Power Doppler ultrasound assessment of follicular vascularity in the early follicular phase and its relationship with outcome of in vitro fertilization

S. M. Shrestha,^{1,2} M. F. Costello,^{1,2} P. Sjoblom,^{1,2,4} G. McNally,³ M. Bennett,¹
S. J. Steigrad,² and G. J. Hughes^{1,2}

Submitted January 3, 2006; accepted January 13, 2006; published online April 22, 2006

Objective: To determine whether ovarian perifollicular blood flow (PFBF) in the early follicular phase (EFP) was associated with treatment outcome.

Design: Retrospective longitudinal cohort study.

Setting: Tertiary referral centre/university hospital.

Patients: Thirty-four women underwent 37 IVF cycles, which resulted in 35 embryo transfers.

Interventions: Serial transvaginal scans using power Doppler ultrasound during the follicular phase. Ovarian PFBF of follicles ≥ 5 mm was subjectively assessed using a modified grading system (grades 0–4).

Main outcome measures: Ovarian PFBF and pregnancy.

Results: Treatment cycles were retrospectively divided into two groups: Group 1 ($n = 20$) had cycles with at least one small (5–10 mm) or medium (11–14 mm) size follicle(s) of high grade (2–4) PFBF on cycle day 5 or 6 or 7; and Group 2 ($n = 17$), had cycles that did not. Group 1 had a significantly higher proportion of high grade large follicles in the late follicular phase (35% vs. 21%) (OR 2.0; 95% CI 1.1–3.7) and higher clinical pregnancy rate (47% vs. 12%) (OR 6.3; CI 1.1–35.7) compared to Group 2.

Conclusion: High grade ovarian PFBF in the EFP during IVF is associated with both high grade PFBF in the late follicular phase and a higher clinical pregnancy rate.

KEY WORDS: Early follicular phase; IVF; perifollicular vascularity; power Doppler ultrasound; pregnancy rate.

INTRODUCTION

Despite many advances in IVF, only about 25% IVF treatment cycles lead to a pregnancy (1). Data from the United Kingdom show that nearly 90% of embryos selected for transfer fail to implant (2). Poor

oocyte/embryo quality and the poor endometrial receptivity are suggested to be the major causes of IVF treatment failure.

A number of ovarian markers of implantation potential that determine the IVF treatment outcome have been introduced. It has been shown that in-vitro characteristics such as the negative expression of 11 Beta-hydroxysteroid dehydrogenase (11 β HSD) by granulosa cells (3), and the increased proliferation (4) and steroidogenic activity (2) of cumulus cells are predictors of conception in IVF.

Similarly, it had been demonstrated that ovarian perifollicular vascularity assessed by power Doppler ultrasound (PDU) on the day of egg pick up is an

¹ School of Women's and Children's Health, University of New South Wales, Sydney, New South Wales, Australia.

² Department of Reproductive Medicine and IVF, Maroubra, New South Wales, Australia.

³ Department of Medical Imaging, Royal Hospital for Women, Barker Street, Randwick, New South Wales 2031, Australia.

⁴ To whom correspondence should be addressed at Fertility Centre Scandinavia Stockholm, Storängsvägen 10, SE-115 42 Stockholm, Sweden; e-mail: peter.sjoblom@fcivf.com.

in vivo ovarian marker of implantation potential (5–7). These authors demonstrated that the clinical pregnancy rate in IVF is significantly higher when the embryos transferred were derived from highly vascularized follicles. This would suggest that angiogenesis during follicular growth is a determinant of outcome in IVF treatment.

Power Doppler ultrasound assessment of ovarian perifollicular vascularity on the day of egg pick up helps to select oocytes which develop into the embryos with better potential for implantation (5–7). While it is useful to know which follicles at egg collection contain good quality oocytes, it would also be useful to identify early characteristics of cycles that produce good quality oocytes.

In a longitudinal assessment of follicular vascularity during IVF stimulation, we observed that a wave of high grade small (5–10 mm) follicles seemingly advanced to high grade medium (11–14 mm) size follicles and in turn to high grade large (≥ 15 mm) size follicles in IVF as the cycle progressed (unpublished data). We therefore hypothesized that in cycles in which there were small and medium sized follicles with high grade ovarian perifollicular blood flow (PFBF) in the early follicular phase there would also be large follicles with high grade ovarian PFBF in the late follicular phase and a higher clinical pregnancy rate in IVF would be seen in these cycles. Hence, the aim of this longitudinal study was to assess whether ovarian PFBF in the early follicular phase is associated with cycle outcome in IVF.

MATERIALS AND METHODS

This retrospective cohort study recruited 34 patients undergoing IVF treatment between May 2002 to March 2003 at IVF-Australia, Royal Hospital for Women, Sydney, Australia. Institutional review board approval of the study was obtained from the South-Eastern Sydney Area Health Service Research Ethics Committee-Eastern Section and the Human Research Ethics Committee at the University of New South Wales. Written consent to participate in the study was obtained from each patient.

All patients underwent one of three different ovarian stimulation protocols: IVF long protocol (IVFLP), IVF short protocol (IVFSP) and IVF gonadotropin releasing hormone (GnRH) antagonist protocol (IVFANT). In the IVFLP, patients were pre-treated with the oral contraceptive pill (OCP) (Brevinor 21, Pharmacia Australia, Rydalmere,

NSW, Australia) from day 5 of the menstrual cycle preceding the treatment cycle for 21 days. Fifteen days after starting the OCP, GnRH agonist was introduced either as a nasal spray (Synarel, Pharmacia Australia, Rydalmere, NSW, Australia) 200 μ g twice daily, or as subcutaneous injection (Lucrin injections, Abbott Australasia, Cronulla, NSW, Australia) 1 mg daily for at least 10 days until pituitary downregulation was confirmed (serum E2 < 120 pmol/l). Follicle stimulating hormone injections (Gonal F, Serono Laboratories, Frenchs Forest, NSW, Australia or Puregon, Organon Laboratories, Lane Cove, NSW, Australia) were then commenced for ovarian stimulation with the starting dose being determined according to the patient's age and the presence or absence of polycystic ovaries (PCO) on ultrasound. In the IVFSP, GnRH agonist was administered from menstrual cycle day 1 and FSH injections were commenced on cycle day 2 or 3. In both of these protocols, daily FSH injections and GnRH agonist were continued until the day of human chorionic gonadotropin (hCG) injection. In the IVFANT protocol, daily FSH injections commenced from cycle day 1 or 2 and continued until the day of hCG injection. A single 3 mg dose of GnRH antagonist (Cetrotide, Serono Laboratories, Frenchs Forest, NSW, Australia) was administered on cycle day 6 or 7. If ovarian follicular growth did not allow ovulation induction with hCG injection on the 5th day after injection of Cetrotide, Cetrotide 250 μ gm was administered once daily beginning 96 h after the injection of Cetrotide 3 mg until the day of ovulation induction.

Intramuscular injection of hCG 10,000 IU was given for all three protocols when at least one follicle was ≥ 18 mm in mean diameter. Transvaginal sonography (TVS) guided ovum pick up was performed 36 h after the hCG injection. The eggs collected were either inseminated (IVF) or injected [Intracytoplasmic sperm injection (ICSI)] with prepared sperm 2–4 h following collection and fertilisation was confirmed 16–18 h later. Embryos were transferred transcervically into the uterine cavity under ultrasound control 2 or 3 days following ovum pick up. Clinical pregnancy was confirmed with the visualisation of a gestational sac on ultrasound at week 6 of amenorrhoea. Pregnancy loss was defined as spontaneous miscarriage or ectopic pregnancy before 12 weeks of gestation.

All patients underwent serial transvaginal Doppler ultrasound scanning from cycle days 5 to 7 and continued every 2 days until hCG injection

using the Eccoccee SSA—340A ultrasound machine (Toshiba Corporation, Japan) with power and colour Doppler facilities, equipped with a 6 MHz curvilinear transvaginal probes. The spatial peak temporal average intensity of ultrasound for B-mode and Doppler examinations was less than 100 mW/cm², which is within the safety limits recommended by the Bioeffects Committee of the American Institute of Ultrasound in Medicine (8). The sensitivity for the detection of blood flow was set at 0.09 cm/s for all the cases. During power Doppler ultrasound colour gain for all the cases was set at the level A3 (10), where A stands for amplitude and A3 denotes highest amount of persistence, 10 shows the level of colour gain when the noise (over-gaining) just disappears. All scans were performed by a single operator (SMS) at the same time each morning (between 7 A.M. and 9.30 A.M.), thus controlling the effects of circadian variation on blood flow (9).

During each ultrasound scanning of each ovary, the power Doppler colour box was positioned over each ovarian follicle and the cross-sectional image of the follicle with the maximum colour indication in the follicular circumference was frozen and then the PFBF was graded. Ovarian PFBF was graded using the modified grading system (Grade 0: 0%, Grade 1: 1–25%, Grade 2: 26–50%, Grade 3: 51–75%, Grade 4: 76–100%) based on Chui *et al.* (5). In other words, Grade 0 follicles do not have any detectable blood flow around the follicular circumference, Grade 1 follicles have blood flow visible in 1–25% of the follicular circumference; Grade 2 follicles have blood flow visible in 26–50% of the follicular circumference, Grade 3 follicles have blood flow visible in 51–75% of the follicular circumference, and Grade 4 follicles have blood flow visible in 76–100% of the follicular circumference. Thereafter, the size of the follicle was calculated from the mean of two maximum diameters. The study follicles were also divided into one of three categories according to the size range: small (5–10 mm), medium (11–14 mm) and large (≥ 15 mm). Similarly, the PFBF grade of all follicles was also categorized into high grade (grades 2–4) or poor grade (grades 0–1).

On each ultrasound assessment day the flow velocity waveforms from the ovarian stromal or intra-ovarian arteries (IOA) of both ovaries, both uterine arteries, and spiral artery were obtained in order to calculate the pulsatility index (PI) and the resistance index (RI). The arteries within the ovarian stroma were visualized with power Doppler technique, en-

suring that no perifollicular vessels were included. The Doppler gate was then positioned over the ovarian stromal vessels and the flow velocity waveforms were traced until at least three waveforms with similar amplitude occurred. A computer generated auto calculation box was positioned over two cardiac cycles and the PI and RI were calculated. Similarly, both uterine arteries and the spiral artery PI and RI were calculated. The uterine arteries were visualized lateral to the internal cervical os and the spiral artery was located either from the intra-endometrial region (if present) or from the sub-endometrial region.

Doppler systems incorporate software packages, which allow various calculations to be made from the spectral display, such as PI and RI. Pulsatility index and RI are measures of resistance to blood flow and are inversely related to blood flow (10, 11). The PI is derived from the difference between the peak systolic velocity (S) and end diastolic velocity (D) divided by mean velocity (Velocity_m) ($S-D/\text{Velocity}_m$) (12). Resistance index is the difference between the peak systolic velocity (S) and end diastolic velocity (D) divided by peak systolic velocity ($S-D/S$). Unlike the PI, the RI is affected by the subject's heart rate (13).

Endometrial thickness was also measured on each ultrasound assessment day. The longitudinal section of the uterus with the best image of endometrium in real time B-mode ultrasound was frozen and the endometrial thickness (mm) was measured as the maximum distance between each myometrial/endometrial interface through the central longitudinal axis of the uterus.

In this study, the early follicular phase was defined as cycle day 5, 6 or 7 and the late follicular phase as the day of hCG injection (trigger day –0) or one or two days before hCG injection (trigger day –1 or –2). The early follicular phase corresponded with the time of first ultrasound assessment whilst the late follicular phase corresponded with the last ultrasound assessment prior to hCG injection.

The treatment cycles were divided into two groups: Group 1 or “good beginners” being cycles that had at least one small or medium size follicle(s) of high grade PFBF in the early follicular phase; and Group 2 or “poor beginners” being cycles that did not have any small or medium size follicle(s) with high grade PFBF in the early follicular phase.

Statistical Analysis

Continuous variables were analyzed using either the unpaired Student's *t*-test (normal data distribution) or Mann Whitney *U* test (skewed data) to compare two means (or medians where appropriate). The data was tested for normal distribution using Kolmogorov–Smirnov (K–S) test. Attempts were made to achieve normal distribution by square root transformation where appropriate. Categorical variables were analyzed using the Chi-square (χ^2) test along with the calculation of odds ratio (OR) with 95% confidence intervals (CI). Either Paired Sample *t*-test (for continuous variables) or Wilcoxon Signed Ranks Test (for categorical variables) was used to assess the intra-observer variability in the measurement of ultrasound variables. *p*-value < 0.05 or 95% CI not containing 1.0 were considered statistically significant. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 11.0.

RESULTS

The study group comprised 34 patients undergoing 37 cycles of IVF treatment, of which 20 cycles were classified as Group 1 and 17 cycles as Group 2. Thirty-five cycles resulted in embryo transfer. A total of 245 small or medium size follicles were studied in the early follicular phase. The distribution of PFBF grades of small and medium size follicles in Group 1 and Group 2 is demonstrated in Fig. 1.

The demographic and clinical data for Group 1 and 2 treatment cycles are seen in Table I. There were no significant differences between the two groups in terms of age, body mass index (BMI), duration of infertility, type of infertility, cause of infertility, parity, presence of polycystic ovaries on ultrasound (PCO), smoking, treatment cycle number and type of IVF treatment stimulation protocol.

Table II demonstrates ovarian stromal blood flow and PFBF between the two treatment groups. The mean of the combined right and left IOA PI and RI was used, as there was no significant difference between these. In the early follicular phase, Group 1 had a significantly lower IOA RI compared to Group 2. However, there was no significant difference in IAO PI. In the late follicular phase, both IOA PI and RI were significantly lower in Group 1 compared to Group 2. A total of 246 large follicles were studied in the late follicular phase and 72 (29%) had high grade PFBF. The proportion of high grade large follicles

Fig. 1. Number of small (5–10 mm) and medium (11–14 mm) size follicles in the early follicular phase (day 5 or 6 or 7) according to perfollicular blood flow (PFBF) grade in (A) Group 1 treatment cycles, that had at least one high grade (2–4) small or medium size follicle(s) in the early follicular phase and in (B) Group 2 treatment cycles, that did not have any high grade small or medium size follicles in the early follicular phase.

in the late follicular phase was significantly higher in Group 1 compared to Group 2 (35% vs. 21%; OR 2.0 and 95% CI 1.1–3.7).

There were no differences in uterine blood flow or endometrial thickness between the two study groups in the late follicular phase (Table III). The mean of right and left uterine artery PI and RI was used, as there was no significant differences between these.

The clinical outcome in the two treatment groups is presented in the Table IV. There were significantly more eggs retrieved in Group 1 compared to Group 2, but there was no difference in mean number of

Table I. Demographic and Clinical Data for Group 1^a and Group 2^b Treatment Cycles

	Treatment cycle category		<i>p</i> -value ^c
	Group 1 (<i>n</i> = 20)	Group 2 (<i>n</i> = 17)	
Age (years)	40.0 (29–43) ^d	38.0 (28–44)	0.878
BMI	23.0 (19–27)	25.0 (20–43)	0.118
Duration of infertility (months)	28.0 (12–96)	24.0 (12–84)	0.902
Type of infertility			0.478
Primary	6 (30) ^e	7 (41)	
Secondary	14 (70)	10 (59)	
Cause of infertility			1.00
Unexplained	6 (30)	5 (29)	
Male factor	5 (25)	5 (29)	
Tubal factor	2 (10)	1 (6)	
Mixed	7 (35)	6 (35)	
Parity			0.717
Nulliparous	13 (65)	12 (71)	
Multiparous	7 (35)	5 (29)	
PCO	10 (50)	5 (29)	0.204
Non-PCO	10 (50)	12 (71)	
Smoking	3 (15)	1 (5.9)	0.609
Treatment cycle number			0.348
1	4 (20)	1 (6)	
2 or more	16 (80)	16 (94)	
Type of stimulation protocol			0.797
IVFLP	9 (45)	7 (41)	
IVFSP	4 (20)	5 (29)	
IVFANT	7 (35)	5 (29)	

Note. *n*: number; BMI: body mass index; PCO: polycystic ovaries on ultrasound; IVFLP: IVF long downregulation protocol with gonadotropin releasing hormone (GnRH) agonist; IVFSP: IVF short downregulation protocol with GnRH agonist; IVFANT: IVF protocol with GnRH antagonist.

^aTreatment cycles, that had at least one small (5–10 mm) or medium (11–14 mm) size follicle(s) with perifollicular blood flow (PFBF) grade 2–4 on cycle day 5 or 6 or 7 (early follicular phase).

^bTreatment cycles, that did not have any small or medium size follicles with PFBF grade 2–4 on cycle day 5 or 6 or 7 (early follicular phase).

^c*p*-value for Mann–Whitney U test or χ^2 -test (*df* = 1) or Fisher's exact test (*df* = 1–3) as appropriate.

^dMedian (range).

^eNumber (%).

eggs fertilized between the groups. Eleven clinical pregnancies occurred, giving an overall pregnancy rate per embryo transfer of 31%. Two cycles were excluded from the analysis, as embryo transfer did not occur due to the presence of the endometrial polyp in one patient, and an increased risk of ovarian hyperstimulation syndrome in the other. The pregnancy rate in Group 1 was significantly higher compared to Group 2 (47% vs. 12%; OR 6.3 and 95% CI 1.1–35.7). There was a nonsignificant trend towards a higher pregnancy loss in Group 2 compared to Group 1. The difference in implantation rate did not reach statistical significance.

Table II. Ovarian Stromal and Perifollicular Blood Flow in Group 1^a and Group 2^b Treatment Cycles

	Treatment cycle category		<i>p</i> -value ^c
	Group 1 (<i>n</i> = 20)	Group 2 (<i>n</i> = 17)	
Early follicular phase ^d			
IOA PI (R/L)	2.99 ± 0.91 ^f	3.27 ± 1.0	0.436
IOA RI (R/L)	1.48 ± 0.15	1.63 ± 0.13	0.010
Late follicular phase ^e			
IOA PI (R/L)	2.69 ± 0.50	3.31 ± 0.87	0.033
IOA RI (R/L)	1.46 ± 0.11	1.59 ± 0.09	0.001
Number of large (≥ 15 mm) follicles with grade 2,3,4 PFBF	52/150 (35) ^g	20/96 (21)	0.020 (OR 2, 95% CI 1.1–3.7)

Note. *n*: number; IOA: intraovarian artery; PI: pulsatility index; RI: resistance index; R: right; L: left; R/L: right plus left; PFBF: perifollicular blood flow.

^aTreatment cycles, that had at least one small (5–10 mm) or medium (11–14 mm) size follicle(s) with perifollicular blood flow (PFBF) grade 2–4 on cycle day 5 or 6 or 7 (early follicular phase).

^bTreatment cycles, that did not have any small or medium size follicles with PFBF grade 2–4 on cycle day 5 or 6 or 7 (early follicular phase).

^c*p*-value for unpaired Student's *t*-test or χ^2 -test (*df* = 1) with odds ratio (OR) and 95% confidence interval (CI) as appropriate.

^dEarly follicular phase = cycle day 5 or 6 or 7, corresponding with the first ultrasound scan.

^eLate follicular phase = day of hCG injection or 1 to 2 days before the hCG injection, corresponding with the last ultrasound scan.

^fMean ± SD.

^gNumber (%).

Reliability Assessment

For intra-observer error, there was no significant difference between the consecutively repeated measurements of ovarian PFBF (Wilcoxon Signed Ranks Test *p* > 0.05), impedances of uterine, spiral and intraovarian arteries and endometrial thickness (Paired Sample *t*-test *p* > 0.05). In addition, there was no inter-observer variability in this study because all the measurements were performed by the same operator (SMS).

DISCUSSION

The introduction of transvaginal colour Doppler ultrasound (CDU) has enabled detailed non-invasive studies of the vascularity of the uterus and ovaries (14–17). However, quantitative assessment of vascularity using CDU has limitations: CDU is less effective than PDU in detecting blood flow at low velocities in microvasculature due to a lower signal-to-noise (S/N) ratio; it is prone to alias; CDU is angle

Table III. Uterine Blood Flow and Endometrial Thickness in Group 1^a and Group 2^b Treatment Cycles in the Late Follicular Phase^c

	Treatment cycle category		<i>p</i> -value ^d
	Group 1 (<i>n</i> = 20)	Group 2 (<i>n</i> = 17)	
Endometrial thickness (mm)	10.76 ± 1.93 ^e	10.52 ± 1.76	0.701
UA PI (R/L)	1.64 ± 0.41	1.69 ± 0.52	0.732
UA RI (R/L)	0.80 ± 0.06	0.79 ± 0.08	0.484
SA PI	2.14 ± 0.55	1.78 ± 0.48	0.106
SA RI	0.80 ± 0.05	0.78 ± 0.10	0.667

Note. *n*: number of treatment cycles; UA: uterine artery; PI: pulsatility index; RI: resistance index; R: right; L: left; R/L: right plus left; SA: spiral artery.

^aTreatment cycles, that had at least one small (5–10 mm) or medium (11–14 mm) size follicle(s) with perfollicular blood flow (PFBF) grade 2–4 on cycle day 5 or 6 or 7 (early follicular phase).

^bTreatment cycles, that did not have any small or medium size follicles with PFBF grade 2–4 on cycle day 5 or 6 or 7 (early follicular phase).

^cLate follicular phase = day of hCG injection or 1 to 2 days before the hCG injection, corresponding with the last ultrasound scan.

^d*p*-value for unpaired Student's *t*-test.

^eMean ± SD.

dependent and can not detect the blood flow that is close to 90° to the ultrasound probe (18, 19). PDU has a higher S/N ratio, which enables better visualization of the microvasculature, and PDU does not alias and it is angle independent. In the present study we assessed the ovarian perfollicular blood flow semi-quantitatively and therefore PDU was chosen.

In this study we found that the ovarian stromal blood flow both in early and the late follicular phase, the proportion of large follicles with high grade PFBF in the late follicular phase and the clinical pregnancy rate were significantly higher in “good beginners” (Group 1) compared to “poor beginners” (Group 2). It is notable that a relatively high pregnancy rate (37%) and a low pregnancy loss rate (11%) were achieved in a relatively old patient cohort (Group 1, median age 40 years). This is the first study demonstrating this association between the ovarian PFBF assessed by PDU in the early follicular phase and the treatment outcome in IVF.

There have been four studies published, all cross-sectional, assessing ovarian PFBF semi-quantitatively using the same grading system (grades 1–4) in IVF treatment, three of which used PDU (5–7), and one CDU (16). Ovarian PFBF was assessed on the day of, but prior to the human chorionic gonadotropin (hCG) trigger in the CDU

Table IV. Ovarian Stromal and Perfollicular Blood Flow in Group 1^a and Group 2^b Treatment Cycles

	Treatment cycle category		<i>p</i> -value ^c
	Group 1 (<i>n</i> = 20)	Group 2 (<i>n</i> = 17)	
Eggs retrieved	9.1 ± 5.9 ^d	5.8 ± 3.8	0.047 ^e
Eggs fertilized	5.7 ± 3.8	4.2 ± 2.6	0.185 ^e
No. of embryos transferred	2.0 ± 0.3	1.9 ± 0.5	0.328
Implantation rate	9/38 (24)	3/30 (10)	0.124 (OR 2.8, CI 0.7–11.4)
Clinical pregnancies per ET ^f	9/19 ^g (47)	2/16 ^g (12)	0.027 (OR 6.3, CI 1.1–35.7)
Pregnancy loss	2/9 (22)	1/2 (50)	0.491

Note. ET: embryo transfer.

^aTreatment cycles, that had at least one small (5–10 mm) or medium (11–14 mm) size follicle(s) with perfollicular blood flow (PFBF) grade 2–4 on cycle day 5 or 6 or 7 (early follicular phase).

^bTreatment cycles, that did not have any small or medium size follicles with PFBF grade 2–4 on cycle day 5 or 6 or 7 (early follicular phase).

^c*p*-value for unpaired Student's *t*-test or χ^2 -test (*df* = 1) with odds ratio (OR) and 95% confidence interval (CI), Mann-Whitney *U*-test, or Fisher's exact test (*df* = 1) as appropriate.

^dMean ± SD.

^eSquare root transformation was taken to achieve normal distribution.

^fTwo cycles not proceeding to embryo transfer were excluded.

^gNumber (%).

study, and on the day of oocyte retrieval in the PDU studies. All studies showed a significant improvement in cycle pregnancy rate (CPR) if embryos resulting from the fertilization of eggs from better perfused follicles were used. In addition, this was confirmed in a recent meta-analysis of the three PDU studies (20).

Chui *et al.* (5) demonstrated that oocytes resulted in significantly higher proportion of triploid embryos if they were derived from follicles with low grade vascularity, compared to if there were derived from follicles with high grade vascularity. Bhal *et al.* (6) demonstrated that the oocyte retrieval rate, maturity and fertilization rate were all significantly higher and the triploidy rate significantly lower in the group with high grade vascularity follicles. Van Blerkom *et al.* (21), demonstrated that oocytes derived from follicles with low oxygen content have a significantly higher frequency of defects in chromosome number, spindle organization, and cytoplasmic structure as well as a significantly reduced ability to develop to the 6–8 cell stage embryo in vitro when fertilized. Such a hypoxic intra-follicular environment could result from a failure of an appropriate microvasculature to develop around the growing follicle,

suggesting a link between ovarian follicular blood flow and oocyte quality.

This could possibly explain the significantly higher clinical pregnancy rate in “good beginners” compared to “poor beginners” (47% vs. 12%; χ^2 p 0.027; OR 6.3 with 95% CI 1.1–35.7) in our study. However, due to multifollicular development, it was not possible for us to follow the individual oocyte of the same follicle from the early follicular phase until the day of egg collection and subsequent embryo transfer.

The significantly higher proportion of large follicles with high grade PFBF in the late follicular phase and significantly higher clinical pregnancy rate in “good beginners” in our study indirectly shows the association between a high grade PFBF in the late follicular phase with a high clinical pregnancy rate, confirming the findings of the other PDU studies (5–7). This study also demonstrated that the mean number of oocytes retrieved was significantly higher in “good beginners” compared to “poor beginners,” supporting the findings by Bhal *et al.* (6).

The grading of ovarian PFBF and the categorization of high grade and low/poor grade PFBF in our study is different to the published three PDU studies (5–7), with follicles with 0–25% PFBF being categorized as grade 1 in the earlier studies, and follicles with 0% PFBF were categorized as grade 0 and follicles with 1–25% PFBF were categorized as grade 1 in our study. Perifollicular blood flow grades 2, 3 and 4 were identical between the earlier studies and our study. A further difference is the classification of high (grades “2 to 4” and “3 to 4,” respectively) and low/poor (grades “0 to 1” and “1 to 2,” respectively) grade ovarian PFBF.

The proportion of grade 3 and 4 follicles was higher in the studies by Chui *et al.* (5), (52%) and Bhal *et al.* (5), (64%) than in our study (between 1 and 3% in small sized follicles, 4 and 16% in medium sized follicles and 7 and 13% in the large sized follicles throughout the follicular phase). The information on proportion of grade 3 and 4 was not available in the PDU study by Borini *et al.* (7). The most likely explanation for this difference in the proportion of grade 3 and 4 follicles between the present study and the other studies is that the ovarian PFBF in the above cited studies (5–7) was assessed on the day of egg pick up (approximately 32–34 h following hCG administration) compared to all PFBF assessments being performed prior to the hCG trigger in our study. Exogenous hCG or endogenous LH surge has been demonstrated to increase angiogenesis of

human ovarian follicles by increasing local vascular endothelial growth factor (VEGF) production (22).

During the follicular development, the ovarian stromal vessels are the primary vessels that supply pre-antral follicles (23–25). A number of studies have showed a relationship between ovarian stromal blood flow early in the cycle and the ovarian responsiveness (11,26–29). A lower IOA PI early in the cycle (day 2 or 3) is associated with a larger number of growing follicles (11) and a larger number of oocytes retrieved (29). Similarly, Bassil *et al.* (27) demonstrated that a lower IOA RI two days before HMG injections started is associated with an increased oocyte retrieval rate. A number of studies have shown that IOA PSV assessed on day 3 (26) or on the day of downregulation (28) is significantly lower in poor responders.

Also in present study we found that a lower IOA RI in the early and the late follicular phase is associated with better vascularisation of follicles and better treatment outcome. However, the IOA PI was not significantly different in the early follicular phase, but it was in the late follicular phase, possibly due to the fact that PI is not as accurate as RI because of the variability inherent in measurement of mean velocity with current software programs (30). We are neither aware of any published studies correlating the ovarian PFBF with the IOA PI or RI both in early and the late follicular phase, nor of studies correlating the early follicular phase ovarian PFBF with the treatment outcome with which to make comparisons.

There are several limitations to the present study. Firstly, there is an absence of data on the correlation between the ovarian PFBF and the oocyte/embryo quality because it was not possible to follow up of the individual oocytes of the same follicle from the early follicular phase until the day of egg collection and subsequent embryo transfer. Secondly, it would have been ideal to perform the TVS examinations on the same cycle day (e.g., on cycle day 6 rather than on days 5, 6, 7) to improve consistency. Unfortunately this was not possible due to logistical reasons. Thirdly, this is a retrospective cohort study and as such it has limitations with regard to the generalizability of the findings. Data were collected in an exploratory study with a view to generate hypotheses and not *a priori* to test hypotheses. However, the pattern of “good beginners” was so distinct and potentially useful that publication of retrospective observational data would be justified in order to provide a basis for further studies of the

issue. Thus, the sample size was not optimized. The ideal design of a future investigation would be a prospective cohort study, as one cannot randomize patients into being good or poor beginners. Such a study with an adequate sample size calculated from the present results is necessary to confirm the findings before changing the clinical practice.

It may then be possible to identify poor prognosis IVF cycles at an early stage of ovarian stimulation. One may consider the early cancellation of such cycles in an attempt to minimize further IVF related procedural risks plus financial and emotional costs. The identification of early markers of follicular/oocyte competence may enable new hypotheses to be developed that may guide the search for important determinants of follicular/oocyte growth and development. This may in turn lead to the development of more successful ovarian stimulation protocols.

CONCLUSION

High grade ovarian perifollicular blood flow in the early follicular phase of IVF treatment cycles is associated with both high grade perifollicular blood flow in the late follicular phase and a higher clinical pregnancy rate.

ACKNOWLEDGMENTS

The authors wish to thank Dr Gillian Heller, Senior Lecturer, Department of Statistics, Macquarie University, Australia for her kind help in statistical analysis. The authors also would like to acknowledge the IVF Australia-Eastern Suburb clinic nurse coordinators Di Craven, Mary O'Neil and Robyn Grant for their kind cooperation.

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