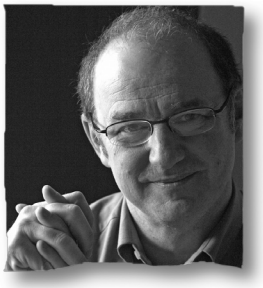
Outlook

Ultrasound and the receptivity of the endometrium



Stephen Killick has worked with subfertile couples for the last 20 years and has run IVF units in both Manchester and Hull. He has performed and published many studies on fertility research, both from the contraceptive and infertility points of view, and is currently Professor of Reproductive Medicine and Surgery at the University of Hull and the newly established Hull York Medical School. He has worked for the World Health Organization in Geneva and at medical clinics in South Africa and the Netherlands. His current major research interest is the use of ultrasound in reproductive medicine.

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Abstract

Ultrasound imaging can be used to assess the endometrium in a number of ways. In particular, time-lapse video recordings can show that the lining of the uterus undergoes rhythmical contractions that vary in strength and intensity throughout the ovarian cycle. These contractions appear to assist in sperm transport at the time of ovulation, but can decrease the chances of a fertilized egg implanting in the uterus if they persist later in the cycle. They are also the cause of ectopic pregnancies occurring in IVF treatment cycles. In order to reduce these uterine contractions and therefore increase the chances of a successful uterine pregnancy, IVF treatment cycles should incorporate minimal stimulation so as to make them as close to natural cycles as possible.

Keywords: *implantation, junctional zone contraction, ultrasound*

Introduction

A failure of implantation remains the main reason why most IVF treatment fails to result in pregnancy. Despite the many advances that have been made to the way in which IVF is performed, implantation rates per embryo stubbornly remain at less than 15%. There is considerable evidence, including for example results from cases of oocyte donation between women of different ages, that tells us that much of this implantation failure is the consequence of lower embryo quality, i.e. related to the 'seed' rather than the 'soil'. However, an unknown proportion must be the consequence of abnormal or suboptimal endometrial development, and the ways in which the receptivity of the endometrium can be assessed are extremely limited.

This short review will look at some of the ways in which ultrasound has been used to assess endometrial receptivity, with particular reference to the possibility of improving implantation in cycles with minimal exogenous steroid stimulation.

Benefits of ultrasound

A cursory search of the scientific literature reveals in excess of 1000 different molecules within the endometrium that researchers have advocated as having the potential to influence implantation. The search for relevant ratios and interrelationships in such a complex milieu, given the obvious *in vitro* limitation of molecular work, is daunting (Aplin, 2006). In contrast, ultrasound examinations can be performed repeatedly *in vivo*, giving us measurements of rates of change and hence ideas of function as well as structure. To date, ultrasound has been used to measure the structural development, the vasculature architecture and, most revealingly, uterine muscular movements.

Endometrial structure as seen by ultrasound

Ultrasound sees the endometrium as a single thin line immediately after menstruation. This then expands under the influence of oestrogen in the follicular phase of the cycle to the typical trilaminar hypoechoic appearance. After ovulation

there is little increase in size. The change to a secretory state is characterized by increasing echogenicity beginning at the periphery of the endometrium and progressing towards the midline over a period of 24–48 h. The mature luteal endometrium appears homogenous and hyperechoic compared with the myometrium (**Figure 1**).

The growth of the endometrium is an obvious way to measure the response to endocrine stimulation, and the endometrium becomes thicker in cycles stimulated with high doses of steroids, but there is no consistent correlation between either the final thickness or the rate of growth and the subsequent chance of implantation (Turnbull *et al.*, 1995). There appears to be a minimal value of about 5 mm double thickness below which implantation rarely occurs, but endometria are rarely this thin except in anovulatory cycles, i.e. the result of an ovarian rather than an endometrial problem. Volume measurements with either three-dimensional ultrasound or magnetic resonance (MR) likewise have shown no correlation with pregnancy rates. One exception to this might be with clomiphene-induced cycles in which several researchers have suggested the thinner the endometrium, the less chance of pregnancy (Turnbull *et al.*, 1995).

The endometrial pattern has been correlated with IVF success rates, with a trilaminar pattern shown to be more favourable than a homogenous luteal pattern at the time of the human chorionic gonadotrophin ovulatory trigger (Sher *et al.*, 1991). The interpretation of these findings is probably that an early luteal appearance is a sign of early progesterone production rather than any inherent fault with endometrial development. A premature rise in serum progesterone has been shown to be associated with lower pregnancy rates in various assisted-reproduction cycles (Randall *et al.*, 1996; Bosch *et al.*, 2003; Ozcakir *et al.*, 2004), although not all authors agree (Givens *et al.*, 1994; Melo *et al.*, 2006). The inconsistency in results might reflect whether the rise in progesterone is early enough and of a high enough level to alter endometrial pathology. Ultrasound may therefore be a more accurate interpreter of premature luteinization than a single serum progesterone estimation.

Endometrial vascularity as seen by ultrasound

Ultrasound can estimate blood flow in a number of ways. Flow in larger vessels, such as the ovarian or uterine arteries, can be evaluated using the pulsatility index (PI) or resistance index (RI). Uterine arterial flow varies as might be expected throughout the normal ovulatory cycle, with the lowest resistance to flow seen during the mid-luteal phase (Tan *et al.*, 1996). Interestingly the changes in flow in both the ovarian and uterine vessels are greater on the side of the pre-ovulatory follicle (Tan *et al.*, 1996). Although as with other endometrial measurements there is a correlation between flow characteristics and serum hormone levels, which exaggerates many of the responses in stimulated cycles, there are conflicting results as to whether PI values during the luteal phase are significantly lower in conception cycles (Chien *et al.*, 1995; Levi-Setti *et al.*, 1995; Tekay *et al.*, 1996; Aytoz *et al.*, 1997; Schild *et al.*, 2001; Chien *et al.*, 2004).

Flow in the smaller neovascular vessels within the endometrium itself might be more relevant to its function, and measurements of the vascular index (VI), flow index (FI) and vascular flow index (VFI) have been used in attempts to predict receptivity. Studies have demonstrated a steady increase in endometrial vascularity until just before ovulation and a subsequent decline afterwards (Raine-Fenning *et al.*, 2004a). The same authors have demonstrated a reduction in endometrial vascularity in patients with unexplained infertility irrespective of serum hormone concentrations (Raine-Fenning *et al.*, 2004b).

It would be expected, somewhat intuitively, that a better endometrial vascularity would result in a greater chance of pregnancy. However, Ng *et al.* (2006) recently measured RI, PI, VI, FI and VFI at the time of oocyte recovery in IVF cycles. Their findings were of a lower endometrial VI and VFI in treatments that resulted in pregnancy. They suggested a greater chance of pregnancy with no endometrial flow. Further work is necessary in this area.

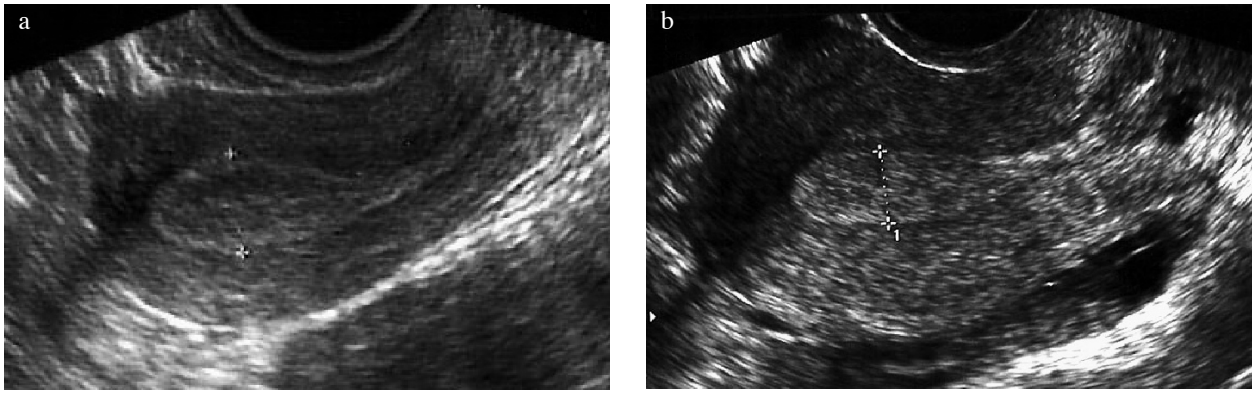

Figure 1. Temporal changes in the appearance of the endometrium as seen by ultrasound throughout the ovarian cycle. The double endometrial width is marked by +……+. Note the thin hypoechoic junctional zone surrounding the endometrium. Histological studies show this to be compacted myometrial tissue. (**a**) trilaminar endometrium as seen at the end of the follicular phase; (**b**): luteal endometrium as seen at about the time that implantation would normally occur.

Junctional zone contractions

Uterine myometrial contractions have been well investigated for their action at the time of parturition and dysmenorrhoea, but time-lapse ultrasound imaging has shown the endometrium to undergo peristaltic-like movements at other times (Birnholz, 1984; Ijland *et al.*, 1996; Kunz *et al.*, 1996). There are no contractile fibres within the endometrium, so these movements must be the result of contractions in the adjacent myometrium. The innermost layer of the myometrium adjacent to the endometrium has a more compact structure than the rest of the myometrium (Tetlow *et al.*, 1999), suggesting a modified function. Different authors ascribe different names to this layer: junctional zone, inner myometrium, subendometrial halo and subendometrial layer are all synonymous. The layer can be viewed by either ultrasound or MR.

Junctional zone contractions (JZC) result in rhythmic movements of the endometrium at a frequency around three times per minute, and creating peristaltic waves travelling at about 3 cm/min (Ijland *et al.*, 1997a). The contractions are most frequent and of a higher amplitude at mid-cycle (Abramowicz and Archer, 1990) when most waves are seen to travel from the cervix to the fundus. The function of JZC at about the time of ovulation would appear to be to assist in sperm transport (Leyendecker *et al.*, 1996). Kunz *et al.* (1996) demonstrated that radiolabelled albumin particles could be transported from the cervix to the Fallopian tube within a minute, which is far faster than a sperm could possibly swim. Moreover, the particles were transported preferentially to the tube adjacent to the dominant follicle.

JZC diminish as the luteal phase progresses, becoming less frequent, of lower amplitude and multidirectional (Kunz *et al.*, 1996; Ijland *et al.*, 1997b). By mid-luteal phase, when implantation would be expected to begin, contractions are hardly present at all in natural cycles. Several studies have now confirmed that increased JZC at the time of implantation appear to act against successful implantation in both natural (Ijland *et al.*, 1997b) and stimulated cycles (Fanchin *et al.*, 1998), although not all the data support this view (Vlaisavljevic *et al.*, 2001). Fanchin *et al.* (1998) (see **Figure 2**) found a significant negative correlation between the frequency of JZC and the subsequent pregnancy rate in IVF cycles. It should be remembered that in stimulated cycles not only are JZC more prominent (Abramowicz and Archer, 1990; Lesny *et al.*, 1998), presumably because of the increased hormone concentrations, but also the embryos are transferred to the uterine cavity slightly earlier in the reproductive cycle when JZC would be expected to be more prominent.

Our group (Lesny and Killick, 2004) has studied JZC and confirmed the negative relationship between them and the chances of pregnancy in stimulated IVF cycles (**Table 1**). A relationship between the physical stimulation during embryo transfer and the frequency of contractions has also been demonstrated (Lesny *et al.*, 1999a; Biervliet *et al.*, 2002). The more traumatic the embryo transfer, the more the endometrium moves, and the less likely the treatment is to result in pregnancy. Ultrasound-positive contrast medium at the time of a mock embryo transfer has been used to demonstrate that JZC can expel embryos from the uterine cavity out through the cervix (fundo-cervical contractions). They can also relocate

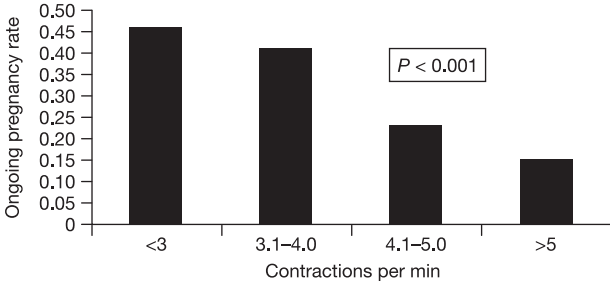

Figure 2. Frequency of junctional zone contractions at the time of embryo transfer in IVF cycles compared with the subsequent ongoing pregnancy rate. Adapted from Fanchin *et al.* (1998).

Table 1. Difference in the pregnancy rate between 50 consecutive patients treated with IVF who had less than five endometrial contractions in the 2 min immediately following embryo transfer as compared with patients who had more than five contractions in the 2 min following embryo transfer (ET).

	<5 JZC per 2 min after ET	*>5 JZC per 2 min after ET*
n	37	13
Clinical pregnancies	11	0
Pregnancy rate /ET[a] (%)	29.7	0

JZC = junctional zone contraction.
[a]$P < 0.05$ by χ^2 test.

embryos from the uterus into the Fallopian tube (cervico-fundal contractions) (Lesny *et al*., 1999b). The first ever IVF pregnancy, which occurred in a stimulated cycle, was a tubal ectopic. It is interesting to note that Bob Edwards and Patrick Steptoe discontinued stimulated cycles after this event, and Louise Brown was conceived during a natural monitored cycle. Ectopic pregnancy rates for stimulated IVF remain in the region of 4% of all conceptions, even after transmyometrial transfer (Biervliet *et al*., 2002) and it seems highly likely that many of the embryos transferred to the uterus find their way into the Fallopian tube as a result of JZC. A proportion of embryos probably return to the uterus before they implant.

In order to improve IVF intrauterine pregnancy rates, ways of decreasing JZC need to be investigated. Strategies might include transferring embryos as late as possible in the cycle when JZC are reduced, performing embryo transfer as atraumatically as possible, and providing minimal hormonal stimulation for oocyte development so as to keep treatment cycles as close to natural cycles as possible.

Pharmaceutical suppression of contractions would be an attractive way forward. Progesterone support of the luteal phase has been shown to be beneficial in IVF cycles despite the multiple corpora lutea already producing high concentrations. This may be because progesterone is suppressive of JZC, and early progesterone support in the form of vaginal progesterone has been shown to decrease JZC at the time of embryo transfer (Fanchin *et al*., 2001). Our group has tried to reduce JZC with isosorbide, rofecoxib and diclofenac (unpublished data) but has not been able to detect a suppressive effect.

Videos of JZC

Viewing this paper online [http://www.rbmonline.com/Article/2859] enables the reader to access a short video of JZC. The video shows a longitudinal image of the uterus in the early luteal phase of the cycle, and it has been speeded up to five times normal speed. Peristaltic movements are seen in the endometrium arising from the cervical area and progressing towards the fundus. Such contractions are thought to enhance sperm transport and to have largely ceased by the time implantation occurs. In stimulated IVF cycles, the contractions are enhanced both by the high hormone levels and the fact that the embryos are transferred to the uterus earlier than in a natural cycle. These contractions lower the overall chances of successful implantation and increase the chances of implantation occurring in the Fallopian tube.

More videos of endometrial contractions can be accessed from a published CD-ROM version (see Lesny *et al*., 1999c).

References

Abramowicz JS, Archer DF 1990 Uterine endometrial peristalsis—a transvaginal ultrasound study. *Fertility and Sterility* **54**, 451–454.

Aplin JD 2006 Embryo implantation: the molecular mechanism remains elusive. *Reproductive BioMedicine Online*. **13**, 833–839.

Aytoz A, Ubaldi F, Tournaye H *et al.* 1997 The predictive value of uterine artery blood flow measurements for uterine receptivity in an intracytoplasmic sperm injection program. *Fertility and Sterility* **68**, 935–937.

Biervliet FP, Lesny P, Maguiness SD *et al*. 2002 Transmyometrial embryo transfer and junctional zone contractions. *Human Reproduction* **17**, 347–350.

Birnholz JC 1984 Ultrasonic visualisation of endometrial movements. *Fertility and Sterility* **41**, 157–158.

Bosch E, Valencia I, Escudero E *et al*. 2003 Premature luteinization during gonadotropin-releasing hormone antagonist cycles and its relationship with *in vitro* fertilization outcome. *Fertility and Sterility* **80**, 1444–1449.

Chien LW, Lee WS, Au HK, Tzeng CR 2004 Assessment of changes in utero-ovarian arterial impedance during the peri-implantation period by Doppler sonography in women undergoing assisted reproduction. *Ultrasound in Obstetrics and Gynecology* **23**, 496–500.

Chien LW, Tzeng CR, Chang SR, Chen AC 1995 The correlation of the embryo implantation rate with uterine arterial impedance in *in vitro* fertilization and embryo transfer. *Early Pregnancy* **1**, 27–32.

Fanchin R, Righini C, De-Ziegler D *et al*. 2001 Effects of vaginal progesterone administration on uterine contractility at the time of embryo transfer. *Fertility and Sterility* **75**, 1136–1140.

Fanchin R, Righini C, Olivennes F *et al*. 1998 Uterine contractions as visualised by ultrasound alter pregnancy rates in IVF and embryo transfer. *Human Reproduction* **13**, 1968–1974.

Givens CR, Schriock ED, Dandekar PV, Martin MC 1994 Elevated serum progesterone levels on the day of human gonadotropin administration do not predict outcome in assisted reproduction cycles. *Fertility and Sterility* **62**, 1011–1017.

Ijland MM, Evers JLH, Dunselman GAJ *et al*. 1997a Relation between endometrial wavelike activity and fecundability in spontaneous cycles. *Fertility and Sterility* **67**, 492–495.

Ijland MM, Evers JL, Hoogland HJ 1997b Velocity of endometrial wavelike activity in spontaneous cycle. *Fertility and Sterility* **68**, 72–75.

Ijland MM, Evers JLH, Dunselman GAJ *et al*. 1996 Endometrial wavelike movements during the menstrual cycle. *Fertility and Sterility* **65**, 746–749.

Kunz G, Beil D, Deininger H *et al*. 1996 The dynamics of rapid sperm transport through the female genital tract: evidence from vaginal sonography of uterine peristalsis and hysterosalpingoscintigraphy. *Human Reproduction* **11**, 627–632.

Lesny P, Killick SR 2004 The uterine junctional zone and its contractions. *British Journal of Obstetrics and Gynaecology* **111**, 1182–1189.

Lesny P, Killick SR, Robinson J *et al*. 1999a Embryo transfer and junctional zone contractions: is it safe to use a tenaculum? *Human Reproduction* **14**, 2367–2370.

Lesny P, Killick SR, Robinson J *et al*. 1999b Case report: ectopic pregnancy after transmyometrial embryo transfer. *Fertility and Sterility* **72**, 357–359.

Lesny P, Killick SR, Tetlow RL *et al*. 1999c Embryo transfer and uterine junctional zone contractions. *Human Reproduction Update* **5**, 87–88 (CD ROM).

Lesny P, Killick SR, Tetlow RL *et al*. 1998 Uterine junctional zone contractions during assisted reproduction cycles. *Human Reproduction Update* **4**, 440–445.

Levi Setti PE, Rognoni G, Bozzo M *et al*. 1995 Color-Doppler velocimetry of uterine arteries in pregnant and nonpregnant patients during multiovulation induction for IVF. *Journal of Assisted Reproduction and Genetics* **12**, 413–417.

Leyendecker G, Kunz G, Wildt L *et al*. 1996 Uterine peristalsis and dysperistalsis as dysfunctions of the mechanism of rapid sperm transport in patients with endometriosis and infertility. *Human Reproduction* **11**, 1542–1551.

Melo MAB, Meseguer M, Garrido N *et al.* 2006 The significance of premature luteinization in an oocyte-donation programme. *Human Reproduction* **21**, 1503–1507.

Ng EH, Chan CC, Tang OS *et al*. 2006 The role of endometrial and subendometrial blood flows measured by three-dimensional power Doppler ultrasound in the prediction of pregnancy during IVF treatment. *Human Reproduction* **21**, 164–170.

Ozcakir HT, Levi R, Tavmergen E, Goker ENT 2004 Premature luteinisation defined as progesterone estradiol ratio >1 on hCH administration day seems to adversely affect clinical outcome in long gonadotropin-releasing hormone agonist cycles. *Journal of Obstetrics and Gynaecology Research* **30**, 100–104.

Raine-Fenning NJ, Campbell BK, Kendall NR *et al*. 2004a Quantifying the changes in endometrial vascularity throughout the normal menstrual cycle with three-dimensional power Doppler angiography. *Human Reproduction* **19**, 330–338.

Raine-Fenning NJ, Campbell BK, Kendall NR *et al*. 2004b Endometrial and subendometrial perfusion are impaired in women with unexplained subfertility. *Human Reproduction* **19**, 2605–2614.

Randall GW, Gantt PA, Gantt D *et al*. 1996 Elevated serum progesterone values at the time of ovulation induction in luteal leuprolide acetate-down-regulated GIFT cycles are associated with decreased clinical pregnancy rates. *Journal of Assisted Reproduction and Genetics* **13**, 459–463.

Schild RL, Knobloch C, Dorn C *et al*. 2001 Endometrial receptivity in an *in vitro* fertilization program as assessed by spiral artery blood flow, endometrial thickness, endometrial volume, and uterine artery blood flow. *Fertility and Sterility* **75**, 361–366.

Sher G, Herbert C, Maasarani G, Jacobs MH 1991 Assessment of the late proliferative phase endometrium by ultrasonography in patients undergoing IVF/ET. *Human Reproduction* **6**, 232–237.

Tan SL, Zaidi J, Campbell S *et al*. 1996 Blood flow changes in the ovarian and uterine arteries during the normal menstrual cycle. *American Journal of Obstetrics and Gynecology* **175**, 625–631.

Tekay A, Martikainen H, Jouppila P 1996 Comparison of uterine blood flow characteristics between spontaneous and stimulated cycles before embryo transfer. *Human Reproduction* **11**, 364–368.

Tetlow RL, Richmond I, Manton DJ *et al*. 1999. Histological analysis of the uterine junctional zone as seen by transvaginal ultrasound. *Ultrasound in Obstetrics and Gynecology* **14**, 188–193.

Turnbull LW, Lesny P, Killick SR 1995 Assessment of uterine receptivity prior to embryo transfer: a review of currently available imaging modalities. *Human Reproduction Update* **1**, 505–514.

Vlaisavljevic V, Reljic M, Gavric–Lovrec V, Kovacic B 2001 Subendometrial contractility is not predictive for *in vitro* fertilization (IVF) outcome. *Ultrasound in Obstetrics and Gynecology* **17**, 239–244.

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