, Boardman et al., 2002; He et al., 2004).

Vasculature in the ovary

Gonadal development

Blood vascular development during gonadogenesis in the mouse first occurs around E11.5 in both sexes, when the indifferent gonad contains a primitive vascular system into which small branches from the mesonephric vessels extend (Coveney et al., 2008). Sex-specific development of the vasculature is critical for divergent morphogenesis of the male gonad. Vasculatization of the presumptive testes involves the migration of endothelial cells from the mesonephros into the gonadal tissue (Martineau et al., 1997; Tilmann and Capel, 1999; Brennan et al., 2002). These cells form a meshwork of smaller branched vessels in the coelomic domain of the XY gonad that resolves into a distinctive coelomic vessel by E13.5 (Coveney et al., 2008), making it morphologically distinguishable from the ovary. Between E12.5 and 13.5, vascular branches extending from the coelomic vessel can be identified along the outside of testis cords and can be identified as the primordial arterial system of the testis by their Notch1 and Ephrinb2 expression (Brennan et al., 2002). In contrast, the XX gonad lacks a coelomic vessel, and XX vascular development does not involve migration of cells from the mesonephros. Rather, vascular development of the XX gonad appears to be the result of proliferation and extension of the branches of the primordial gonadal vasculature (Tilmann and Capel, 1999; Brennan et al., 2002; Bullejos et al., 2002; Coveney et al., 2008). At E13.5, dense networks of vessels are found in close proximity to the strings of germ cells known as oogonia cords or germ line cysts (Bullejos, Bowles and Koopman, 2002). Less is known about the role for vasculature during primordial follicle assembly and early post-natal ovarian development; however, it appears that there is no major gene regulation of the angiogenic growth factors Vegfa, Vegfl and Vegfr2 during this period which occurs post-natally (Brown et al., 2010). Recently, a distinct role for prosangiotic activity of the VEGF family has been implicated in early follicle activation as well as angiogenesis in the post-natal ovary (McFee et al., 2009). The lymphatic vasculature has been reported in ovaries of a wide range of species including human (Brodowska et al., 2007; Vainionpaa et al., 2007; Shin et al., 2008), primates (Xu and Stouffer, 2009), rodents (Brown et al., 2006, 2010), ruminants (Morris, 1966; Findlay et al., 1986), flying fox (Pow and Martin, 1995) (also see Fig. 1), pig (Anderson, 1926), cat (Ricciardi et al., 1989), dog (Marroni et al., 1992), rabbit (Otsuki et al., 1987) and reptiles (Calderon et al., 2004). Recently, we described the presence of lymphatic vessels in the developing mouse ovary (Brown et al., 2010). The lymphatic vasculature developed post-natally, concurrent with the first wave of estrogenic follicle growth, and in association with the expression of Vegfc, Vegfd and Vegfr3. Vessels were first apparent in the hilus (stalk) of the ovary at post-natal day (P) 8.5 and were later localized to the stroma, in close proximity to growing follicles by P 12.5, indicating that lymphatic vasculogenesis establishes a new network migrating from extra-ovarian vessels into the ovary at this time. A capillary network forms throughout the ovary but capillaries were not present directly around follicles at any stage (Brown et al., 2010). This work was recently supported by the use of three-dimensional imaging of a PROX1-EGFP reporter, confirming post-natal development arising from existing vessels in the extra-ovarian rete (Svingen et al., 2012). Together these studies demonstrate that cells migrate into the ovary in the final stages of follicle formation, making the infiltration of other cell types that contribute to the granulosa or theca populations also feasible. Indeed, migration of putative pre-theical cells from the mesonephros has been suggested (Ungeqvist and Yao, 2012).

The difference in both the temporal and spatial localization and development of the blood and lymphatic vasculatures leads us to speculate about their function and interaction at this early stage. The significant lag time in the lymphatic vasculature suggests that there is no strong requirement for the lymphatic vasculature in early ovarian development/function, and that it is unlikely that fluid homeostasis, immune surveillance or hormone uptake are significant prior to P 12.5 when the lymphatic network is first fully formed and the first wave of folliculogenesis is at the secondary stage with oocytes not yet capable of maturation.
Figure 1: Schematic representation of lymphatic differentiation. Prior to embryonic day (E)9 in the mouse, embryonic veins express both lymphatic vascular endothelial hyaluronan receptor 1 (LYVE1) and vascular endothelial growth factor receptor 3 (VEGFR3). Following the expression of Prospero homeobox protein 1 (PROX1), a budding event occurs resulting in the initial development of the lymphatic vasculature. At this time, the blood vasculature loses the expression of both LYVE1 and VEGFR3, which are lymphatic-specific markers in mature vasculature. Following E14.5, the more mature lymphatic vasculature gains the expression of additional markers including tyrosine kinase with Ig and EGF homology domains-2 (TIE2), neuropilin 2 (NRP2) and secondary lymphoid chemokine (SLC), whilst the mature blood vasculature expresses VEGFR1, VEGFR2, TIE2, cluster of differentiation 31 (CD31) and cluster of differentiation 34 (CD34), differentiating the two-vascular lineages.

Adams et al. (2002). It could be speculated that the establishment of an ovarian lymphatic network in association with the first wave of follicular activity suggests that there is an interaction between follicle growth signals and lymphangiogenesis, and that lymphatic function is important for growing follicles.

Folliculogenesis

Ovarian angiogenesis involves remodelling of the blood vasculature established in embryos, occurs concurrently with folliculogenesis and continues throughout follicle growth and luteinization. Follicles prior to the pre-antral stage (primordial through secondary follicles) have no vascular network of their own and rely on the diffusion of oxygen and nutrients from stromal vessels (Fraser and Duncan, 2005; Fraser, 2006). At the time antrum formation begins, a wreath-like structure of vessels forms around the follicle, consisting of two concentric networks of vessels within the thecal layers, and is closely linked with growth and development of the follicle (Reynolds et al., 1992). Established blood vasculature delivers an increasing supply of gonadotrophins, growth factors, oxygen, lipids and steroid precursors required for folliculogenesis and oocyte maturation, as well as removing waste products arising from active growth and metabolism by follicles. The vessels increase in size and number but never penetrate the basement membrane, thus keeping the granulosa layers and oocyte completely avascular until ovulation (reviewed in Fraser, 2006). The formation of a robust vascular network has been linked to follicle survival and atresia (Kosaka et al., 2007) as well as production of high developmentally competent oocytes. In mono-ovular species selection of a dominant follicle may be dependent on the development of a robust vascular network with increased vascular permeability (Barboni et al., 2000). Development of follicular vasculature is augmented by FSH derived from the pituitary, which stimulates the production of Vegfs in the ovary (Arrujo et al., 2011). The combination of hypoxia and gonadotrophins acts to further induce Vegf transcription (Alam et al., 2009; Klipper et al., 2010; Tam et al., 2010). This local Vegf production in turn potentiates the response of granulosa cells to FSH (Doyle et al., 2010).

Follicles that fail to acquire satisfactory vascular support usually undergo atresia. Vascular loss is associated with the earliest stages of follicular atresia (Greenwald, 1989). Inhibition of VEGFA or VEGFR2 during the late follicular phase and the peri-ovulatory period in rodents or primates has drastic effects on ovarian function. During the late follicular phase, VEGFR2 neutralization results in disruption of the endocrine and autocrine changes necessary for follicular maturation and ovulation, demonstrating the role for vasculature in follicle survival (Zimmermann et al., 2003).

Follicular vascular status has been implicated in human pregnancy success. Flow index, or qualitative blood cell displacement, in follicles chosen for monofollicular IVF transfers was positively associated with implantation and pregnancy rate (Lozano et al., 2007), whilst oocytes collected from hypoxic follicles were associated with a higher frequency of chromosomal abnormalities (Van Blerkom et al., 1997). In women undergoing IVF, a high rate of blood flow associated with individual follicles correlates with improved competence of that oocyte to form a viable pregnancy (Bhal et al., 1999; Borini et al., 2001; Costello et al., 2005).

While not all stages of follicle growth and development have been assessed in all species, it would appear there is significant species variation with regard to the lymphatic vasculature surrounding growing follicles. In the mouse ovary, lymphatic vessels are found within the stromal compartment, in the cortex and surrounding growing follicles, with the largest vessels located within the medullary region in close association with the blood vasculature but the thecal layer appears to lack a network of capillary vessels (Brown et al., 2010; Svingen et al., 2012) (Fig. 2). We could find no evidence that ovarian blood vasculature was affected by Adams1 deficiency; however, others have reported altered CD31-positive vessel density in Adams1 null mouse ovaries (Shozu et al., 2005). Alternatively, in primates lymphatic endothelial cells and vessels have been described within the stroma and thecal layer of pre-ovulatory follicles (Xu and Stouffer, 2009).

We recently demonstrated that normal ovarian lymphatic development is dependent on the protease ADAMTS1 (Brown et al., 2006) (Fig. 3). ADAMTS1 (a disintegrin and metalloproteinase with thrombospondin motifs 1) is a widely expressed extracellular protease. Within the ovary Adams1 is expressed by granulosa cells and regulated by FSH
During folliculogenesis (Doyle et al., 2004), and then rapidly induced by the ovulatory LH surge in a progesterone receptor-dependent manner (Robker et al., 2000; Russell et al., 2003; Doyle et al., 2004). Mice null for Adams l lack lymphatics in the prepubertal mouse ovary but not in other organs, including liver and skin (Brown et al., 2006). The ovarian lymphatic defect can be partially restored by hormonal stimulation with the FSH mimetic, eCG (Brown et al., 2010). In both normal and Adams l-deficient ovaries eCG was also capable of increasing lymphatic vessel diameter, indicative of increased lymphatic function. Changes in the vasculature were associated with FSH-mediated increases in expression of lymphatic growth factors Vegfc and Vegf3 as well as their receptor, Vegfr3. These studies demonstrated that the lymphatic system is responsive to hormones that modulate folliculogenesis. Very recently, lymphatic vessels were described in the zebrafish ovary, and as having a peculiar structure, namely encompassing arterioles in their lumen, while no details pertaining to their location with respect to intraovarian structures were described (Shimoda and Isogai, 2012).

The lymphatic vasculature is also present in the ovarian bursa, the membrane around the ovary, in a number of poly-ovular species, including guinea pig, golden hamster and European beaver (Shinohara et al., 1987; Dobroszynska and Janiszewska, 1998; Sui and Li, 2001). Lymphatic stomata are present within the inner layer of the ovarian bursa and their function can be modified by treatment with exogenous hormones, including PMSG, hCG and androgens, as well as during pregnancy (Sui and Li, 2003). In golden hamster, it would appear that the bursal lymphatics develop following the closure of the bursa, are rarely present at the first ovulation and are commonly present after the fourth ovulation (Shinohara et al., 1987). These lymphatic vessels may be involved in re-absorption of fluid released from the ovary during ovulation which may, speculatively, participate in hormone circulation associated with ovulation. Certainly, lymphatics have been shown to be a route of hormone efflux from the ovary (Findlay et al., 1986).

**Corpus luteum development and function**

After ovulation, follicles form a highly vascularized CL, which secretes large quantities of progesterone to maintain uterine competence for pregnancy. During ovulation, the basement membrane separating the avascular granulosa layers of the follicle and the vascularized thecal and stromal layers breaks down. The capillaries become fenestrated and rapidly sprout, invading the granulosa cell layer and proliferating extensively (Lei et al., 1991; Christenson and Stouffer, 1996; Ricke et al., 1999). Blood vascular remodelling within the ovary is often compared with that of the most rapidly growing tumours and the CL establishes one of the highest rates of blood vessel density and flow of any organ (Niswender and Nett, 1988). Immune cell infiltration of the developing CL is thought to promote angiogenesis through production of prostaglandins and interleukin 8 (Miyamoto et al., 1999, 2003). In golden hamster, it would appear that the bursal lymphatics develop following the closure of the bursa, are rarely present at the first ovulation and are commonly present after the fourth ovulation (Shinohara et al., 1987). These lymphatic vessels may be involved in re-absorption of fluid released from the ovary during ovulation which may, speculatively, participate in hormone circulation associated with ovulation. Certainly, lymphatics have been shown to be a route of hormone efflux from the ovary (Findlay et al., 1986).

**Figure 2** Vasculature in the remodelling mouse ovary. The blood and lymphatic vasculature of developing (post-natal day 10) (A and B; 60 x magnification), growing (C and D; 40 x magnification), pre-ovulatory (E and F; 40 x magnification) follicles and the CL (G and H; 60 x magnification) of mouse ovaries. Lymphatic vasculature (A, C, E, G) was stained (brown) for LYVE1 whilst blood vasculature was stained for Von Willebrand Factor (VWF, brown) (B, D, F, H) as previously described (Brown et al., 2010). In the developing ovary, the lymphatic vasculature moved in from the extra-ovarian tissue and is present in some small vessels (A) whilst the blood vasculature is present throughout the ovary, and found in close proximity to all growing follicles (B). During the period of follicle growth, the lymphatic vasculature has infiltrated the ovary; however, it is closely associated with large vessels of the blood vasculature, and whilst associated with growing follicles, is absent from the theca (C), while the blood capillaries are found within the theca at this stage (D). At the peri-ovulatory stage, the lymphatic vasculature, whilst in close association with the basement membrane of the follicles, remains large and closely associates with the blood vasculature (E), while there is a rich blood microvasculature in the theca (F). The mouse CL is devoid of lymphatic vasculature (G) but rich in blood capillaries (H).
throughout the luteal phase in marmosets also caused marked decreases in luteal angiogenesis and a fall in plasma progesterone (Dickson et al., 2001; Wulff et al., 2001c). Each of these interventions supports the conclusion that VEGFA plays essential, non-redundant roles in the cyclic vascular remodelling required for structural and functional development of the CL. Additional factors further contribute to the formation of functional vessel networks in new luteal vasculature, a key example being the inhibition of delta-like ligand 4 in marmoset, which caused increased luteal endothelial density but reduced progesterone secretion and eventual premature involution of the CL (Fraser et al., 2012). The lymphangiogenic growth factors VEGFC and VEGFD and their expression, regulation and functional significance within the CL has not been explored to date. Interestingly, in the cow, the conceptus recognition protein interferon tau has been proposed to amplify lymphangiogenesis via induced VEGFC signalling at the time of CL rescue and when CL function is elevated to maintain pregnancy (Nitta et al., 2011). There is significant species variability in lymphatic vasculature of the CL. In rats, one report suggests that lymphatic vessels are found in the peripheral zone of the CL in later stages of development (Ichikawa et al., 1987), whilst a second report suggests that vessels were not present in the ovary at first ovulation but were present in the peripheral zone of the CL following ovulation and subsequently in the central connective tissue nest (Ichikawa et al., 1987). In rabbit, lymphatic capillaries were observed among the theca lutein but not granulosa lutein cells 3 days following hCG stimulation; this persisted until Day 14, with vessels shown to contain macrophages and degenerated luteal cells (Otsuki et al., 1987). In sheep, a diffuse network of highly permeable lymphatic vascular ducts have been described in the CL during the luteal phase and early pregnancy (Morris and Sass, 1966). Most recently, lymphatic endothelial cells and vessels were described within the primate CL, with their number and staining intensity of LYVE1 increasing from early to mid-luteal phase, remaining elevated thereafter, and associated with the expression of both VEGFC and VEGFR3, which increased in the early to mid-luteal phase and decreased during the latter stages of luteal life (Xu and Stouffer, 2009). Interestingly, these events seem to be associated with the peak in ADAMTS1 gene expression and protein production in the primate ovary, further solidifying a possible role for ADAMTS1 in ovarian lymphangiogenesis (Young et al., 2004; Peluffo et al., 2011). There appears to be no lymphatic vessels in the CL of mice when examined at 6 months of age in naturally cycling ovaries or in superovulated ovaries (Brown et al., 2010).

There are a number of possible reasons for the differences in CL vasculature reported between species, ranging from functional requirement to detection techniques. Only recently have markers, such as LYVE1, Podoplanin, PROX1 and VEGFR3, become available for the molecular characterization of the lymphatic vasculature and further studies using such markers may help to clarify some of the discrepancies in the literature. It is also possible that species differences exist, particularly with respect to mono- and poly-ovular species, or alternatively, functional differences, such as the longer lifespan in ruminant, pig and human, compared with rodent CL, may be one reason for this difference. While it is still not entirely clear what the function of the lymphatic vasculature is within the CL, it is likely to mimic that seen in other tissues, playing critical roles in fluid homeostasis, hormone transport and immune cell trafficking. Studies in pig and sheep examined lymph flow from the ovary and the constituents of this lymph fluid. Lymph flow from the ovine ovary is highest when active CLs are present, and higher (per unit weight) than any other organ measured, whilst lymph flow at estrus was minimal.
Blood and lymphatic vasculature in the ovary

Vasculature in pathogenesis of ovarian disease

In addition to characterization of the function of VEGFA in normal ovarian physiology, a growing body of evidence suggests that dysregulation of VEGFA is associated with both polycystic ovary syndrome (PCOS) and ovarian hyperstimulation syndrome (OHSS). PCOS is an endocrine disorder associated with androgen excess and ovulatory dysfunction, affecting 6–7% of women of reproductive age worldwide (Escobar Morreale, 2008). The ovarian pathophysiology of PCOS includes inappropriate accumulation of fluid-filled ovarian cysts and abnormal hyervascularization of the theca (Abbott et al., 2002). Recently PCOS has been associated with dysregulation of granulosa cell, serum and follicular fluid VEGFA (Agrawal et al., 2008; Abd El Aal et al., 2005; Artini et al., 2006). Serum levels of VEGFA have been shown to be higher in patients with PCOS, and this is associated with increased ovarian vascularity on Doppler (Borini et al., 2001). Laparoscopic ovarian drilling (LOAD), a surgical intervention used to induce ovulation in women with PCOS, decreases ovarian blood flow velocity, an outcome which has been proposed to be linked with the decrease in the prevalence of OHSS following LOAD in patients with PCOS (Farquhar et al., 2012). Follicular fluid VEGFA levels have also been associated with follicle maturation, oocyte quality and fertilization competence in patients with PCOS (Bokal et al., 2005); however, more research in this area is required to better understand these relationships. Furthermore, excess generation of VEGFA in response to controlled ovarian stimulation during assisted reproduction technology (ART) is thought to promote/exacerbate OHSS (McClure et al., 1994; Neulen et al., 1995). OHSS is an exaggerated response to ovulation induction therapy and exogenous gonadotrophins in particular, which occurs in ~1% of cases, resulting in symptoms of extravascular fluid retention, hypervolaemia and the pathological development of OHSS (Wang et al., 2002; Gomez et al., 2003; Pau et al., 2006).

The fundamental molecular cause of OHSS is still debated. Certainly an excessive ovarian response to LH initiates it and a range of evidence supports VEGFA-mediated vascular hyperpermeability and hence excessive release of vascular fluid as a key contributor (reviewed in Soares et al., 2008). VEGFA/VEGFR2 are known to be increased in response to gonadotrophins in most species (rats, mouse, humans, primates). The increased capillary permeability and ascites is predominantly linked to the ovary (Blumenfeld et al., 1997) and both total and free levels of VEGFA are higher in the luteal phase of hyperstimulated women (Pau et al., 2006). In vitro assays have identified hCG and VEGFA as candidates for increased vascular permeability, and suggest the action occurs via modulation of VE-cadherin, altering gap junction structure (Bates et al., 1999). Women undergoing IVF that are less susceptible to OHSS have higher natural antagonist (sVEGFR1) levels (Pau et al., 2006), while women with high levels of sVEGFR have been reported to have poor ovarian response following stimulation (Neulen et al., 2001). Whether there are deficiencies in abundance or function of ovarian lymphatics causing reduced reabsorption of fluid in OHSS is yet to be properly examined.

Ovarian lymphatic dysfunction has been proposed to be involved in the development of massive ovarian edema (MOE). MOE occurs most commonly in teenage girls, after the onset of menarche, and causes accumulation of fluid within the ovary, predominantly in the stroma (Yilmaz et al., 2005). MOE has been reported to occur simultaneously with retroperitoneal lymphoma, metastatic gastric carcinoma and metastatic uterine cervical carcinoma, and is thought to arise as a result of ovarian lymphatic vascular blockage (Krasvic et al., 2004; Dalloul et al., 2007). In patients receiving ART, and more specifically clomifene citrate, a selective estrogen receptor modulator, MOE is thought to arise from blockage of both the blood and lymphatic vasculature (Kawaguchi et al., 2008). Given the effects of clomifene citrate to increase circulating gonadotrophins, and the known effect of gonadotrophins on the lymphatic vasculature and lymphatic growth factors, it is a strong possibility that the vasculature is being directly affected by the changes in hormonal milieu.

The ovarian lymphatic vasculature is known to provide a trafficking network for metastatic cells derived from tumours of the ovary. Malignant dysgerminoma, a highly metastatic and aggressive germ cell-derived tumour, disseminates to draining lymph nodes via the ovarian lymphatic vasculature (Kasenda et al., 2009). Extensive lympho-vascular permeation as well as bilateral pelvic node metastasis has been described in ovarian endometrioid adenocarcinoma (Alam et al., 2009). Ovarian lymphatic vasculature has also been proposed to be the route of metastasis for malignant germ cell tumours (Kumar et al., 2008), ovarian clear cell carcinoma (Takano et al., 2008), metastatic ovarian adenocarcinoma (Martel et al., 2003; Sato et al., 2008), ovarian teratoma (Djordjevic et al., 2007), sex cord-stromal tumours (Duncan et al., 2008), advanced small cell carcinoma (Karwar et al., 2008) and ovarian granulosa cell tumours (Schmidt et al., 2008). These metastases are most commonly described in the nodes known to drain the ovary, the pelvic, para-aortic and iliac lymph nodes (Klipper et al., 2010).

Additionally, a small number of publications have described the presence of lymphangioiigenic growth factors VEGFC and VEGFD in a number of ovarian cancer types. VEGFD abundance was associated with stage, intra-tumoural lymphatic vessel abundance, tumoural lymphatic invasion, lymph node metastasis and shorter survival. The combination of VEGFD, intratumoural lymphatics and lymphatic invasion were all prognostic factors for survival (Tam et al., 2010). VEGFC and VEGFD expression in human ovarian carcinoma (serous papillary carcinoma, endometrioid carcinoma, ovarian mucinous) was associated with high tumour grade, clinical stage, presence of ascites and lymph node metastasis (Bolat et al., 2008). Recently, experiments in mouse models of ovarian cancer have identified leaky, dysfunctional lymphatic vasculature, associated with the presence of growth factor-producing immune cells (Jeon et al., 2008) and implicating the lymphatic vasculature in the pathological onset of ascites.
**Conclusion and future directions**

The ovarian blood vasculature is important for follicle activation, growth and survival as well as production of high-competence oocytes and generation of a CL capable of maintaining pregnancy. The exact function of the ovarian lymphatic vasculature is less apparent, yet it is likely to play critical roles in fluid homeostasis and hormone trafficking, and in combination with the blood vasculature, has been linked to a number of ovarian fluid-homeostatic disorders, including PCOS, OHSS and MOE. An improved understanding of the lymphatic vascular structure and function within the human ovary would likely shed light on whether there was further involvement in ovarian pathology. Through animal studies, a more detailed understanding of the regulation of ovarian blood and lymphatic systems, and of their aetiolog and relationship with the endocrine system of the ovary, will inform further investigations into their functional or spatial deficiencies in patients with these clinical conditions. The first evidence is emerging of a malleable lymphatic system and a model for regulation of normal adult lymphangiogenesis, and may one day be utilized to regenerate damaged vessels and cure lymphatic diseases and disorders. Current therapies for lymphatic disease are focused on physical therapy, compression bandages and manipulation of osmolarity, none of which are curative. The advent of modulators of blood and lymphangiogenic mechanisms may provide new clinical tools to manage lymphatic disease more effectively.

**Authors’ roles**

H.M.B. and D.L.R. contributed equally to all aspects of manuscript preparation. The authors alone are responsible for the content and writing of the paper.

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**Conflict of interest**

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