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Editorial

The critical importance of ovarian angiogenesis

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The vascular system develops through two distinct pathways known as vasculogenesis and angiogenesis. The former involves formation of vascular networks from endothelial progenitor cells (Käßmeyer *et al.* 2009), while angiogenesis is the extension of new blood vessels from pre-existing ones (Robinson *et al.* 2009). The establishment and optimal functioning of the vasculature is an essential component throughout the body and the ovary is no exception. Blood vessel growth is tightly regulated requiring the timely balance between pro- and anti-angiogenic growth factors as well as the complex, intimate interplay between endothelial cells and various other cell types (e.g. immune cells and pericytes). In this Research Front on ovarian angiogenesis, we present four reviews and one original article that reveal the latest research and current hypotheses on the key stages of vascular development in the ovary.

Remarkably little was known about how the vasculature in the fetal ovary is formed, until recently. In the first paper of this Research Front, the latest insights are highlighted by McFee and Cupp (2012). Intriguingly, ovarian vasculature in the embryo appears to develop through a vasculogenic process rather than the typical angiogenesis that occurs in embryonic testes. Moreover, the vascular patterning is preferentially aligned along the neuronal network template and thus could be influenced by neuronal growth factors (Anderson *et al.* 2002). McFee and Cupp (2012) also suggest that the ability of germ cell/oocytes to proliferate, develop and form primordial follicles is potentially influenced by fetal ovarian medulla blood supply since these cells lack a direct blood supply. Indeed, follicle assembly initially originates near to the medullary vasculature (Sawyer *et al.* 2002). This would imply that the inappropriate development of an ovarian vasculature could result in improper prenatal folliculogenesis. This could then adversely affect fertility of both women and animals.

The VEGF system is very complex with the existence of numerous pro- and anti-angiogenic isoforms, receptors and signalling partners (Gabhann and Popel 2008). McFee and Cupp (2012) provide a scholarly account of their importance in the regulation of fetal ovarian development. They speculate that the temporal differences in expression of pro-angiogenic 'a' and anti-angiogenic 'b' VEGFA isoforms might explain the different patterns of vascularisation in the fetal ovary and testes (McFee and Cupp 2012). Interestingly, Qiu *et al.* (2012) recently showed that overexpression of VEGF165b reduced follicular development and number of ovulations and more importantly, this was associated with lower fertility in mice. Collectively, this further emphasises that optimal ovarian function requires an appropriate balance between pro- and anti-angiogenic factors.

It is well established that hypoxia-induced expression of VEGFA plays a pivotal role in stimulating angiogenesis during tumour development. However, the exact role of hypoxia in ovarian angiogenesis has remained elusive for a long time. In the second review of this Research Front, Meidan *et al.* (2012) discuss the role of the transcription factor, hypoxia inducible factor 1∝/β (HIF1∝/β), which is critical for ovulation in mice (Kim *et al.* 2009). The current evidence indicates that HIF1∝ is upregulated and translocates to the nucleus of luteinising granulosa cell during the ovulatory window although this upregulation is only short-lived. It is likely that, at least in part, the LH surge upregulates the HIF1∝ in luteinising follicular cells (van den Driesche *et al.* 2008). This feature of HIF1∝ regulation could be unique to the ovary. It is also increasingly apparent that any induction of HIF1∝ stimulates VEGFA expression in luteal cells (Zhang *et al.* 2011). What is less well known is the role of hypoxia and/or HIF1∝ in the regulation of FGF2. This certainly requires investigation since FGF2 and HIF1∝ expression profiles are closely matched in the bovine CL (Robinson *et al.* 2007; Nishimura and Okuda 2010). Furthermore, it is becoming increasing apparent that FGF2 is a key factor controlling endothelial cell sprouting during the follicle-luteal transition in cattle (Laird *et al.* 2012). Accurate measurement of tissue hypoxia is challenging and will continue to hinder the elucidation of the role hypoxia plays in ovarian angiogenesis. The use of novel, live imaging positron emission tomography (PET) technologies using biomarkers such as ^{18}F-labelled fluoromisonidazole (^{18}F-MISO) as used in tumour biology (Mendichovszky and Jackson 2011) will help to address this challenge. Furthermore, such technologies might increase our understanding of the role of hypoxia in disorders such as luteal deficiency.

It has been long-recognised that ovulation has numerous characteristics of an inflammatory response. However, the role of immune cells in the regulation of ovarian angiogenesis has been neglected, until recently and these findings are highlighted by Shirasuna *et al.* (2012) in the third review. While there are some definite species differences, there is a pool of evidence that immune cells (e.g. neutrophils, macrophages and/or lymphocytes) infiltrate into the developing CL. This is likely due to

increased vascular permeability and stimulation by pro-inflammatory signals (e.g. interleukin 8 and prostaglandin E2; (Jiemtaweeboon *et al.* 2011)) but also might be regulated by angiogenic factors (Shirasuna *et al.* 2012). There is increasing evidence that these immune cells are active players in luteal angiogenesis. For example, neutrophils stimulate the formation of luteal endothelial cell capillary-like structures (Jiemtaweeboon *et al.* 2011). Whilst, Turner *et al.* (2011) recently demonstrated that macrophages play a crucial role in maintaining vascular integrity in mice. This concept is further supported by the observations that bone marrow-derived vascular progenitor cells and macrophages contribute to neovascularisation during CL formation (Kizuka *et al.* 2012). The role of macrophages is potentially the most important since these cell can be differentiated into the 'tissue remodelling' M2 phenotype by the luteal microenvironment. In turn, M2 macrophages then produce various pro-angiogenic factors including VEGFA, VEGFC and FGF2 to promote angiogenesis. Collectively, this highlights the need for more research to better understand the cell–cell communication that occurs between immune cells and endothelial cells, while at the same time not forgetting the role of pericytes (Robinson *et al.* 2009).

A dysfunctional vasculature is often an underlying abnormality observed in the pathological ovary (e.g. excessive vasculature in ovarian hyperstimulation syndrome (OHSS) and diminished follicle reserve is associated with inadequate ovarian vascularisation). In the final review, Duncan and Nio-Kobayashi (2012) discuss the potential targeting of angiogenic molecular pathways as novel, clinical approaches in the management of the pathological ovary. It is clear from *in vivo* studies where different angiogenic pathways have been manipulated that there are several pathways, which play fundamental roles in regulating angiogenesis at different stages of ovarian development. For example, VEGFA, the delta-Notch system, and angiopoietin-2 are critical for functional luteal angiogenesis (Fraser *et al.* 2000; Xu and Stouffer 2005; Fraser *et al.* 2012), while the anti-angiogenic factor thrombospondin 1 (TSP1) suppressed pre- and early antral follicular development (Garside *et al.* 2010). Targeting angiogenic mechanisms in the treatment of OHSS has progressed the most, with strategies focussing on reducing the synthesis of VEGFA (e.g. minimising the LH surge or dopamine agonists) rather targeting VEGFA directly. Perhaps, the most exciting opportunities are in the promotion of follicular and/or luteal function by increasing and/or maintaining the blood supply and thereby improving fertility. This promotion, rather than, inhibition of angiogenesis will have several challenges but the current *in vivo* evidence does suggest this concept is viable (e.g. (Ginther *et al.* 2005; Friedman *et al.* 2012)). Angiogenic-based treatments for ovarian disorders remain in their infancy but have great potential.

This Research Front issue of *Reproduction*, *Fertility and Development* provides a timely series of reviews that address our current knowledge about the regulation of vascular function in ovary in humans and farm animals. They also highlight emerging areas of research that will improve our knowledge about this complex area of biology.

References

Anderson, R. A., Robinson, L. L. L., Brooks, J., and Spears, N. (2002). Neurotropins and their receptors are expressed in the human fetal ovary. *J. Clin. Endocrinol. Metab.* **87**(2), 890–897. doi:10.1210/JC.87.2.890

Duncan, W. C., and Nio-Kobayashi, J. (2012). Targeting angiogenesis in the pathological ovary. *Reprod. Fertil. Dev.* **25**, 362–371. doi:10.1071/RD12112

Fraser, H. M., Dickson, S. E., Lunn, S. F., Wulff, C., Morris, K. D., Carroll, V. A., and Bicknell, R. (2000). Suppression of luteal angiogenesis in the primate after neutralization of vascular endothelial growth factor. *Endocrinology* **141**(3), 995–1000. doi:10.1210/EN.141.3.995

Fraser, H. M., Hastings, J. M., Allan, D., Morris, K. D., Rudge, J. S., and Wiegand, S. J. (2012). Inhibition of delta-like ligand 4 induces luteal hypervascularization followed by functional and structural luteolysis in the primate ovary. *Endocrinology* **153**(4), 1972–1983. doi:10.1210/EN.2011-1688

Friedman, O., Orvieto, R., Fisch, B., Felz, C., Freud, E., Ben-Haroush, A., and Abir, R. (2012). Possible improvements in human ovarian grafting by various host and graft treatments. *Hum. Reprod.* **27**(2), 474–482. doi:10.1093/HUMREP/DER385

Gabhann, F. M., and Popel, A. S. (2008). Systems Biology of Vascular Endothelial Growth Factors. *Microcirculation* **15**(8), 715–738. doi:10.1080/10739680802095964

Garside, S. A., Henkin, J., Morris, K. D., Norvell, S. M., Thomas, F. H., and Fraser, H. M. (2010). A thrombospondin-mimetic peptide, ABT-898, suppresses angiogenesis and promotes follicular atresia in pre- and early-antral follicles in vivo. *Endocrinology* **151**(12), 5905–5915. doi:10.1210/EN.2010-0283

Ginther, O. J., Gastal, E. L., Gastal, M. O., and Beg, M. A. (2005). In vivo effects of pregnancy-associated plasma protein-A, activin-A and vascular endothelial growth factor on other follicular-fluid factors during follicle deviation in mares. *Reproduction* **129**(4), 489–496. doi:10.1530/REP.1.00555

Jiemtaweeboon, S., Shirasuna, K., Nitta, A., Kobayashi, A., Schuberth, H. J., Shimizu, T., and Miyamoto, A. (2011). Evidence that polymorphonuclear neutrophils infiltrate into the developing corpus luteum and promote angiogenesis with interleukin-8 in the cow. *Reprod. Biol. Endocrinol.* **9**, 79. doi:10.1186/1477-7827-9-79

Käßmeyer, S., Plendl, J., Custodis, P., and Bahramsoltani, M. (2009). New insights in vascular development: Vasculogenesis and endothelial progenitor cells. *Anat. Histol. Embryol.* **38**(1), 1–11. doi:10.1111/J.1439-0264.2008.00894.X

Kim, J., Bagchi, I., and Bagchi, M. (2009). Signaling by hypoxia-inducible factors is critical for ovulation in mice. *Endocrinology* **150**, 3392–3400. doi:10.1210/EN.2008-0948

Kizuka, F., Tokuda, N., Takagi, K., Adachi, Y., Lee, L., Tamura, I., Maekawa, R., Taketani, T., Tamura, H., Suzuki, T., Owada, Y., and Sugino, N. (2012). Involvement of bone marrow-derived vascular progenitor cells in neovascularization during formation of the corpus luteum in mice. *Biol. Reprod.* **87**, 55. doi:10.1095/BIOLREPROD.112.099960

Laird, M., Woad, K. J., Hunter, M. G., Mann, G. E., and Robinson, R. S. (2012). Fibroblast growth factor 2 induces the precocious development of endothelial cell networks in bovine luteinising follicular cells. *Reprod. Fertil. Dev.* **25**, 372–386. doi:10.1071/RD12182

McFee, R. M., and Cupp, A. S. (2012). Vascular development in the ovary: potential roles of VEGFA isoforms. *Reprod. Fertil. Dev.* **25**, 333–342. doi:10.1071/RD12134

Meidan, R., Klipper, E., Zalman, Y., and Yalu, R. (2012). The role of hypoxia in ovarian angiogenesis. *Reprod. Fertil. Dev.* **25**, 343–350. doi:10.1071/RD12139

Mendichovszky, I., and Jackson, A. (2011). Imaging hypoxia in gliomas. *Br. J. Radiol.* **84**, S145–S158. doi:10.1259/BJR/82292521

Nishimura, R., and Okuda, K. (2010). Hypoxia is important for establishing vascularization during corpus luteum formation in cattle. *J. Reprod. Dev.* **56**(1), 110–116. doi:10.1262/JRD.09-162E

Qiu, Y., Seager, M., Osman, A., Castle-Miller, J., Bevan, H., Tortonese, D. J., Murphy, D., Harper, S. J., Fraser, H. M., Donaldson, L. F., and Bates, D. O. (2012). Ovarian VEGF(165)b expression regulates follicular development, corpus luteum function and fertility. *Reproduction* **143**(4), 501–511. doi:10.1530/REP-11-0091

Robinson, R. S., Nicklin, L. T., Hammond, A. J., Schams, D., Hunter, M. G., and Mann, G. E. (2007). Fibroblast growth factor 2 is more dynamic than vascular endothelial growth factor A during the follicle-luteal transition in the cow. *Biol. Reprod.* **77**(1), 28–36. doi:10.1095/BIOLREPROD.106.055434

Robinson, R. S., Woad, K. J., Hammond, A. J., Laird, M., Hunter, M. G., and Mann, G. E. (2009). Angiogenesis and vascular function in the ovary. *Reproduction* **138**(6), 869–881. doi:10.1530/REP-09-0283

Sawyer, H. R., Smith, P., Heath, D. A., Juengel, J. L., Wakefield, S. J., and McNatty, K. P. (2002). Formation of ovarian follicles during fetal development in sheep. *Biol. Reprod.* **66**(4), 1134–1150. doi:10.1095/BIOLREPROD66.4.1134

Shirasuna, K., Shimizu, T., Matsui, M., and Miyamoto, A. (2012). Emerging roles of immune cells in luteal angiogenesis. *Reprod. Fertil. Dev.* **25**, 351–361. doi:10.1071/RD12096

Turner, E. C., Hughes, J., Wilson, H., Clay, M., Mylonas, K. J., Kipari, T., Duncan, W. C., and Fraser, H. M. (2011). Conditional ablation of macrophages disrupts ovarian vasculature. *Reproduction* **141**(6), 821–831. doi:10.1530/REP-10-0327

van den Driesche, S., Myers, M., Gay, E., Thong, K. J., and Duncan, W. C. (2008). HCG up-regulates hypoxia inducible factor-1 alpha in luteinized granulosa cells: implications for the hormonal regulation of vascular endothelial growth factor A in the human corpus luteum. *Mol. Hum. Reprod.* **14**(8), 455–464. doi:10.1093/MOLEHR/GAN040

Xu, F. H., and Stouffer, R. L. (2005). Local delivery of angiopoietin-2 into the preovulatory follicle terminates the menstrual cycle in rhesus monkeys. *Biol. Reprod.* **72**(6), 1352–1358. doi:10.1095/BIOLREPROD.104.037143

Zhang, Z. H., Yin, D. Z., and Wang, Z. C. (2011). Contribution of hypoxia-inducible factor-1 alpha to transcriptional regulation of vascular endothelial growth factor in bovine developing luteal cells. *Anim. Sci. J.* **82**(2), 244–250. doi:10.1111/J.1740-0929.2010.00832.X