Assessment of the ovarian volume, number and volume of follicles and ovarian vascularity by three-dimensional ultrasonography and power Doppler angiography on the HCG day to predict the outcome in IVF/ICSI cycles

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OBJECTIVE: The aim of this prospective study was to investigate whether ovarian blood flow is related to embryological parameters and whether it could be a predictor of outcomes of IVF/ICSI. METHODS: Eighty infertile women underwent ovarian stimulation with gonadotrophins after a long protocol with GnRH agonists. The ovarian volume (OV), number of follicles (NF) and follicular volume (FV) of all follicles >10 mm and vascularization index (VI), flow index (FI) and vascularization–flow index (VFI) were obtained by three-dimensional (3D) ultrasonography and power Doppler angiography (PDA) on the day of HCG administration. These parameters were tested for their relation with IVF laboratory parameters. RESULTS: The OV, FV, VI, FI and VFI were significantly greater in the pregnant group. The NF and FV were the only independent predictors of the number of oocytes retrieved, mature and fertilized, and the number of embryos developed and their cumulative embryo score. Nevertheless, the number of grade 1 embryos depends on the NF and the VI. The ovarian FI and the number of transferred grade 1 embryos can predict gestation in 76% of IVF patients. A low FI and non-grade 1 embryo transferred are also associated with an increased pregnancy loss. CONCLUSION: 3D ultrasonography and PDA allow for an easier ovarian assessment in IVF cycles. The predictive value of IVF outcome suggests a high clinical usefulness of this new technique.

Key words: blood flow/embryo quality/IVF–ICSI outcome/oocyte quality/3D ultrasound

Introduction

Ovarian blood flow appears to play a crucial role in the development of ovarian follicles when stimulated with gonadotrophins in IVF cycles. More important, follicular blood flow is capable of influencing or mediating oocyte maturation, its potential ability to fertilize and develop and embryo quality (Nargund et al., 1996a,b; Van Blerkom et al., 1997; Bhal et al., 1999; Coulam et al., 1999; Mercé, 2002).

Until recently, follicular blood flow has been evaluated by bidimensional pulsed Doppler (Nargund et al., 1996a,b; Van Blerkom et al., 1997; Coulam et al., 1999) and the perifollicular blood flow by colour mapping (Van Blerkom et al., 1997; Bhal et al., 1999; Coulam et al., 1999; Mercé, 2002). The perifollicular peak systolic velocity before the HCG administration was significantly related to the possibility of retrieving oocytes and developing embryos of high preimplantation potential (Nargund et al., 1996a,b). By evaluating the percentage of perifollicular colour mapping circumference by power Doppler, it was concluded that follicles showing a high degree of vascularity produce a significantly higher number of mature oocytes and have high fertilization rate (Bhal et al., 1999; Mercé, 2002). Oocytes obtained from follicles with good perifollicular blood flow show a lower frequency of abnormalities in the chromosomal arrangement within the metaphase II spindle, greater dissolved oxygen content and a high concentration of vascular endothelial growth factor (VEGF) (Van Blerkom et al., 1997). Moreover, oocytes from those follicles with a lower degree of vascularity develop embryos with a high rate of triploids (Bhal et al., 1999). All these explain why the pregnancy rate is higher when the transferred embryos come from oocytes with highly vascularized follicles (Bhal et al., 1999; Coulam et al., 1999; Mercé, 2002).

Assessment of the ovarian blood flow by pulsed Doppler or bidimensional colour Doppler has important methodological disadvantages. When using pulsed Doppler, the follicular blood flow is obtained from a single perifollicular artery subjectively chosen by the explorer. Moreover, because it is impossible to determine the insonation angle, working with blood velocities is highly unreliable. Perifollicular colour mapping with power Doppler is a more reliable technique because...
of its high sensitivity and less angle dependence. Nonetheless, it is assumed that colour mapping from a single perifollicular vascular plane represents the whole follicular blood flow. Recently, three-dimensional (3D) power Doppler angiography (PDA) has been incorporated in the study of the ovarian blood flow (Järvelä et al., 2002, 2003a, 2004; Kupesic and Kurjak, 2002; Pan et al., 2002, 2003, 2004; Kupesic et al., 2003; Vlaisavljevic et al., 2003; Wu et al., 2003). This new technique combines the advantages of the colour power Doppler and 3D ultrasound. The 3D power Doppler can depict all the ovarian and follicular blood vessels and calculate the vascularization index (VI) (representing the percentage of vessels) and the flow index (FI) (representing the blood flow intensity) from the whole ovarian volume (OV) explored.

The ovarian blood flow study by 3D PDA shows good intraobserver and interobserver reproducibility (Järvelä et al., 2003b; Raine-Fenning et al., 2003, 2004; Mercé et al., 2005). Currently, published reports regarding the utility of 3D PDA indices as predictors of the ovarian response remain contradictory (Kupesic and Kurjak, 2002; Järvelä et al., 2003c; Kupesic et al., 2003; Mercé et al., 2006). A high FI from the ovarian stroma after pituitary suppression is associated with high numbers of retrieved oocytes and high fertilization and pregnancy rates (Kupesic and Kurjak, 2002; Kupesic et al., 2003). After FSH stimulation, higher VI and vascularization flow index (VFI) have been demonstrated in a group of women achieving pregnancy (Järvelä et al., 2004).

The aim of this study was to evaluate the ovarian blood flow in IVF cycles after stimulation with gonadotrophins. We investigated whether 3D PDA indices are related to embryological parameters used in the IVF laboratory and to the outcome of the IVF cycle.

Subjects and methods

Patients

We prospectively evaluated 80 women attending the Assisted Reproduction Unit in the International Ruber Hospital for IVF/ICSI treatment from September 2003 to March 2005. The study protocol was approved by the local ethics committee, and an oral informed consent was obtained from all patients. Women with polycystic ovaries, endometriomas or any kind of functional or organic ovarian cysts or uterine myomata were excluded from the study. There were three cases with a single ovary because of a prior surgical removal for benign conditions (i.e. simple cyst). All women included had a normal level of basal FSH (<10 mIU/ml) during the early follicular phase, had a regular menstrual cycle and were non-smokers.

IVF protocols

Each patient was evaluated in a single treatment cycle. A standard long GnRH agonist protocol for IVF/ICSI treatment was used. Pituitary down-regulation was achieved with subcutaneous GnRH agonist triptorelin (Decapeptyl, Ipsen Pharma, Barcelona, Spain) from the 21st day of the previous cycle with a 0.1 mg/day dosage. Pituitary suppression was evaluated 14 days after the beginning of the treatment and confirmed by the presence of small antral follicles (diameter 2–6 mm), an endometrial thickness <5 mm and serum estradiol (E2) level lower than 50 pg/ml. After the pituitary suppression was confirmed, ovarian stimulation treatment was started with 150 IU/day of subcutaneous recombinant FSH (Puregon, Spanish Organon, Madrid, Spain). The FSH dosage was adjusted according to the follicle response monitored by serial ultrasound scans and E2 serum levels. In 17 cycles, it was associated with highly purified HMG (Menopur, Ferring SA, Madrid, Spain) trying to improve the follicular growth. When the average diameters of the three leading follicles were at least 18 mm, as measured by ultrasound, 250 IU of recombinant HCG (Ovitrelle, Serono Laboratories, Madrid, Spain) was administered as a single subcutaneous injection. A minimum of three dominant follicles on the day of HCG administration were required to indicate the follicular aspiration procedure.

Serum E2 concentrations were measured using an automated quantitative test that combines a competition method with a final fluorescent detection (VIDAS Estradiol II, BioMerieux SA, Lyon, France). The analytical detection limit of the E2 was 9 pg/ml. The within-run reproducibility varied from 2.2% to 7.5% coefficient of variation according to different levels of hormone. The between-run reproducibility was between 3.2% and 9.5% coefficient of variation depending on hormone level.

Oocyte retrieval was performed by transvaginal ultrasonography around 36 h after HCG administration. Oocytes were categorized as mature if the coronal cells were still apposed to the oocyte but the cumulus had expanded into a fluffy mass. Oocytes were then fertilized in vitro using IVF or ICSI. ICSI procedure was applied in all or part of mature oocytes recovered in each case. Cell-stage embryos were graded and scored according to a morphological classification adapted from Lens and Rijnders (1996). Grading was as follows: grade 1 (score 4), when embryos have similarly shaped blastomeres and with <10% of embryo fragmentation; grade 2 (score 3), if there are similar blastomeres with <20% of embryo fragmentation; grade 3 (score 2), when embryos have similar or dissimilar blastomeres with <30% of embryo fragmentation; grade 4 (score 1), when there are scarcely recognizable blastomeres and more than 30% of fragmentation. An individual score and cumulative embryo score (CES) were calculated by multiplying the morphological score with the number of blastomeres (Steer et al., 1992). Embryo transfer was performed through an ultrasound-guided technique 48 or 72 h after oocyte retrieval, and one to three embryos were replaced.

All patients received 600 mg of micronized progesterone (Progeffik, Lab. Effik SA, Madrid, Spain) intravaginally daily from ovum pickup day. To diagnose pregnancy, serum β-HCG concentration was determined 14 days after embryo transfer. Serial transvaginal ultrasound scans confirmed a normal or abnormal pregnancy. For the purposes of this study, we have only considered clinical pregnancies defined by the presence of one or more gestational sacs with living embryos or the histological confirmation of gestational products in miscarriages.

3D ultrasonography and PDA

All the 3D ultrasound and PDA examinations were carried out by a single observer (L.T.M.) on the day of HCG administration. All the patients were explored in a gynaecological position using the Voluson 730 system (Iberian Kretztechnik, Madrid, Spain) equipped with a vaginal multifrequency (from 3 to 9 mHz) volume transducer, which has a 146° field of view.

First, we carried out an examination of the uterus and ovaries in the B mode. All the follicles with a mean diameter (MD) >10 mm were considered. Every follicle was displayed on the screen on the plane of its maximum diameter. Longitudinal and transverse maximum diameters were measured, and MD was calculated. Follicular volume (FV) was determined for each follicle according to the sphere formula: FV (ml) = 4.1888 × (MD/2 (cm))³. The uterine maximum diameters and endometrial thickness were measured in the longitudinal uterine plane.
The power Doppler window was placed on the maximum longitudinal plane of both ovaries, including the whole ovarian surface. The following Doppler predetermined characteristics were applied in every patient: normal colour quality (normal resolution and middle level of photogram index), colour gain from –3.8 to –3.4, pulse repetition frequency (PRF) of 600 Hz and a wall motion filter of 50 Hz. When an adequate-power Doppler signal was achieved, we placed the 3D box to acquire the volume from the region of interest (ROI). According to the ovarian size, the volumetric sector angle was adjusted between 60° and 90°, and 3D power Doppler data sets were obtained using a medium-quality mode. Volume acquisition interval oscillated between 10 and 15 s for each ovary. All patients were requested to remain as still as possible, and the probe movements were avoided during the acquisition time. If ‘flash’ artefacts appeared owing to a patient’s bowel movements, the volume was reacquired until a satisfactory image was achieved. The volumes were stored on the equipment hard disk, transferred to compact disks and studied later in a personal computer.

All the stored volumes were analysed by the same investigator (L.T.M.). The virtual organ computer-aided analysis (VOCAL) imaging program was used to calculate the OV and 3D power Doppler indices (Figures 1 and 2). Using the manual mode, the contour of the different ovarian slices was traced by taking 15° rotational steps by using the longitudinal plane as the work pattern (Mercé et al., 2005, 2006).

3D power Doppler indices were calculated using the histogram facility. VI is the number of colour voxels in the volume studied, symbolizing in this way the number of vessels arriving to the organ, expressed as a percentage. FI is the mean colour value of the colour voxels, thus representing the average blood flow intensity, expressed as a whole number ranging from 0 to 100. VFI integrates both vascularization and blood flow (tissue’s perfusion). It is also expressed as a whole number ranging from 0 to 100, and it represents the mean colour value of grey and colour voxels in the studied ROI.

The main outcome measurements were OV, number of follicles (NF) with an MD larger than 10 mm, total FV (that is calculated by adding up all the FVs from all the follicles larger than 10 mm), VI, FI and VFI. Even though no significant differences between both ovaries were demonstrated for the above parameters, the sum of two side measurements was used for statistical analysis.

We have previously assessed the intraobserver and interobserver reproducibility for OV, antral follicle count and power Doppler 3D indices by 3D ultrasonography and PDA (Mercé et al., 2005). The intraobserver correlation coefficient for volume measurements was 0.988, for antral follicle count 0.964, for VI 0.917, for FI 0.913 and for VFI 0.908.

**Statistical analysis**

Statistical analysis of the data was performed with Statistical Package for Social Sciences (SPSS) software for Windows, version 11.5. Normal distribution of the data was assessed with the Kolmogorov–Smirnov test. Comparison was carried out by analysis of variance (ANOVA) for normally distributed data and by Mann–Whitney U-test for skewed data. The $\chi^2$ and Fisher’s exact test was used to compare categorical data.

![Figure 1](https://academic.oup.com/humrep/article-abstract/21/5/1218/987118)

Figure 1. Assessment of the ovarian volume by the virtual organ computer-aided analysis (VOCAL) imaging program. Using the manual mode, the contour of the different ovarian slices was traced by rotational steps every 15° taking the longitudinal plane as the work pattern.
Three-dimensional power Doppler to predict IVF outcome

where appropriate. Correlation between 3D ultrasonography and PDA parameters and IVF laboratory data was assessed by the Pearson method or Spearman’s rank method, according to data distribution. Multiple regression analysis in a stepwise procedure was applied to determine which sonographic and PDA variables were independent predictors of different biological variables. Logistic regression analysis was performed taking 3D PDA and follicular volume and quality of transferred embryos as IVF outcome predictors. A $P$-value (two tailed) less than 0.05 was taken as significant.

Results

We evaluated a single IVF/ICSI cycle in 80 infertile women. The patients’ mean age was $34.0 \pm 3.5$ years (range 27–41). The median duration of infertility was 3 years (range 1–11). Infertility was primary for 67.5% of the women and secondary for the rest. The infertility causes were male factor in 29 cases, tubal factor in 18 cases, mixed causes in 13 cases and unknown etiology in 20 cases. Embryo transfer could not be carried out in three cycles because of a fertilization failure. There were 38 clinical pregnancies (47.5%), among which there were 10 twins (26.3%) and no triplets. Eight patients presented with a miscarriage (21.1%), and two of them were twin gestations.

Table I includes the comparisons among the general and clinical characteristics of the patients, the doses of gonadotrophins, the days of treatment and the $E_2$ level on the HCG administration day related to the outcome of the IVF/ICSI cycle. The duration of the infertility is the only variable showing significant differences between pregnant and non-pregnant groups. The number of oocytes retrieved, mature and fertilized, total number of embryos, grade 1 embryos and cumulative embryo scores (CES) of all the developed embryos on the transfer day were significantly higher in women getting pregnant (Table II). There were no differences between the pregnant and non-pregnant groups on the embryo transfer day (day 2 or day 3) or in the number of embryos transferred. In contrast, in women who got pregnant, a significantly higher number of grade 1 embryos (2 versus 1, $P = 0.001$) were transferred, and the embryos had a superior CES ($45.18 \pm 26.36$ versus $31.59 \pm 20.11$, $P = 0.013$).

Table III compares the OV, NFs and total FV of follicles >10 mm in MD with the VI, FI and VFI on the day of HCG administration according to the IVF/ICSI outcome. Except for the NFs, all the ultrasound and PDA variables were significantly higher in the group of women who got pregnant.

The total OV, the NFs and the total FV were significantly correlated with the VI ($r = 0.288, P = 0.011; r = 0.343, P = 0.002$;
Table I. General and clinical characteristics in relation to IVF/ICSI outcome

<table>
<thead>
<tr>
<th></th>
<th>Pregnant (n = 38)</th>
<th>Non-pregnant (n = 42)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age (years) (mean ± SD)</td>
<td>33.87 ± 3.36</td>
<td>34.12 ± 3.59</td>
<td>0.749</td>
</tr>
<tr>
<td>Duration of infertility (years) [median (range)]</td>
<td>2 (1–7)</td>
<td>3 (1–11)</td>
<td>0.031</td>
</tr>
<tr>
<td>Type of infertility [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>25 (46.3)</td>
<td>29 (53.7)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>13 (50.0)</td>
<td>13 (50.0)</td>
<td>0.814</td>
</tr>
<tr>
<td>Cause of infertility [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male factor</td>
<td>16 (55.2)</td>
<td>13 (44.8)</td>
<td></td>
</tr>
<tr>
<td>Tubal factor</td>
<td>11 (61.1)</td>
<td>7 (38.9)</td>
<td></td>
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<tr>
<td>Mixed factor</td>
<td>5 (38.5)</td>
<td>8 (61.5)</td>
<td>0.179</td>
</tr>
<tr>
<td>Unexplained</td>
<td>6 (30.0)</td>
<td>14 (70.0)</td>
<td></td>
</tr>
<tr>
<td>Total dosage of FSH (IU) median (range)</td>
<td>1713 (950–7200)</td>
<td>1800 (600–9000)</td>
<td>0.342</td>
</tr>
<tr>
<td>Days of FSH treatment [median (range)]</td>
<td>11 (8–14)</td>
<td>11 (7–16)</td>
<td>0.996</td>
</tr>
<tr>
<td>Total dosage of LH (IU)* (mean ± SD)</td>
<td>2142.86 ± 1443.21</td>
<td>2137.50 ± 1642.12</td>
<td>0.995</td>
</tr>
<tr>
<td>Days of LH treatment* (mean ± SD)</td>
<td>10.86 ± 1.07</td>
<td>9.90 ± 4.20</td>
<td>0.568</td>
</tr>
<tr>
<td>E2 on HCG day (pg/ml) (mean ± SD)</td>
<td>2852.21 ± 1161.51</td>
<td>2369.64 ± 1058.08</td>
<td>0.055</td>
</tr>
</tbody>
</table>

*aTransfer was not performed in three cases.

Table II. Biological parameters and IVF laboratory data in relation to IVF/ICSI outcome

<table>
<thead>
<tr>
<th></th>
<th>Pregnant (n = 38)</th>
<th>Non-pregnant (n = 42)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of retrieved oocytes (mean ± SD)</td>
<td>11.21 ± 5.05</td>
<td>8.24 ± 5.11</td>
<td>0.011</td>
</tr>
<tr>
<td>Number of mature oocytes (mean ± SD)</td>
<td>8.18 ± 3.71</td>
<td>5.55 ± 4.17</td>
<td>0.004</td>
</tr>
<tr>
<td>Number of fertilized oocytes (mean ± SD)</td>
<td>5.89 ± 2.85</td>
<td>4.00 ± 3.35</td>
<td>0.008</td>
</tr>
<tr>
<td>Number of embryos on transfer day (mean ± SD)</td>
<td>5.71 ± 2.78</td>
<td>4.00 ± 3.21</td>
<td>0.014</td>
</tr>
<tr>
<td>Number of grade 1 embryos on transfer day (median [range])</td>
<td>2 (0–7)</td>
<td>1 (0–5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CES of all the embryos on transfer day [median (range)]</td>
<td>76 (4–316)</td>
<td>28 (12–146)</td>
<td>0.002</td>
</tr>
<tr>
<td>Day of embryo transfer [n (%)]**</td>
<td>8.82</td>
<td>5.05</td>
<td>0.305</td>
</tr>
<tr>
<td>Day +2</td>
<td>21 (44.7)</td>
<td>26 (55.3)</td>
<td></td>
</tr>
<tr>
<td>Day +3</td>
<td>17 (56.7)</td>
<td>13 (43.3)</td>
<td></td>
</tr>
<tr>
<td>Number of transferred embryos [median (range)]</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
<td>0.351</td>
</tr>
<tr>
<td>Number of transferred grade 1 embryos [median (range)]</td>
<td>2 (0–2)</td>
<td>1 (0–1)</td>
<td>0.001</td>
</tr>
<tr>
<td>CES of the transferred embryos (mean ± SD)</td>
<td>45.18 ± 26.36</td>
<td>31.59 ± 20.11</td>
<td>0.013</td>
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</table>

CES, cumulative embryo score.

Table III. Three-dimensional ultrasonography and power Doppler angiography parameters on the HCG day in relation to IVF/ICSI outcome

<table>
<thead>
<tr>
<th></th>
<th>Pregnant (n = 38)</th>
<th>Non-pregnant (n = 42)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian volume (ml)</td>
<td>62.89 ± 21.31</td>
<td>51.37 ± 21.22</td>
<td>0.020</td>
</tr>
<tr>
<td>Number of follicles</td>
<td>14.39 ± 6.28</td>
<td>11.71 ± 6.28</td>
<td>0.070</td>
</tr>
<tr>
<td>Total follicular volume (ml)</td>
<td>35.76 ± 11.60</td>
<td>30.14 ± 11.76</td>
<td>0.035</td>
</tr>
<tr>
<td>Vascularization index</td>
<td>24.73 ± 8.11</td>
<td>18.44 ± 10.25</td>
<td>0.003</td>
</tr>
<tr>
<td>Flow index</td>
<td>68.65 ± 8.39</td>
<td>62.20 ± 9.93</td>
<td>0.003</td>
</tr>
<tr>
<td>Vascularization index</td>
<td>8.82 ± 3.15</td>
<td>6.44 ± 3.75</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation.

r = 0.317, P = 0.004, respectively) and the VFI (r = 0.294, P = 0.009; r = 0.371, P = 0.001 and r = 0.315, P = 0.004, respectively). Nevertheless, the FI does not significantly correlate with the OV (r = 0.167, P = 0.147), the NFs (r = 0.215, P = 0.055) and the total FV (r = 0.170, P = 0.132).

Table IV summarizes the correlation coefficients among the 3D sonographic and PDA parameters and the laboratory results. All the coefficients reach a statistical significance, although the highest was between the NFs and the number of oocytes retrieved (r = 0.862) and between the total FV and the number of oocytes recovered (r = 0.808). Subsequently, a multiple regression analysis according to a stepwise procedure was applied to determine which ultrasonographic and PDA variables were independent in the prediction of the biological and embryological parameters under study. The NF was the only independent parameter to predict the number of oocytes retrieved (r = 0.856) and the CES of the embryos on the transfer day (r = 0.532) (Table V). The total FV is the only predictive variable of the number of mature oocytes (r = 0.703), the number of fertilized oocytes (r = 0.644) and the number of developed embryos on the transfer day (r = 0.612). The number of grade 1 embryos on the transfer day was predicted by the NFs and the VI (r = 0.45) (Table VI).

Table VII summarizes the results of the logistic regression analysis over the total FV, the VI, the FI, the VFI, the number of grade 1 embryos transferred and the CES of all the transferred embryos in the prediction of the IVF/ICSI outcome. The number of grade 1 embryos and the FI were the best predictors, followed by the total FV, the VFI, the VI and the CES of the transferred embryos. By applying this model, pregnancy was predicted in 29 of 38 (76.3%) patients, and there was an
unfavourable outcome in 28 of 39 patients (71.8%). In 14 patients with an FI <60, the pregnancy rate was 14.3% and the pregnancy loss rate 50%. When the FI was between 60 and 70 (39 women), the pregnancy rate was 41% and the miscarriage rate 37.5%. In 27 patients with an FI >70, the pregnancy rate reached 74.1% and pregnancy loss rate 5% ($\chi^2 = 14.495$, $P = 0.001$ for the pregnancy rate and $\chi^2 = 6.713$, $P = 0.035$ for the pregnancy loss rate).

When no grade 1 embryo was transferred (20 women), the pregnancy rate was 30% and the miscarriage rate 66.7%. A single grade 1 embryo was transferred in 23 patients, and the pregnancy rate was 34.8% with no miscarriages. In 34 women, more than one grade 1 embryos were transferred, and the pregnancy rate reached 70.6% and the miscarriage rate was only 16.7% ($\chi^2 = 11.084$, $P = 0.004$ for the pregnancy rate and $\chi^2 = 9.922$, $P = 0.007$ for the pregnancy loss rate). We found no significant relationship between the FI or the number of grade 1 embryos transferred and the multiple pregnancy rates. The transfer day and the total number of

A single grade 1 embryo was transferred in 23 patients, and the pregnancy rate was 34.8% with no miscarriages. In 34 women, more than one grade 1 embryos were transferred, and the pregnancy rate reached 70.6% and the miscarriage rate was only 16.7% ($\chi^2 = 11.084$, $P = 0.004$ for the pregnancy rate and $\chi^2 = 9.922$, $P = 0.007$ for the pregnancy loss rate). We found no significant relationship between the FI or the number of grade 1 embryos transferred and the multiple pregnancy rates. The transfer day and the total number of
embryos transferred are not significantly related with the pregnancy rate, the number of miscarriages or the number of twin gestations.

Discussion
To the best of our knowledge, this is the first study to assess the relationships between the ovarian blood flow evaluated by 3D PDA and the biological and embryological parameters from the IVF laboratory. We have determined which parameters predict more accurately the cycle outcome in 80 infertile women undergoing IVF/ICSI treatment.

PDA should overcome limitations of pulsed Doppler and bidimensional colour Doppler in the assessment of the ovarian stromal and follicular blood flow (Kupesic and Kurjak, 2002; Järvelä et al., 2004). By processing the power Doppler signal, this technique is able to detect all the blood vessels in an ROI (as the ovary) and calculate their blood flow. The software VOCAL calculates three vascularity indices: the VI, the FI and the VFI, reflecting vascular density, blood flow and tissue perfusion, respectively (Raine-Fenning et al., 2003). 3D power indices show a great reproducibility in the assessment of the ovarian blood flow, although there are some differences depending on the methodology applied (Järvelä et al., 2003b; Raine-Fenning et al., 2003, 2004; Mercé et al., 2005). With our method of examination, we have recently demonstrated that the functional state of the ovary, basal after pituitary suppression or stimulated after gonadotrophin treatment, has no influence over the reliability of the vascularity indices. Moreover, because of the very good interobserver agreement to calculate power Doppler indices and because the 3D volumes can be processed offline, it is possible to perform examination and take measurements at different times and by different explorers. This is an added advantage of this new technique (Mercé et al., 2005, 2006).

We have demonstrated that the OV, the NFs >10 mm and the FV of all the follicles >10 mm on the day of HCG administration are significantly correlated with the number of oocytes retrieved, the number of mature oocytes, number of fertilized oocytes and the number and quality of the embryos developed until the day of transfer (Table IV). Nonetheless, when a multiplet regression according to a stepwise method was applied, the only independent variable to predict the number of oocytes recovered and the CES of the embryos developed on the transfer day is the NFs on the HCG day. The number of mature oocytes, the number of fertilized oocytes and the number of embryos are significantly dependent on the total FV (Table V). These results are in agreement with those previously provided by bidimensional ultrasound (Wittmaack et al., 1994; Dubey et al., 1995; Bergh et al., 1998; Salha et al., 1998). In one study that utilized over 9933 follicles from 400 patients, no statistical correlation was found between the follicular diameter and the rate of oocyte recovery (Salha et al., 1998). On the contrary, the fertilization rate was lower when the FV was <1 ml (approximately 12 mm) (Salha et al., 1998). Similarly, follicles <2 ml or 16 mm show lower rates of fertilization and pregnancy in conventional IVF cycles probably because of the higher percentage of immature oocytes (Dubey et al., 1995; Bergh et al., 1998). Neither the division rate nor the embryo quality is influenced by the FV (Wittmaack et al., 1994; Salha et al., 1998). All these results lead us to think that there must be other mediators influencing the potential development of the oocytes.

The VI, FI and VFI are significantly related to the biological and embryological parameters from the IVF laboratory, although showing lower correlation coefficients (Table IV). Nevertheless, only the VI, FI is, along with the NFs, an independent predictor of the number of grade 1 embryos developed on the embryo transfer day (Table VI). Therefore, according to this result, the embryo quality depends on the ovarian stromal and follicular vascularization. The VI represents the percentage of vessels from the whole ovary, although on the day of HCG administration the ovarian vessels are mainly integrated by those nurturing the follicular walls. Initially, a peak systolic velocity >10 cm/s was directly related to the probability of obtaining grade 1 and 2 embryos (Nargund et al., 1996b).

Subsequently, by evaluating the percentage of vessels surrounding the follicles using power Doppler, it was concluded that the recovery, maturity and fertilization rates of the oocytes were significantly higher, and the triploid rate was significantly lower in those follicles showing a high vascularization degree. Nevertheless, the embryo morphology was not related to the perifollicular colour mapping (Bhal et al., 1999). There is evidence that poorly vascularized follicles have a low follicular oxygen concentration that is related to a high frequency of chromosomal abnormalities and defective embryos (Van Blerkom et al., 1997). We have confirmed that the percentage of perifollicular vascularization in follicles <15 mm was significantly related to the maturity and fertilization rates of the oocytes (Mercé, 2002). Nonetheless, all these results were obtained assuming that only one artery from the follicular wall, or a single perifollicular colour mapping, represents the blood flow to the whole follicle, therefore introducing a bias that is difficult to assess.

Data in Tables I–III compare the pregnant and non-pregnant groups with the aim of determining which parameters are significantly different and, therefore, whether they could be used as predictors of the IVF cycle outcome. Among the general characteristics, only the duration of infertility was significantly shorter, and there were no significant differences with age, type or aetiology of infertility, the doses and length of the gonadotrophin treatment or the E2 level on the day of HCG administration. On the contrary, the sonographic parameters (OV and total FV) and the 3D PDA indices (VI, FI and VFI) were significantly higher in the pregnant group as compared with the non-pregnant group (Table III). By evaluating 56 women undergoing IVF and embryo transfer, Kupesic and Kurjak (2002) demonstrated that the FI from the ovarian stroma after pituitary suppression is significantly higher in women getting pregnant compared with those who do not. For these authors, an augment in the age of the woman is associated with a poor ovarian response, represented by a small OV, a low follicular count and a low index of ovarian stromal blood flow (Kupesic et al., 2003).

In our study, we have found that the sonographic parameters of OV, NFs and FV of all the follicles >10 mm are significantly related to the VI and VFI but not with the FI. Nonetheless, we do not find differences in the age of pregnant and non-pregnant
groups. Pan et al. (2003, 2004) have reported that the VI, FI and VFI on the HCG administration day are higher in the group of women hyperresponders to the gonadotrophin treatment (Pan et al., 2003) and lower in the poor responder group as compared with a normal response (Pan et al., 2004). Järveli et al. (2004) studied the ovarian flow by 3D power Doppler in 60 infertile women (42 with normal ovaries and 12 with polycystic ovaries) on the day of pituitary suppression, the day of HCG administration and the day of oocyte retrieval. These authors observed that the VI and VFI were higher in the pregnant women as compared with the non-pregnant women only on the day of HCG administration after FSH stimulation (Järvelä et al., 2004).

The biological and embryological parameters of the IVF laboratory until the day of embryo transfer have also been significantly higher in the pregnant group. Nevertheless, in the present series, the outcome of the IVF cycle is not conditioned by the transfer day or the number of embryos transferred. The quality of the transferred embryos appears to be an important factor that predicts the outcome of the IVF cycle because the number of grade 1 embryos transferred as well as the CES of the transferred embryos was significantly higher in the pregnant group as compared with the non-pregnant group. The number of cells and the fragmentation pattern are certain predictors of a positive outcome of the IVF cycle, although some other additional parameters could be used to assess the embryo quality (Desai et al., 2000). An embryo scoring system based on the uniformity of the size of the blastomeres, on the presence of cellular fragmentation and on the patient’s age provided a reduction of high-order multiple pregnancies not affecting the rates of pregnancy or delivery in an IVF programme (Peterson et al., 2004). A retrospective multivariate analysis of 642 cycles with no more than two embryos transferred demonstrated that the best predictors of ongoing pregnancy and multiple pregnancy are the developmental stage and the morphological score and the age of the patient (Hunault et al., 2002). Quantitative scores such as the CES (Steer et al., 1992) and mean score of transferred embryos (MSTE) (Terriou et al., 2001) have been proposed to evaluate the quality of the transferred embryos and to predict the IVF outcome.

To study the predictors of the IVF outcome, we performed a logistic regression analysis including only those variables showing significant differences between the pregnant and non-pregnant groups: total FV, VI, FI and FVI, the number of grade 1 transferred embryos and CES of the embryos transferred. As summarized in Table VII, the number of grade 1 transferred embryos and the FI are the best predictors. This model is capable of predicting a successful IVF outcome in 76% of patients. Kupesic and Kurjak (2002) observed that the best predictors of IVF outcome on the day of pituitary suppression were the number of antral follicles and the FI of the ovarian stroma. Their model predicted a pregnancy in 50% of patients. When the FI was <11, there were no pregnancies; patients with an FI between 11 and 13 had a 47.2% pregnancy rate, whereas patients with an FI >13 had a 50% pregnancy rate. According to our results, an ovarian FI <60 or the transfer of non-grade 1 embryos is significantly associated with a lower pregnancy rate (14.3% and 30%, respectively) and with a greater number of miscarriages (50% and 66.7%, respectively). On the contrary, an FI >70 or the transfer of more than one grade 1 embryo is significantly related to a higher pregnancy rate (74.1% and 70.6%, respectively) and to a lower rate of pregnancy loss (5% and 16.7%, respectively).

In summary, 3D ultrasound and PDA is a suitable technique to assess easily all the sonographic parameters and the ovarian vascularity on the day of HCG administration in a short period and in only one examination. Our results show that the number and volume of all the follicles >10 mm are the only independent predictors of the number of oocytes retrieved, mature and fertilized, as well as the number of divided embryos and the CES of all the embryos on the transfer day. Nevertheless, the embryo quality, as assessed by the number of grade 1 embryos, depends not only on the NFs but on the ovarian VI as well. In the present series, the best predictors of IVF outcome are the ovarian FI and the transfer of grade 1 embryos. Besides, these variables predict the possibility of a pregnancy loss. 3D ultrasound and PDA are becoming a technique of high clinical utility in IVF programmes.

References


Järvelä IY, Sladkevicius P, Kelly S, Ojha K, Campbell S and Nargund G (2003a) Effect of pituitary down-regulation on the ovary before in vitro fertilization as assessed by the number of grade 1 embryos, depends not only on the NFs but on the ovarian VI as well. In the present series, the best predictors of IVF outcome are the ovarian FI and the transfer of grade 1 embryos. Besides, these variables predict the possibility of a pregnancy loss. 3D ultrasound and PDA are becoming a technique of high clinical utility in IVF programmes.

References


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