Advances in ultrasound assessment in the establishment and development of pregnancy

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Current data demonstrate that angiogenesis in the ovaries and uterus is an essential component of both follicular and luteal phases of menstrual cycle, tightly correlating with the levels of bioactive substances such as hormones, growth factors and interleukins. Ultrasound is used principally to demonstrate follicular growth, a receptive triple layer endometrium and to exclude pathologies such as fibroids and ovarian tumours. However, the development of new technologies such as CDI, CPA, 3D-US, 3D-CPA is now set to expand the role of ultrasound in the assessment of the processes in the ovaries, uterus and early pregnancy. There is growing evidence that studies of peri-follicular vascularity will predict the development of a healthy oocyte and subsequently an embryo. Endometrial blood flow studies with conventional CDI and the newer techniques of CPA and 3D-CPA will be important in predicting endometrial receptivity. Ovarian stromal vascularity appears to correlate with vascular endothelial growth factor (VEGF) levels and high vascularity is associated with PCO and a risk of ovarian hyperstimulation syndrome. 3D-CPA may improve our ability to assess ovarian and endometrial vascularization and blood circulation, to diagnose tubal patency. Increasingly, 3D ultrasound is being applied to diagnose the pathology of early singleton and multiple pregnancies.

The female reproductive system is unique in that this is the only site in the uninjured adult body where angiogenesis (i.e. the formation of new blood vessels) occurs in a repetitive cyclic fashion. It is taking place in the ovary during follicular development and corpus luteum formation, and in the endometrium during proliferative phase of menstrual cycle. Soon after formation of the follicular antrum, the follicle acquires a vascular sheath in the theca layer. The establishment of the vascular sheath, particularly the expansion of the inner capillary plexus of the theca interna, co-occides with a period of rapid growth and differentiation of the follicle. The granulosa and theca cells of ovarian follicles are sources of angiogenic activity, which appear to be under the control of gonadotropins. At about the time of follicular rupture, vessels of the theca begin to sprout and...
penetrate the membrana granulosa; then, they anastomose to form a
dense network, ultimately bringing each lutein cell into close approxi-
mation to the vascular system. It has been shown that the rates of
follicular and luteal blood flow are among the highest in the body (5–10
and 10–30 ml/min/g, respectively) as compared to normal renal flow of
approximately 4–8 ml/min/g\(^2\). Endometrial growth and development in
response to systemic concentrations of ovarian steroids are well known.
The rate of blood flow to uterine tissues also varies throughout the men-
strual cycle and during the pregnancy\(^4\).

Details of angiogenic events in the ovary and endometrium have been
pivotal in understanding folliculogenesis and implantation. Therefore, by
coupling various ultrasound technologies to high frequency transvaginal
probes it is possible to perform detailed studies of ovarian and endometrial
vasculature which is important for the establishment and development of
pregnancy.

Recently, the introduction of colour Doppler imaging (CDI), colour
power angiography (CPA) and three-dimensional ultrasound (3D-US) has
increased the range of diagnostic information on angiogenesis within the
ovary and endometrium, and morphological conditions in the uterus and
Fallopian tubes.

Doppler ultrasound makes use of the Doppler effect where the changes
of frequency (frequency shift) caused when an ultrasound beam interacts
with moving erythrocytes in blood vessels can be measured and displayed.
This Doppler shift can be displayed either as a colour map of the blood
vessels within the organ being studied or as a flow velocity waveform
(spectral Doppler) which allows the measurements of absolute velocity
(cm/s) and resistance to flow (pulsatility index). New modalities such as
colour power angiography use the amplitude of the Doppler signals which
represents the density of erythrocytes within the vessels being studied. This
provides a more sensitive display of vasculature within an organ.

Three-dimensional ultrasonography (3D-US) has been used to assess the
uterine anatomy and to detect congenital anomalies of uterus. 3D-US differs
from conventional 2D scanning in that a volume of echoes is captured
instead of the usual 2D ‘slices’. This volume can be obtained by equipment
which can capture the volume from a free-hand sweep (positional informa-
tion being provided by an electromagnetic sensor) or by means of an
automatic capture of the volume by the transducer itself which moves in
two planes. This latter device is quicker and more accurate and will obtain
a volume within 5–20 s. When the volume has been obtained, it can be
analysed in several different ways.

**Re-slicing**

This means that volume can be analysed with traditional 2D slices but
now all planes can be obtained, even the plane at right angles to the surface
of the probe which cannot be obtained by conventional imaging. An example of the value of this was demonstrated by Jurkovic et al, who showed that this technique could display the coronal plane of the uterus making it useful in detecting major congenital anomalies and intra-uterine defects.

**Volume measurements**

More accurate measurements of the volume especially of irregular structures can be obtained by using multiplanar reformatted sections method and built-in software. This has been shown to improve the measurements of ovarian, follicular, uterine and endometrial volumes. Improved volume measurements provide better staging of endometrial cancer. However, the usefulness of those improved volume measurements have yet to be demonstrated in reproductive medicine.

**Surface rendering**

By means of shading and thresholding, surface rendered views can provide impressive 3D images of structures, especially if there is a solid/fluid interface. Impressive images can be obtained of the early embryo, but unique images can also be obtained of ovarian cysts, especially those with vegetations and submucous fibroids, particularly if hydrosonography is used. One of the most innovative applications of the surface rendering in gynaecology is its use to demonstrate tubal patency by obtaining three-dimensional views of flow along the Fallopian tube by 3D-CPA.

**Colour power angiography**

CPA is monochromatic and does not provide directional information, but its sensitivity to low flow makes this more useful to study tissue perfusion. Quantitative analysis of the blood flow in the predefined volume is available by implementing the colour histogram mode, the results of which are expressed as various indices. The indices are calculated by the built-in or separate computer using specially developed software.

**Vascularization index (VI)**

This describes the vessel density in the selected volume (colour voxels/[colour voxels + grey scale voxels]).

**Flow index (FI)**

This describes the intensity of blood flow in these vessels (weighted colour voxels/colour voxels).
Vascularization-flow index (VFI)

Vascularization-flow index reflects to some extent perfusion of the selected volume (weighted colour voxels/[colour voxels + grey scale voxels]).

Assessment of uterine receptivity

At present, ultrasonographic parameters of endometrial receptivity have a strong negative value in setting some minimum criteria, although their value as prognostic indicators for implantation following embryo transfer has yet to be proven. Good uterine perfusion, as shown by good diastolic flow and low resistance to flow indices, was correlated with conception following assisted reproduction treatment. Several authors demonstrated statistically significant difference in the measurements of uterine artery pulsatility index (PI) between those women who became pregnant and those who did not after IVF treatment. Steer et al suggested that measurement of mean uterine PI on the day of embryo transfer to assess endometrial receptivity is a good method of assessing uterine receptivity in women undergoing IVF-ET treatment. A mean PI of greater than 3.0 before embryo transfer could predict up to 35% of failures to become pregnant. Similar results have been obtained by other authors in studies investigating uterine PI and implantation rates whether on the day of follicular aspiration or on the day of human chorionic gonadotrophin (hCG) injection. However, some authors were not able to demonstrate any predictive value of uterine artery PI in terms of uterine receptivity in IVF patients. The uterine artery blood flow impedance on the day of embryo transfer was unrelated to the risk of the conceptus ending in spontaneous abortion or ectopic pregnancy. This finding implies that poor uterine blood flow does not increase the risk of adverse pregnancy outcome, if pregnancy was achieved.

Velocities and resistance indices were not correlated with endometrial thickness. However, combination of triple layered endometrium (thickness more than 8 mm) and of low vascular impedance in the uterine arteries (PI less than 3.0) have been suggested as reliable ultrasound markers for endometrial receptivity. The uterine scoring system which included multiple ultrasonographic and colour Doppler parameters on day 22 of the menstrual cycle prior IVF treatment cycle appeared to be a useful predictor of implantation.

Assessment of subendometrial blood flow has been of interest recently. Using conventional CDI, the degree of penetration of vessels into the endometrium correlated with the success of implantation. Absent subendometrial vascularity correlated with failure of implantation. Yang and coworkers used power Doppler imaging in assessment of endometrial vascularization by measuring the coloured area in the endometrium. Those women with an intra-endometrial coloured area <5 mm² achieved
significantly lower implantation rates (8.1% versus 20.2%, P = 0.003 than those with area >5 mm\(^2\). Therefore, the number of transferred embryos should be determined on the basis of the quantity of intra-endometrial vascularity as well as the endometrial thickness, so as to improve the reproductive outcome for those with poorly vascularized endometrium, and to reduce the potential risk of multiple pregnancies for those containing adequate intra-endometrial vascularity\(^{24}\). Three-dimensional CPA quantitative assessment of blood circulation gives a deeper insight into evaluation of endometrial receptivity. On the day of pituitary suppression, Schild et al\(^{25}\) found significant differences in subendometrial blood flow and vessel density between women who became pregnant and those who failed to become pregnant in an in vitro fertilisation programme. Surprisingly, they found lower 3D indices of volume flow in conception cycles compared with non-conceptional cycles, suggesting that a lesser degree of intra-uterine vascularization and perfusion at the beginning of ovarian stimulation indicated a more favourable endometrial milieu. Possibly, this reflected a better functional pituitary suppression following GnRH agonist administration which increases the chances of successful implantation.

Various regimens of medications such as low dose aspirin, heparin and NO (nitric oxide) have been found to improve endometrial receptivity due to their promoting effects on uterine perfusion. Low-dose aspirin treatment in patients undergoing in vitro fertilisation cycles significantly improved ovarian responsiveness, uterine and ovarian blood flow velocity, subsequent implantation and pregnancy rates in IVF patients as shown in a large randomized, double-blind study\(^{26}\). In studies of women who had increased uterine artery PI in a previous IVF cycle, administration of NO donor\(^{27}\) or cGMP promoter (sildenafil)\(^{28}\) have been shown to improve uterine and endometrial blood flow which may lead to improvement of an in vitro fertilisation outcome. Further studies are needed to understand and evaluate the endometrial vascular pattern both in spontaneous and stimulated ovarian cycles using conventional CDI and also the 3D-CPA technique, as well as safety of the agents used to promote better endometrial vascularization.

Fallopian tubes are important part in the chain of pregnancy establishment. Normally they have to be patent, which means hollow through the entire tubal length. Hysterosalpingo-contrast sonography (HyCoSy) using a positive contrast has been widely used to provide a rapid ultrasound-based test of tubal patency\(^{29}\). This method can be incorporated into the set-up of the initial investigation for infertility patients. However, a number of difficulties in tubal visualisation are encountered. Due to tubal tortuosity, the entire tube can rarely be seen in a single scanning plane and the echo-contrast medium is usually observed in small sections. In theory, by manipulating the probe, the contrast agent can be followed...
to the fimbrial end of the tube where free spill may be observed; but, in reality, this is infrequently achieved because the contrast agent has the same echogenicity as the surrounding bowel. Three-dimensional ultrasound helps to overcome these problems, because it enables the capture of a volume which should include the full length of the tube. Power Doppler which is sensitive to slow flow makes it possible to detect the flow of contrast medium along the tube up to the fimbrial end where free spill is identified. It allows the storage and retrospective analysis of the information acquired. More studies are required to estimate the cost-effectiveness of this procedure and to establish whether it can replace conventional diagnostic hysteroscopy and laparoscopy in the diagnosis of tubal patency.

The reported low specificity in the various studies, ranging between 15–44%, obviously indicates that uterine receptivity is one among several different factors contributing to implantation. Studies using donor oocytes have generally demonstrated equivalent implantation and pregnancy rates among reproductive young and older recipients, suggesting that oocyte and embryo quality are paramount in successful implantation. However, good embryos can derive only from eggs in normally developing follicles.

**Studies of ovarian stromal blood flow**

Adequate ovarian blood circulation is an important precondition for normal ovarian function. Blood flow patterns are different in the polycystic ovaries as demonstrated by Doppler ultrasound assessment than those in the normal ovaries. Zaidi et al have shown that ovarian stromal blood flow velocity is significantly higher (P <0.001) in women with polycystic ovaries (PCO) compared to women with normal ovaries. The reason for this increased stromal flow is unknown, but it may be related to high levels of angiogenic cytokines in the theca cell layer. It has been shown that women with PCO have a higher ovarian stromal PSV and serum concentration of VEGF than those with normal ovaries. It is well known that women with PCO are more susceptible to ovarian hyperstimulation syndrome (OHSS) and increased stromal flow may predict an increased susceptibility to this condition. VEGF is used to assess ovarian response during infertility treatment and a significant rise in serum VEGF concentration after hCG administration appears to be one of the most important predictors of OHSS.

Stromal flow velocities have also been used to study ovarian responsiveness in IVF treatment. Engmann et al studied 81 women with normal baseline serum FSH levels and normal ovaries; the ovarian stromal blood flow velocities measured after pituitary suppression appeared to be an independent predictor of ovarian response. In this study, patients with peak systolic velocity (PSV) greater than 10 cm/s has a better ovarian...
response and had a higher clinical pregnancy rate than those with PSV <10 cm/s. However, some of the studies\textsuperscript{16,17} have failed to demonstrate any significant difference in the intra-ovarian PI between those women who became pregnant and those who did not following an \textit{in vitro} fertilisation treatment. This may be because PI is a less sensitive indicator of angiogenesis than PSV.

Using three-dimensional power Doppler imaging, prominent changes of the vascularization and blood circulation in the dominant ovary during normal menstrual cycle were found. In midluteal phase, the vascularization index, flow index and vascularization-flow index in the dominant ovary were, respectively, 6.6-, 1.3- and 9.2-folds higher than in the non-dominant ovary\textsuperscript{39}. This way of assessment of ovarian vascularization in normal menstrual cycles and pathological conditions may be more sensitive and more accurate in diagnosing the changes than colour and pulsed Doppler measurements.

\section*{Evaluation of follicular function}

Campbell \textit{et al}\textsuperscript{40} first reported that indices of blood flow in the wall of the leading follicle could be monitored by transvaginal ultrasonography with colour Doppler imaging and spectral Doppler over the peri-ovulatory period. They showed that peak systolic velocity (PSV) around the follicle was a better indicator of angiogenesis than resistance (pulsatility index, PI). There was a significant rise in PSV from the time of the LH surge indicating a marked increase in blood flow during peri-ovulatory period\textsuperscript{40}. There may be relationship between low follicular blood velocity and luteinised unruptured follicle (LUF) in spontaneous cycles\textsuperscript{41}.

Follicular vascularity in the stimulated ovary during \textit{in vitro} fertilisation and embryo transfer (IVF-ET) treatment has also been studied. Analysis of these data showed that there was a significant relationship between the follicular PSV immediately before ultrasound-guided follicular aspiration, oocyte recovery and subsequent production of good quality pre-implantation embryos. There was a 70\% chance of producing a grade I or II embryo if the follicular PSV was greater than 10 cm/s but only 18\% if no blood flow was detected\textsuperscript{42}. The results of the study also showed that there was no direct relationship between follicular volume or flow resistance as indicated by the PI before the administration of human chorionic gonadotrophin (hCG). There was, however, a significant positive correlation between the detection of a follicular flow velocity waveform within a given follicle and the recovery of an oocyte. The factorial increase in PSV after hCG administration was significantly higher in follicles that subsequently produced good quality embryos\textsuperscript{43}. Chui and coworkers\textsuperscript{17} developed the grading system of assessing the
percentage of follicular circumference in which flow was identified from a single cross-sectional slide. Poor follicular blood flow was significantly associated with poor outcome, and successful pregnancies occurred more frequently in those women with good blood flow\(^{17,44,45}\). A strong relationship between follicular vascularity and the cumulus activity \textit{in vitro} was found\(^{46}\). Similar findings were observed in the stimulated intra-uterine insemination cycles when the grade of vascularized dominant follicles (larger than 16 mm in diameter) was recorded. As in IVF cycles, there was significant association between pregnancy rates and live birth rates and good blood flow\(^{47}\). The findings are consistent with the suggestion that changes in follicular vascularity may initiate biochemical events, which are essential within the follicular environment. There have been reports showing an association between oxygen concentration in follicular fluid\(^{48,49}\), or the oxygen consumption or adenosine triphosphate (ATP) content of an oocyte\(^{50,51}\), and the production of a good pre-implantation embryo. Moreover, the dissolved oxygen content of the follicle has been shown to be related to colour Doppler qualitative patterns and vascular endothelial growth factor and subsequently associated with developmental competence of the corresponding oocyte\(^{52}\).

The current findings of an association between follicular PSV and subsequent clinical pregnancy are consistent with the assumption that follicular blood flow may be associated with events essential for successful establishment of pregnancy. The knowledge gained from studies on follicular vascularity may be useful for designing future studies to understand physiological and biochemical events during folliculogenesis and to improve success rates of infertility treatment.

**Assessment of early pregnancy**

Key chronological landmarks in early human development seen on transvaginal sonography are still the same despite the developing ultrasound technique. The normal gestational sac can be first seen in the endometrium on transvaginal sonography 5 weeks after the last menstrual period (3 weeks after conception). It reaches the size of about 10 mm in diameter. Yolk sac becomes identifiable a few days later. The embryo adjacent to yolk sac and fetal heart pulsations (about 125 bpm) are first seen at 6 weeks. From then until 14 weeks, the growth of the embryo is measured from its crown-rump length (CRL). At 6 weeks of gestation, embryonic CRL is about 3 mm. At 8 weeks of gestation, embryo CRL is about 16 mm, heart rate 175 bpm and fetal body movements can now be observed. Recently, 3D ultrasound scanning in the first trimester was applied to visualize the surface of anatomical structures of the embryo and early fetus. The results of the investigations suggested that 3D ultrasonography is
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becoming an important modality in future embryological and early fetal research and detection of embryonic and fetal developmental disorders.\textsuperscript{53-56} 3D power Doppler imaging has the potential to study process of placentation and fetal cardiovascular development.

Over the last few years, intervillous blood circulation has been widely studied during the first trimester of pregnancy.\textsuperscript{57-59} Findings were in concordance with histomorphological evidence that spiral arteries begin to open directly into the intervillous space during the second month of gestation.\textsuperscript{57,60} The number of areas with the established intervillous circulation increases gradually with embryonic and placental growth. These findings indicate that establishment of the intervillous circulation is a continuous process rather than an abrupt event at the end of the first trimester. 3D power Doppler seems to be useful in visualizing intervillous blood circulation in the developing placenta.\textsuperscript{56}

Colour Doppler measurements failed to show the difference in uteroplacental circulation between patients with threatening miscarriage with a living embryo and those with normal pregnancy.\textsuperscript{61,62} It was speculated that vascular injury is possibly so minimal that it cannot be detected by current Doppler techniques. However, it appeared useful to select the patients who should undergo conservative or surgical management of first trimester miscarriages,\textsuperscript{63} which were already imminent. Perhaps application of 3D power Doppler imaging in the studies of early pregnancy miscarriages will help to obtain more clinically useful results.\textsuperscript{56}

Perinatal morbidity and mortality of twin gestations is higher among monochorionic in comparison with dichorionic twins.\textsuperscript{64} Therefore, early diagnosis of multiple pregnancy is very important because it allows mothers and obstetricians to anticipate various multiple pregnancy related problems. Data in which both zygosity and chorionicity were determined support the observation that chorionicity rather than zygosity determines outcome.\textsuperscript{65} Using high resolution, first trimester transvaginal sonography chorion and amnion types have been assessed in a number of large prospective studies.\textsuperscript{64,66} If only one embryo is found in each gestational sac, the number of amnion and chorion sacs is equal. If two or more embryos are seen within any chorionic sac, the number of amnion sacs can be determined usually at about 8 weeks of pregnancy, because the earlier amniotic sac contains a small amount of fluid and membranes are too thin for visualisation. Mono-amniotic twins will be surrounded by a single amnion. Pregnancies are classified as dichorionic if there is a single placental mass and there is extension of placental tissue into the base of the inter-twin membrane, the 'lambda' sign. Pregnancies are classified as monochorionic if there is a single placental mass in the absence of the lambda sign.\textsuperscript{67,68} Three-dimensional ultrasound scanning was described as a useful method to study inter-relationships and contacts of twin and triplet embryos and fetuses.\textsuperscript{55,69} By rotating and translating the planes...
inside the acquired volume containing the whole gestational sac, two yolk sacs and two embryos could be clearly visualized and precise diagnosis of chorionicity and amnionicity can be achieved.

The significantly adverse outcome of monochorionic twins is attributed mainly to the presence of communicating placental vascular anastomoses. Up to 35% of monochorionic twin gestations are complicated by twin-to-twin transfusion syndrome, which can be essentially disregarded after ultrasonographic diagnosis of a dichorionic gestation.

Conjoined twins is the most frequently reported first trimester structural anomaly unique to twins. The commonest type of conjoined twins is thoracopagus, where the twins are joined at the thorax; 3D transvaginal sonography has been used to depict thoraco-omphalopagus. Although it was not essential, 3D scanning aided diagnosis by using multiplanar imaging as well as spatial surface animation. As a rule, due to the unclear prognosis associated with conjoined twins, whenever a mono-amniotic twin gestation is diagnosed, the possibility of conjoined twins should be considered.

There are few data available on detection of fetal structural anomalies by high resolution transvaginal scans in early pregnancy in unselected populations. Detection rates reported in the literature are reaching up to 65%. Sonographic recognition of congenital anomalies depends on knowledge of normal fetal development, ultrasound equipment used, and natural histories of the particular disorders. Scans during the first stage of pregnancy will miss some fetal anomalies because they appear later in pregnancy or might be undetectable at this stage. This is a scope of whole separate review. One thing is worth mentioning: 3D ultrasound appears useful in diagnosing fetal anomalies during the first trimester of pregnancy.

References

1. Richards JS. Maturation of ovarian follicles actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol Rev 1980; 60: 51–89
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16 Tekay A, Martikainen H, Jouppila P Blood flow changes in uterine and ovarian vasculature, and predictive value of transvaginal pulsed colour Doppler ultrasonography in an in-vitro fertilisation programme *Hum Reprod* 1995, 10 688-93


28 Sher G, Fisch J. Vaginal sildenafil (Viagra): a preliminary report of a novel method to improve uterine artery blood flow and endometrial development in patients undergoing IVF. *Hum
Human reproduction: pharmaceutical and technical advances

Reprod 2000; 15: 806–9


31 Abdalla HI, Wren ME, Thomas A, Korey L. Age of the uterus does not affect pregnancy or implantation rates; a study of egg donation in women of different ages sharing oocytes from the same donor. Hum Reprod 1997; 12: 827–9


44 Bhal PS, Pugh ND, Chui DK et al. The use of transvaginal power Doppler ultrasonography to evaluate the relationship between perifollicular vascularity and outcome in in-vitro fertilization treatment cycles. Hum Reprod 1999; 14: 939–45


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60. Carter AM. When is the maternal placental circulation established in man? *Placenta* 1997; 18: 83


