Endometrial angiogenesis: Physiology and clinical implications

Introduction

Angiogenesis is the process of formation of new blood vessels from existing ones (in this it differs from vasculogenesis, the de novo formation of blood vessels in the embryo). With the exception of the female reproductive tract, healthy mature human tissues do not display angiogenesis to any significant extent, except in response to trauma. In the ovary and uterus, on the other hand, extensive vascular remodelling and formation of new vessels occurs on a regular cyclical basis and forms the bedrock of preparations for the implantation of the early embryo. Research into normal human fertility and its regulation is likely therefore to benefit from an elucidation of the processes involved in angiogenesis. In addition, pathological conditions relating to the reproductive tract appear in some cases to involve aberrant angiogenesis, and future research may lead to new therapeutic measures that may become effective treatments for these conditions. Finally, female reproductive biology provides a unique window into angiogenesis, and knowledge derived from this area is likely to add substantially to our knowledge of this major physiological and pathological process.

Physiology

Uterine blood supply comes from the uterine arteries, which give rise to arcuate arteries. From these arise the radial arteries, which divide at the endo-myometrial junction into straight arterioles supplying the basal layer of the endometrium and spiral end-arterioles supplying the functional layer. Arterioles in the basal layers have a vascular smooth muscle coat in circular and longitudinal layers, which stains positive for smooth muscle actin and myosin heavy chains. Smooth muscle and pericytes are reduced in the superficial layer and the most superficial vessels consist only of endothelial cells.[1] The vessels form a capillary plexus under the luminal epithelium which is drained by a system of veins. Endothelial cells in the functional layer show cyclical variation in proliferative activity, while proliferation in basal layer endothelium does not vary with the menstrual cycle. Importantly, endothelial cells that are not associated with pericytes or vascular smooth muscle coat are more susceptible to variations in local levels of vascular endothelial growth factor (VEGF) and undergo apoptosis (programmed cell death) on withdrawal of VEGF more readily than endothelium that has established a pericytic or smooth muscle connection.[2]

In general, angiogenesis can occur by means of sprouting, intussusception (a process whereby new vessels are formed without breaching the vessel wall) or vessel elongation, with a_vb_3 integrin being a marker for vessel sprouts. This has not been identified in the endometrium, suggesting that intussusceptive angiogenesis predominates in this site, in contrast to angiogenesis in relation to tumours which is mainly by sprouting. The regulation of endometrial angiogenesis is believed to involve locally produced substances in addition to endocrine factors that affect blood vessel growth. The most important of these is probably the VEGF family of proteins, acting as extremely potent stimulants of endothelial cell mitosis. Six members of the VEGF family have been identified; VEGF-A is the best studied and is known to exist in five isoforms derived by alternative splicing of the products of the VEGF gene located on the short arm of chromosome 6. There are three specific VEGF receptors (VEGFR-1, -2 and -3), all of which are members of the tyrosine kinase-receptor family.[4] VEGFR-1 and VEGFR-2 are expressed on human endometrial endothelial cells.[5] Human endometrium synthesises all five isoforms of VEGF-A.[6] In the proliferative phase, in situ hybridisation and immunohistochemistry studies demonstrate VEGF-A expression in glandular epithelial cells and stromal cells. After ovulation, VEGF-A expression declines in the stromal cells, but continues in surface epithelial cells[7] and secretion of VEGF-A is directed towards the luminal surface.[8]

Ovarian sex steroids would be obvious candidates for regulating endometrial VEGF expression. Indeed, oestrogen response elements have been identified in the VEGF gene and in vitro evidence shows stimulation of VEGF expression by physiological concentration of oestradiol.[9] Levonorgestrel implants also appear to increase VEGF levels in the endometrium, associated with increased density of microvessels.[10] However, the main regulator of VEGF expression appears to be local oxygen tension. Hypoxia is a powerful inducer of VEGF, either by increasing production of VEGF mRNA[11] or by reducing VEGF mRNA destruction. Changes in VEGF levels interact with altered expression of VEGF receptors in determining growth or apoptosis of endometrial endothelium. In the proliferative phase, endothelial cells stain strongly for VEGFR-2, whereas after ovulation VEGFR-1 predominates. This shift is important because of the differential effects of VEGF binding to its receptors: binding to VEGFR-1 causes the cells to migrate but not proliferate, whereas VEGFR-2 binding leads to endothelial cell proliferation.[2]

Other endometrial factors have been identified which may regulate angiogenesis.[2] Angiopoietin-1 is produced by vascular smooth muscle and binds to receptors on the endothelium, promoting vascular stability and inhibiting apoptosis. Angiopoietin-2 appears to have an opposite effect. Three members of the fibroblast growth factor family are found in the endometrium and may be synergistic with VEGF. The actual angiogenic state of the endometrial endothelium probably represents a balance between these factors and other proteins known to inhibit angiogenesis including thrombospondin, endostatin, angiostatin, platelet factor 4 and transforming growth factor b. Thrombospondin production is upregulated by progesterone, indicating a role for sex steroids in maintaining the balance between stimulation and inhibition of angiogenesis in the endometrium. An important role is also played by tissue matrix metalloproteinases (MMPs), which degrade the extracellular matrix and permit migration of endothelial cells essential for angiogenesis to occur. MMP production in endometrial stromal cells is downregulated by progesterone and increases with progesterone withdrawal.

Clinical implications

The clinical implications of the recent advances in knowledge concerning endometrial angiogenesis are only beginning to be addressed. Exogenous steroids alter endometrial VEGF levels, although simple correlations between VEGF expression and the abnormal bleeding occasionally produced by contraceptive or hormone

replacement steroids have not been found. Patients using progestogen-only methods of contraception show hysteroscopic evidence of altered angiogenesis, together with increased microvascular density. Reduced endometrial perfusion in Norplant users (leading to local hypoxia and an upregulation of VEGF expression) may underlie these morphological abnormalities and their presumed clinical correlate of abnormal uterine bleeding.[12] An increased proliferative tendency has been described in endothelial cells from women with objective menorrhagia, compared to women with normal menstrual blood loss, accompanied by reduced vascular smooth muscle proliferation in the spiral arterioles. Increased angiogenic potential in the peritoneal cavity may be an essential part of the pathogenesis of endometriosis.[2]

It is clear that basic research into angiogenesis in the female reproductive tract has deepened our understanding of reproductive physiology and is beginning to add another level of complexity to what is known about common clinical conditions.[2] Future work will focus on elaborating these clinical correlations and exploring the use of targeted therapies using factors that alter angiogenesis to treat these conditions. Soluble VEGF receptors, tyrosine kinase inhibitors and inhibitors of MMPs may all be expected to inhibit angiogenesis in vivo. The future carries the potential of using these or similar therapies to treat abnormal bleeding, inhibit the progression of endometriosis and prevent implantation.

Statements on funding and competing interests

Funding. None identified

Competing interests. Stephen Smith is a Director of Metris Therapeutics Limited and as such receives Director's fees.

Raj Mathur, MRCOG, MD

Subspecialty Trainee, Department of Reproductive Medicine, The Rosie Hospital, Cambridge CB2 2SW, UK

Stephen K Smith, FRCOG, MD

Professor, University Department of Obstetrics and Gynaecology, The Rosie Hospital, Cambridge, CB2 2SW, UK

References

1 Kohnen G, Campbell S, Jeffers MD, et al. Spatially regulated differentiation of endometrial vascular smooth muscle cells. *Hum Reprod* 2000; **15**: 284–292.

2 Smith SK. Regulation of angiogenesis in the endometrium. *Trends Endocrinol Metab* 2001; **12**: 147–151.

3 Rogers PA, Lederman F, Taylor N. Endometrial microvascular growth in normal and dysfunctional states. *Hum Reprod Update* 1998; **4**: 503–508.

4 Neufeld G, Cohen T, Gengrinovitch S, et al. Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J* 1999; **13**: 9–22.

5 Meduri B, Bauseri P, Perrot-Applanat M. Expression of vascular endothelial growth factor receptors in the human endometrium: modulation during the menstrual cycle. *Biol Reprod* 2000; **62**: 439–447.

6 Charnock-Jones DS, Sharkey AM, Rajput-Williams J, et al. Identification and localisation of alternately spliced mRNAs for vascular endothelial growth factor in human uterus and estrogen regulation in endometrial carcinoma cell lines. *Biol Reprod* 1993; **48**: 1120–1128.

7 Torry D S, Holt VJ, Keenan JA, et al. Vascular endothelial growth factor expression in cycling human endometrium. *Fertil Steril* 1996; **66**: 72–80.

8 Hornung D, Lebovic DI, Shifren JL, et al. Vectorial secretion of vascular endothelial growth factor by polarised human endometrial epithelial cells. *Fertil Steril* 2000; **69**: 909–915.

9 Zhang L, Rees MC, Bicknell R. The isolation and long-term culture of normal human endometrial epithelium and stroma. Expression of mRNAs for angiogenic polypeptides basally and on oestrogen and progesterone challenges. *J Cell Sci* 1995; **108**(Pt 1): 323–331.

10 Lau TM, Affandi B, Rogers PAW. The effects of levonorgestrel implants on vascular endothelial growth factor expression in the endometrium. *Mol Hum Reprod* 1998; **5**: 57–63.

11 Maltepe E, Schmidt JV, Baunoch D, et al. Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature* 1997; **386**(6623): 403–407.

12 Hickey M, Fraser IS. Clinical implications of disturbances of uterine vascular morphology and function. *Baillieres Clin Obstet Gynaecol* 2000; **14**: 937–951.

A woman's right to choose ... counselling!

Recently, an anonymous patient sued the National Health Service (NHS) because she wasn't offered counselling after an abortion. Nor was she warned that psychological complications could follow the operation. This legal action raises issues of vital concern to patients, counsellors, the NHS and taxpayers.

Few people relish abortion. Even pro-choice supporters regard it as 'the lesser of two evils'. But while other countries remain divided on the issue, the legal consensus in Britain has ruled that safe medical abortion ought to be available. In the process, however, abortion has sometimes come to be regarded as a 'routine' procedure. This is a serious mistake. Leaving aside the moral debate, an abortion is a totally different experience from having your appendix out. All personnel treating the patient should know this. The psychological issues involved are multiple and profound.

As soon as a woman is aware of any unwanted or accidental pregnancy she is forced to contemplate the immense consequences of any choice she makes:

1. She continues with the pregnancy and rears her child with or without help from the father.
2. She continues with the pregnancy and has the baby adopted.
3. She terminates the pregnancy.

In the first and second cases, there will be a child. She would become the mother. This act would determine many events for the rest of her days. The child would need a name and it would automatically be related to a wider group of people in at least two different families. Many of the relationships in that woman's life, and by definition the future life of the child, would be altered at a stroke. If she continues with the pregnancy and then places her baby for adoption, she has to deal with the issues of detaching from baby. It is likely that she will have all the recognised symptoms of a major bereavement. Third, if she aborts, she may feel full of conflict.

For most women having an abortion is not an easy option but one that is arrived at through painful dialogue with themselves and probably with their partner. Because of this there is often strong ambivalence in the woman leading to resentment, anger and sadness. The idea of a child might awaken many deep-seated anxieties within the mother, especially if she has difficult memories from her own childhood. Or the pregnancy's timing might come to represent a form of doom. One woman said to us: 'It's the story of my life. I always desperately wanted a baby; but not now, and not with him'.

Few patients 'mourn' their appendix when it is removed. Many women who choose abortion still 'mourn' the lost possibilities of the life that will not be. Several years ago the privately funded Post Abortion Counselling Service was