Perifollicular vascularity as a potential variable affecting outcome in stimulated intrauterine insemination treatment cycles: a study using transvaginal power Doppler

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BACKGROUND: The aim of the present study was to assess any potential relationship between perifollicular vascularity and outcome in an in-vivo environment following human chorionic gonadotrophin (HCG) administration.

METHODS: A total of 182 unselected consecutive patients undergoing stimulated intrauterine insemination (IUI) cycles was recruited where the perifollicular vascularity of follicles ≥16 mm was studied using a subjective grading system and transvaginal power Doppler ultrasonography, 36 h after HCG administration. RESULTS: A total of 601 follicles was studied. The incidence of follicles showing high-grade perifollicular vascularity (3 and 4) was higher than those with low-grade vascularity (1 and 2) (80 versus 20%). Treatment cycles were divided according to uniformity of vascularity grades of follicles ≥16 mm on the day of IUI [55% all high (3/4) grade; 33% mixed (1/2 and 3/4) and 12% all low (1/2) grade]. The mean age and duration of subfertility were significantly higher (P < 0.05), whereas the number of follicles ≥16 mm pre/post HCG, serum oestradiol and incidence of ultrashort gonadotrophin-releasing hormone (GnRH) agonist use were all significantly lower (P < 0.05) in treatment cycles with uniformly low follicular vascularity grades compared with mixed or uniformly high-grade cycles. However, on subjecting the data to multiple logistic regression analysis, the only independent variables that affected pregnancy rates appeared to be serum oestradiol (OR 1.28, 1.01–1.62) and high-grade follicular vascularity (OR 2.41, 1.08–5.40). CONCLUSION: These data would suggest that perifollicular vascularity has an important role to play in the outcome of IUI cycles, and that power Doppler has the potential to refine the management of assisted reproduction treatment cycles.

Key words: intrauterine insemination/perifollicular vascularity/power Doppler/stimulated cycles/ultrasonography

Introduction

The rationale for intrauterine insemination (IUI) with washed spermatozoa involves bypassing the cervical mucus barrier, resulting in an increased gamete density at the site of fertilization (Ombelet et al., 1997). Better washing procedures are believed to enhance sperm fertilizing ability both in vitro and in vivo (Aitken and Clarkson, 1987), which may lead to higher success rates after IUI in male factor causes (Martinez et al., 1993; Ombelet et al., 1995). The use of gonadotrophins in IUI is now widespread, and has been shown to improve significantly the odds of pregnancy (Hughes, 1997). However, significant risks of ovarian hyperstimulation syndrome—and in particular multiple pregnancy—remain (Ombelet et al., 1996). Despite this, IUI has significant cost savings and is less invasive, without necessarily a reduction in effectiveness, compared with other forms of assisted reproduction treatment such as IVF or gamete intra-Fallopian transfer (GIFT) in particular for non-tubal infertility (Robinson et al., 1992; Peterson et al., 1994; Dodson, 1995).

It has long been recognized that neovascularization may be of prime importance in the growth and selection of ovulatory follicles, in addition to the subsequent development and function of the corpus luteum (Anderson, 1926; Bassett, 1943). Studies of ovarian vascular morphology showed that the capillary network of preovulatory follicles was more extensive than that of other follicles (Clarke, 1900; Anderson, 1926), consequently proposing that initiation and maintenance of follicular growth depends on development of the follicular microvasculature.

Several studies have examined the cyclical variations of female pelvic haemodynamics and angiogenesis using Doppler ultrasound (Long et al., 1989; Scholtes et al., 1989). Since then, transvaginal pulsed Doppler has been used quite extensively to assess uterine and ovarian blood flow patterns in ART cycles (Kurjak et al., 1991; Strohmer et al., 1991; Steer et al., 1992).
More recently, an increasing number of publications have suggested that the use of colour flow imaging may assist in the management of ART cycles (Lunenfeld et al., 1996; Nargund et al., 1996; Bassil et al., 1997).

Power Doppler or colour angio-ultrasonography has been generating increasing clinical interest since its introduction five years ago (Arenson and Allinson, 1994). Recent studies have shown that power Doppler has the potential to improve the depiction of intra-organ vasculature especially in the liver, kidneys, brain and testis (Meire and Farrant, 1994; Babcock et al., 1996) as it has a three-fold increase in sensitivity compared with conventional colour Doppler imaging at detecting low-velocity flow (Rubin et al., 1994; MacSweeney et al., 1996). Despite the fact that the application of power Doppler in gynaecology remains in its infancy, recent studies suggest potential applications especially in relation to the assessment of uterus (Serafini et al., 1997) and perifollicular vascularity (Chui et al., 1997; Bhal et al., 1999), and outcome in IVF treatment cycles.

Statistical models have been reported for predicting success rates in IUI which suggested that follicle number, endometrial thickness, duration of subfertility and semen parameters were the most significant variables in predicting outcome (Tomlinson et al., 1996). The application of Doppler ultrasound in IUI has tended to relate uterine perfusion to outcome (Tsai et al., 1996; Thoma et al., 1997), and few data exist on ovarian vascularity in these treatment cycles. The aim of the present study was to assess any potential relationship between perifollicular vascularity following human chorionic gonadotrophin (HCG) administration and outcome in an in-vivo environment using the subjective grading system and power Doppler ultrasonography which was previously applied in an in-vitro setting (Bhal et al., 1999).

Materials and methods

This was a prospective cross-sectional study of unselected consecutive stimulated IUI treatment cycles carried out at the Cardiff Assisted Reproduction Unit (CARU) between 1996 and 1998. All patients had had tubal patency tests in the form of laparoscopy and dye hydrotubation or hysterosalpingogram, to confirm the presence of at least one patent Fallopian tube. A total of 182 treatment cycles was studied. The main exclusion criteria included couples with a severe male factor where the sperm wash preparation was <1×10^6 motile spermatozoa on the day of IUI. Pregnancy rates in CARU among this group of patients were significantly lower, in the order of 11% per cycle (P.S.Bhal, personal communication), which would not permit a proper evaluation of the influence of follicular vascularity on pregnancy. Patients recruited into the study provided both written and verbal consent to partake in this study, which was approved by the BroTaf Local Research Ethics Committee.

Pituitary desensitization was achieved with 250–500 µg (0.25–0.5 ml) of buserelin (Suprefact®; Hoechst UK Limited, Hounslow, UK) administered as a s.c. injection, starting on day 2 after a menstrual period commenced. Two protocols were used routinely: an ultrashort protocol with s.c. buserelin on days 2–4; or a short protocol with s.c. buserelin from day 2 until the administration of HCG. Patients underwent pelvic ultrasonography scanning to exclude pelvic abnormalities such as ovarian cysts, before commencing ovarian stimulation. Patients with a history of polycystic ovarian disease, endometriosis or previous ovarian hyperstimulation syndrome were given long pituitary desensitization (500 µg buserelin) from day 21 of the cycle preceding treatment. In these patients, once pituitary down-regulation was confirmed (by scanning/serum oestradiol concentration), the daily dose of buserelin was reduced to 250 µg until the day of HCG administration.

Ovarian stimulation in order to achieve follicular development was carried out using exogenous gonadotrophins in the form of human menopausal gonadotrophin (HMG; Fergonal®; Serono Laboratories, Welwyn Garden City, UK; Humegon® or Normegon®; Organon Laboratories, Cambridge, UK) or urofolliotrophin (Metrodin High Purity®; Serono Laboratories). The standard starting dose for stimulation was 150 IU FSH daily from day 3, and this was continued until an appropriate response was obtained. More recently, with the advent of recombinant FSH the commencing dose was reduced to 100 IU FSH (Puregon®; Organon Laboratories; Gonaf F®; Serono Laboratories).

Transvaginal Doppler assessment (Toshiba 140A and 270 machines; Zoetermeer, Netherlands) was performed by one of the authors (P.S.B., N.P. and S.O’B.). 32–36 h following the administration of HCG (Profasi®; Serono Laboratories, Woking, UK) when the lead follicle was ≥16–18 mm in diameter. A 6 MHz curvilinear transvaginal probe was used with the velocity range, wall filter, and colour gain being standardized for both scanners, and all scans performed. The lead or largest follicle, together with all follicles ≥16 mm in diameter, were graded using power Doppler based on a subjective grading system that had been previously described (Chui et al., 1997; Bhal et al., 1999). This grading system was based on the percentage of perifollicular circumference (in 25% increments) that depicted an echo signal and ranged from grades 1–4 (grades 1 or 2 being low grade, and 3 or 4 being high grade). The reproducibility of perifollicular vascularity assessment had previously been analysed using kappa (κ) values which showed high inter-observer agreement (Bhal et al., 1999). Further parameters measured included endometrial thickness, and mean uterine and ovarian stromal pulsatility indices (PI). Following the Doppler scan, IUI using prepared/washed semen (husband/donor) was carried out. All patients received luteal support in the form of i.m. progesterone (Gestone®; Ferring, Langley, UK) on an alternate-day basis, from the day of IUI for 14 days. Serum βHCG estimation was carried out 2 weeks after insemination; if this was positive, luteal support was continued until 14 weeks gestation. Early pregnancy losses included ectopic pregnancies and clinical miscarriages or missed abortions by 8 weeks gestation.

In terms of evaluating outcome in relation to follicular vascularity, treatment cycles were divided into three groups. This was based on the vascularity grades of cohort of follicles ≥16 mm on the day of IUI which were all high grade (only 3 or 4), mixed grades (1/2 and 3/4) or all low grade (only 1 or 2). The data were evaluated in this way, as it was not possible to be precise as to which follicle was responsible for producing the oocyte that would subsequently fertilize and implant in cycles that were subjected to controlled ovarian stimulation.

Statistical analysis

A one-way analysis of variance (ANOVA) was applied to assess statistical differences between mean values (as data were assumed to be normally distributed) of the three groups (all high-, all low- or mixed-grade vascularity). A Bonferroni correction was also applied to ascertain specific differences between these groups. A χ² or Fisher’s exact test was applied on proportions of outcome rates related to vascularity grades, with P < 0.05 considered significant. Appropriate t-tests were applied to study differences in means between singleton and multiple pregnancies. Multiple logistic regression analysis was
Some 55% (n = 101) of cycles had uniformly high-grade vascular perfusion, while 33% (n = 60) and 12% (n = 21) had either mixed or uniformly low-grade perfusion. The mean age and duration of subfertility were significantly higher (P < 0.05) in the group of treatment cycles where all follicles ≥16 mm had low-grade vascular perfusion compared with the mixed and high-grade population (Table I). Mean baseline serum FSH concentrations also tended to be higher in low vascular cycles compared with high- or mixed-grade cycles, but this difference was not significant (Table I). Other patient characteristics, including parity or type of subfertility, were similar between all three vascularity cohorts. The incidence of treatment cycles using an ultrashort GnRH agonist protocol was significantly lower in the low-grade treatment cycle population as opposed to the other two groups (Table I). However, the mean duration and dose of FSH stimulation were comparable between all three groups.

Mean serum oestradiol concentration before HCG administration, and the number of follicles (≥16 mm) post HCG were significantly lower (P < 0.05) in the low-grade group when compared with the mixed- and high-grade treatment cycles (Table I). Mean serum LH concentration on the day of HCG administration, endometrial thickness and utero-ovarian pulsed Doppler indices on the day of IUI were similar in all three vascular perfusion groups (Table I). There was no statistically significant difference in semen parameters in the three groups; neither was there any significant difference in the incidence of cycles where donor semen was used between different vascular perfusion groups (Table I).

There were 42 pregnancies, giving a pregnancy rate of ~23% per cycle. The overall early pregnancy loss rate was ~24%, and the multiple pregnancy rate was ~33%, with 11 sets of twins and three sets of triplets. There was one ectopic pregnancy. The relationship between outcome and follicular vascularity was evaluated based on the vascularity characteristics of the lead cohort of follicles as the majority of these cycles were multifollicular with follicles ≥16 mm all having the capacity to produce oocytes that can be fertilized. Therefore, patients were stratified into three cohorts or groups of treatment cycles with uniformly high/low/mixed follicular vascularity (Table II). Cycles with follicles of uniformly high-grade vascularity were associated with significantly higher pregnancy rates (31%) than cycles with mixed-grade vascular perfusion follicles (18%), with no pregnancies occurring in the group with low-grade vascularity. The incidence of multiple pregnancies was twice as high in the group with uniformly high-grade compared with mixed-grade follicular vascular perfusion (39 versus 18%). This was true despite the fact that the mean number of follicles around the time of ovulation (≥16 mm) was similar in both the singleton and multiple pregnancy groups (singleton versus multiple: 3.6 ± 0.3 versus 3.8 ± 0.3; unpaired t-test, P = NS). Early pregnancy loss rates appeared to be negatively correlated to the vascularity of the cohort, with the lower rates being associated with uniformly high-grade follicular vascularity compared with mixed-vascularity cohorts (16 versus 45%). However, these differences in pregnancy loss and multiple pregnancies were not statistically significant. There were 26 cycles that had a solitary follicle >16 mm in
Table I. Data on patient characteristics, stimulation regime, ovarian and endocrine response and semen parameters in relation to treatment cycle vascularity grades in intrauterine insemination (IUI) study population

<table>
<thead>
<tr>
<th>Cohorts of vascularity grades</th>
<th>All high (n = 101)</th>
<th>Mixed (n = 60)</th>
<th>All low (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of female partner (years)</td>
<td>33.0 ± 0.4</td>
<td>34.4 ± 0.6</td>
<td>35.7 ± 1.3**c</td>
</tr>
<tr>
<td>Duration of subfertility</td>
<td>5.3 ± 0.4</td>
<td>6.3 ± 0.4</td>
<td>7.5 ± 0.9**c</td>
</tr>
<tr>
<td>Baseline FSH (IU/l)</td>
<td>5.0 ± 0.2</td>
<td>4.8 ± 0.2</td>
<td>5.7 ± 0.8</td>
</tr>
<tr>
<td>Multiparity (n)</td>
<td>19 (19)</td>
<td>8 (13)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>Primary infertility (n)</td>
<td>60 (59)</td>
<td>42 (70)</td>
<td>15 (71)</td>
</tr>
</tbody>
</table>

Stimulation regime
- GnRH agonist protocol
  - Ultrashort (n) | 90 (89) | 53 (88) | 12 (57)b |
  - Short (n) | 6 (6) | 4 (7) | 7 (33) |
  - Long (n) | 5 (5) | 3 (5) | 2 (10) |

Day of HCG administration
- Serum oestradiol (×10⁶ pmol/l) | 3.0 ± 0.1 | 3.1 ± 0.2 | 1.5 ± 0.2**d |
- Serum LH (U/l) | 2.4 ± 0.4 | 2.0 ± 0.3 | 3.9 ± 0.7 |
- No. of follicles >16 mm | 2.4 ± 0.1 | 2.6 ± 0.1 | 1.5 ± 0.2**d |

Day of IUI
- No. of follicles >16 mm | 3.3 ± 0.1 | 3.7 ± 0.2 | 1.6 ± 0.2**d |
- Endometrial thickness (mm) | 11.9 ± 0.2 | 12.1 ± 0.3 | 12.5 ± 0.5 |
- Mean uterine artery PI | 2.4 ± 0.04 | 2.3 ± 0.07 | 2.5 ± 0.12 |
- Mean ovarian stromal PI | 0.88 ± 0.01 | 0.89 ± 0.03 | 0.91 ± 0.04 |

Semen parameters
- Sperm wash Count (×10⁶/ml) | 18.1 ± 2.0 | 21.3 ± 2.8 | 14.2 ± 2.8 |
- A/B progression (%) | 76.0 ± 1.1 | 72.5 ± 1.8 | 71.9 ± 2.0 |
- Cycles where donor semen used (n) | 32 (31) | 12 (20) | 6 (29) |

Values are mean ± SE unless otherwise indicated.
Values in parentheses are percentages.
*ANOVA: all had P > 0.05, except ***, where P < 0.05, with Bonferroni correction for significant differences between all grades (a) or between low and mixed/high only (b) grades.
**Denotes χ² (2 degrees of freedom) for comparison of proportions, with b denoting P < 0.05; otherwise not significant.

Table II. Clinical pregnancy and early pregnancy loss rates of treatment cycles assuming that ovulatory oocytes had resulted from the three cohorts of follicular vascularity grades >16 mm in diameter

<table>
<thead>
<tr>
<th>Ovulatory oocytes assumed to have derived from follicles with varying cohorts of vascularity:</th>
<th>All high grade (3/4 only)</th>
<th>Mixed grade (1/2/3/4)</th>
<th>All low grade (1/2 only)</th>
<th>χ²/Fisher’s Exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant cycles</td>
<td>31 (31)</td>
<td>11 (18)</td>
<td>0</td>
<td>P &lt; 0.05 (d.f. = 2)</td>
</tr>
<tr>
<td>Multiple pregnancies</td>
<td>12/31 (39)</td>
<td>2/11 (18)</td>
<td>–</td>
<td>P = NS (d.f. = 1)</td>
</tr>
<tr>
<td>Pregnancy losses</td>
<td>5/31 (16)</td>
<td>5/11 (45)</td>
<td>–</td>
<td>P = NS (d.f. = 1)</td>
</tr>
<tr>
<td>Ectopic pregnancies</td>
<td>1/31 (3)</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Non-pregnant cycles</td>
<td>70</td>
<td>49</td>
<td>21</td>
<td>–</td>
</tr>
<tr>
<td>No. of cycles</td>
<td>101</td>
<td>60</td>
<td>21</td>
<td>Total = 182</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.
d.f. = degrees of freedom.

As this study population was heterogeneous in terms of treatment protocols and causes of subfertility, a multivariate analysis in the form of logistic regression was applied to variables that were significantly different between the three vascularity cohorts. This showed that two factors which independently affected outcome of treatment were serum oestradiol concentration and high-grade perifollicular vascularity (Table III).
high-grade vascularity follicles. This was also noted with be postulated that the changes which affect follicle numbers, et al. from such follicles for transfer in the IVF study (Bhal et al., 1996). One of these groups (Tsai et al., 1996) found that a mean uterine artery PI (UAPI) of ≥3 was associated with no pregnancies occurring, and the ongoing pregnancy rate was lower in the group where the mean UAPI was 2–3 compared with those where PI values were <2 (Tsai et al., 1996). Findings from the current study contrast with those of others (Tsai et al., 1996), as it was not possible to demonstrate that uterine perfusion (based on mean UAPI values) was any different among the three vascularity cohorts. This difference may relate to the timing of the scan, since in the current study it followed HCG administration, whereas others (Tsai et al., 1996) made the measurements prior to HCG administration.

In the first studies of periovulatory intra-ovarian blood flow in women undergoing IUI, significantly lower intra-ovarian PI values were found to be associated with higher pregnancy rates (Thoma et al., 1997). Again, this contrasts with data from the current study. Pulsed Doppler intra-ovarian stromal indices were not different in all three vascularity cohorts. However, intra-ovarian perfusion (as assessed with power Doppler) of individual follicles ≥16 mm did seem independently to affect pregnancy rates.

It is now recognized that advancing maternal age (Frederick et al., 1994; Campana et al., 1996; Brezetchka and Buyalos, 1997; Legro et al., 1997; Sahakyan et al., 1999) and duration of subfertility (Tucker et al., 1990; Tomlinson et al., 1996; Noujua-Huttunen et al., 1999) have a major impact as important predictive variables in the success of IUI treatment cycles. It remains unclear whether this relationship between follicular vascularity and age, as well as duration of subfertility in the current study, was a cause or effect situation. It is likely to be the latter, especially as younger women presenting to fertility clinics are likely to have had a shorter duration of subfertility.

Age-related decline in fecundity is thought primarily to be due to oocyte senescence rather than to ageing of the uterus, as suggested by investigations with donor oocytes (Sauer et al., 1992). Others have suggested that this decline is the result of physical or chemical changes imparted by advancing chronological age that lead to a hardening of the zona pellucida and hence affect implantation (Cohen et al., 1990). More recently, others (Scheffer et al., 1999) showed that the mean antral follicle count in women with proven natural fertility declines with increasing age, and this might be a reflection on the size of the remaining primordial follicle pool. Therefore, it could be postulated that the changes which affect follicle numbers, oocyte or embryo quality in relation to age/duration of subfertility might affect follicular vascularity in a similar manner.

Oestradiol concentration has been documented to be correlated with birth rate in IUI treatment cycles (Dickey et al., 1991), and this is confirmed in the current study. As

### Table III. Multiple logistic regression analysis of variables found to be significantly different in Table I with respect to pregnancy as the dependent variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-grade follicular vascularity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.42 (1.08–5.40)</td>
</tr>
<tr>
<td>Use of ultrashort GnRH agonist</td>
<td>1.50 (0.51–4.41)</td>
</tr>
<tr>
<td>No. of follicles pre HCG</td>
<td>1.0 (0.59–1.68)</td>
</tr>
<tr>
<td>No. of follicles post HCG</td>
<td>1.06 (0.72–1.58)</td>
</tr>
<tr>
<td>Serum oestradiol conc. on day of HCG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.28 (1.01–1.62)</td>
</tr>
<tr>
<td>Age of female partner</td>
<td>0.93 (0.85–1.02)</td>
</tr>
<tr>
<td>Duration of subfertility</td>
<td>0.99 (0.89–1.10)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Indicates variables that were independently related to outcome with 
P < 0.05.

HCG = human chorionic gonadotrophin.

**Discussion**

IUI has long been established as an alternative to other forms of assisted reproduction techniques (Treadway et al., 1990; Tucker et al., 1990), with several clinical prognostic indicators such as number of follicles and semen quality for success rates in IUI (Tomlinson et al., 1996; Hughes, 1997). The question being asked in the current study was whether perifollicular vascular perfusion could influence outcome, particularly in stimulated IUI cycles, and therefore provide another prognostic indicator for success.

In the current study, outcome was correlated with the characteristics of a cohort of follicles ≥16 mm because the largest or ‘dominant’ follicle is not always the first to rupture at ovulation in multifollicular assisted reproduction treatment cycles (Andersen et al., 1995). Studies have also shown that oocyte maturity may be achieved at a mean follicular diameter of 15–16 mm in HMG-stimulated cycles (Silverberg et al., 1991). Although follicles <16 mm have the potential to ovulate viable oocytes, they are likely to be more immature (Trounson et al., 1998), with lower fertilization capabilities (Dubey et al., 1995; Bergh et al., 1998). However, the current study showed that follicle size was independent of vascularity grade in this study population. This contrasts with data from the IVF cycles, which showed a correlation between increasing follicular diameter and higher-grade vascularity (Bhal et al., 1999). One reason for this apparent difference could be that both follicle populations were dissimilar with regards to their selection process for inclusion into both studies. The current study only targeted follicles ≥16 mm, whereas in the IVF study (Bhal et al., 1999) follicle selection was based on the ‘best vascularity’. The former is therefore more likely to have a cohort of follicles with similar diameters than with the IVF study follicles; hence, the independence of size in the IUI study population.

Data from the current study showed that the overall incidence of follicles ≥16 mm would appear to favour the production of high-grade vascularity follicles. This was also noted with the data from a previous study (Bhal et al., 1999). The incidence of cycles with uniformly low-grade follicles having IUI was considerably lower than those where embryos derived from such follicles for transfer in the IVF study (Bhal et al., 1999; 12 versus 21%). It would be difficult to draw any conclusions with regard to the reasons for this difference, as these are two very different study populations. However, one possible explanation was that the inclusion of patients with a tubal factor in the IVF population where the cause of tubal pathology whether due to inflammation, infection or surgery might well affect ovarian vascular perfusion.

The potential relationship between vascular perfusion and outcome in IUI cycles has so far been confined to pulsed Doppler studies of the uterine artery (Tsai et al., 1996; Thoma et al., 1997). One of these groups (Tsai et al., 1996) found that a mean uterine artery PI (UAPI) of ≥3 was associated with no pregnancies occurring, and the ongoing pregnancy rate was lower in the group where the mean UAPI was 2–3 compared with those where PI values were <2 (Tsai et al., 1996). Findings from the current study contrast with those of others (Tsai et al., 1996), as it was not possible to demonstrate that uterine perfusion (based on mean UAPI values) was any different among the three vascularity cohorts. This difference may relate to the timing of the scan, since in the current study it followed HCG administration, whereas others (Tsai et al., 1996) made the measurements prior to HCG administration.

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Oestradiol concentration has been documented to be correlated with birth rate in IUI treatment cycles (Dickey et al., 1991), and this is confirmed in the current study. As
the number of mature oocytes generally correlates with the oestradiol concentration in assisted reproduction treatment cycles (Mitchell et al., 1996), it is not surprising that a higher number of mature follicles will yield higher oestradiol concentrations. However, the reason for this difference in follicle number and oestradiol concentration, especially when the dose and duration of FSH stimulation in all three groups of treatment cycles were similar, is unknown. It is unlikely that oocyte maturity was a factor, as vascularity grading of follicles ≥16 mm was independent of follicle size, and serum LH concentrations between all three cohorts of vascularity grades did not differ. One suggestion might be that the group which produced follicles that were uniformly of low-grade vascularity were apparently ‘low recruiters’ to ovarian stimulation. This could be related to the findings that women in this group tended to be older, and with a longer duration of subfertility. In addition, there was a correlation between mean baseline FSH concentrations and low-grade vascularity, albeit non-significantly. This suggests that the postulated link between reduced ovarian reserve and responsiveness to gonadotrophin stimulation with elevated basal FSH concentrations noted by others (Balasch et al., 1996) might have an ovarian vascular component. Furthermore, although it has been found (Pellicer et al., 1998) that oocyte and embryo quality were no different in younger or older ‘low responders’ to ovarian stimulation, the blood flow in these group of patients appeared to be poorer compared with those of ‘normal responders’. However, a poor response was not incompatible with high-grade follicular vascularity, as demonstrated by those monofollicular cycles where high-grade vascular perfusion was associated with pregnant outcome.

Data on the use of GnRH agonists in assisted reproduction have been conflicting. Studies using GnRH agonists in IUI or direct i.p. sperm insemination have suggested that it can improve pregnancy rates (Rammer and Friedrich, 1998; Minoura et al., 1999), whereas others have suggested that it made no difference in an unselected subset population (Dodson et al., 1991) and was not cost-effective (Noujua-Huttunen et al., 1997). In addition, the use of long-term down-regulation has been advocated by some authors (Tan et al., 1994; Tasdemier et al., 1995), whereas others have suggested that the ultrashort protocol is just as effective in terms of affecting oocyte quality and clinical outcome (Acharya et al., 1992; Strohmer et al., 1997). Moreover, it has been suggested that exposure to GnRH agonist therapy has a direct negative effect on intra-ovarian blood flow by creating a hypo-oestrogenic milieu (Bassil et al., 1997). Animal studies have shown that GnRH agonist has a direct inhibitory action on oestradiol-induced enzymes involved in cell proliferation (Reddy et al., 1985; Medeiros et al., 1988). The ultrashort GnRH agonist protocol may create less of a hypo-oestrogenic environment than the short or long protocol, and this explains its association with higher intra-ovarian/perifollicular vascular perfusion.

The developmental competence of oocytes (Van Blerkom et al., 1997; Huey et al., 1999) and subsequent embryos (Chui et al., 1997; Bhal et al., 1999; Coulam et al., 1999) have been associated with follicular vascularity which could have accounted for the differences in pregnancy rates between the vascularity groups. The availability of higher-grade embryos as opposed to higher numbers of embryos as noted from IVF study populations (Bhal et al., 1999; Coulam et al., 1999) might explain the difference in the multiple pregnancy rates between the groups cycles with high- and mixed-grade vascularity. This is despite the fact that the mean number of follicles (≥16 mm) were similar in both vascularity and pregnancy (singleton/multiple) groups.

Following ovulation, follicles develop as the corpora lutea. The blood flow regulation of the corpus luteum has been shown to be important in spontaneous pregnancies at between 5 and 16 weeks gestation (Jauniaux et al., 1992). It might be postulated therefore, that corpora lutea blood flow may also have similar high- and low-grade vascularity. The hypothesis could further be extended that although neovascularization takes place in the luteal phase, the extent to which it occurs may be dependent on the quality of prior follicular vascular perfusion. Important steroids, peptides and other vascular growth factors that are needed to maintain a pregnancy in its early stages (Devroey et al., 1990; Ferrara et al., 1998) might be adversely affected by reduced- or low-grade luteal neovascularization. The latter has been shown to be the phenomenon in cases of missed abortions (Alcazar et al., 1996), and may be similarly responsible for a higher pregnancy loss rate in cycles with mixed-grade compared with uniformly high-grade follicular vascularity.

The viability of pregnancies is clearly dependent on the competence of embryos (Bhal et al., 1999). It has been suggested (Moor et al., 1998) that the key to oocyte maturation and embryo viability resides in the follicle cell compartment rather than the oocyte itself. Others (Barroso et al., 1999) found that vascular endothelial growth factor, nitric oxide and leptin appear to be markers of follicular hypoxia and suboptimal embryo development. This group questioned whether fluctuations in these regulatory factors determine or reflect changes in the follicular microenvironment affecting the oocyte and its subsequent developmental potential. It has also been suggested that the embryos themselves influence corpus luteal function (Johnson et al., 1993). Therefore, we postulate that high-grade follicles produce high-grade embryos, which could have a positive influence on the subsequent corpora lutea and, therefore, pregnancy outcome.

Perifollicular vascular perfusion appears to be an important factor in determining the outcome of stimulated IUI cycles, and may have clinical implications in assisted reproduction therapy. As there were no pregnancies in the group of women with uniformly low-grade vascularity, the identification of these cycles would be valuable in terms of counselling them with regard to the potential outcome in that cycle. Ideally, the identification of these women (who may also be ‘low recruiters’) earlier in the cycle (before HCG) would be helpful. This could allow the cancellation of treatment after careful counselling, on the basis of perifollicular vascular perfusion, and could be cost-effective, both financially and emotionally. However, further longitudinal data would be needed before this form of prospective management of treatment cycles could be applied clinically. The risk of multiple pregnancies and
their implications on the health service is also well recognized. Since there was a higher multiple pregnancy rate in stimulated IUI cycles with uniformly high-grade follicular vascularity, perhaps these cycles in particular should be considered for follicle reduction or even cancellation. The former may potentially reduce the number of developmentally competent oocytes that have a higher capability of producing more viable embryos for implantation.

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