

Ultrasound derived indices of follicular blood flow before HCG administration and the prediction of oocyte recovery and preimplantation embryo quality

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The principal aim of the study was to relate ultrasound-derived indices of blood flow in individual follicles on the day of, but before, the administration of human chorionic gonadotrophin (HCG) to the subsequent recovery of oocytes and the production of preimplantation embryos. Data were obtained from 21 women (aged 29–43 years) with bilateral tubal occlusion, who were undergoing treatment by in-vitro fertilization (IVF) and embryo transfer. Transvaginal ultrasonography with colour Doppler imaging and pulsed Doppler spectral analysis were used to measure follicular volume and derive indices of blood flow. The end-points for each follicle were the volume, peak systolic velocity (PSV), pulsatility index (PI), and the recovery or non-recovery of an oocyte, the subsequent production or non-production of a preimplantation embryo and the morphological grade of each embryo. A total of 94 follicles were studied; 74 oocytes were recovered (79%) and 40 embryos (33 grade I or II) were produced. There were four clinical pregnancies (pregnancy rate 25.0% per transfer, 19.0% per patient). There was a significant correlation between whether or not follicular blood flow was detected and whether or not an oocyte was recovered ($P < 0.05$, χ^2 test). The values for volume and PI were not clinically useful. The PSV (cm/s, mean \pm SD) was higher in follicles that were associated with the production of an embryo (12.7 ± 5.9) compared with those that were not (8.5 ± 5.0 ; $P < 0.05$, Student's t -test). The probability of producing a grade I or grade II embryo was 75% if the PSV was ≥ 10 cm/s. The corresponding value was 40% if the PSV was < 10 cm/s and 24% if blood flow was not detected (i.e. PSV < 3 cm/s). There was a significant increase ($P < 0.05$, Student's t -test) in the PSV before aspiration in those follicles associated with the subsequent production of an embryo. We conclude that the value for PSV, before the administration of HCG, can be used to identify follicles

with a high probability of producing an oocyte and a high grade preimplantation embryo. The information may also be used to time the administration of HCG to achieve the optimum number and quality of embryos for patient management.

Key words: colour Doppler imaging/embryo quality/follicular blood flow/oocyte recovery/transvaginal ultrasonography

Introduction

There is still a need for the introduction of procedures that will improve the efficacy and reduce the cost of treating infertile couples by in-vitro fertilization (IVF) and embryo transfer after ovarian stimulation by gonadotrophins and human chorionic gonadotrophin (HCG) (Tan *et al.*, 1992; Neumann *et al.*, 1994). The availability of a test procedure for the identification of mature, healthy follicles could be used to time the administration of HCG for oocyte recovery, IVF and embryo transfer. Alternatively, the most appropriate follicles could be identified from a large cohort for oocyte recovery, IVF and embryo transfer, and for embryo cryopreservation for use in subsequent cycles. There are preliminary data which suggest that the size and number of follicles as determined by ultrasonography are related to oocyte recovery, fertilization and cleavability (Wittmaack *et al.*, 1994).

An alternative or complementary test could involve an assessment of follicular blood flow. We have shown previously from a study of spontaneous ovarian cycles using transvaginal ultrasonography and colour Doppler imaging that there is an increase in the maximum peak systolic velocity (PSV) in vessels supplying the dominant follicle before rupture and presumed ovulation (Bourne *et al.*, 1991; Collins *et al.*, 1991; Campbell *et al.*, 1993). The same technique has been used to study patients undergoing ovarian stimulation for IVF and embryo transfer, and the results have shown that a derived vascularity index (i.e. the number of follicles with detectable flow velocity waveforms/total number of follicles) from ovaries containing a combined total of 20 or more follicles is correlated with the oocyte recovery rate (Oyesanya *et al.*, 1996). Furthermore, we have shown that an increase in the PSV in vessels supplying individual follicles immediately before follicle aspiration is significantly correlated with whether or not an oocyte is obtained and the morphological quality of the subsequent preimplantation embryo (Nargund *et al.*, 1996).

This report is concerned with a further analysis of data from the study by Nargund *et al.* (1996) and addresses the use of ultrasound-derived indices of follicular maturity (volume and blood flow) on the day of, but before, the administration of

HCG, for assessing the probability of oocyte recovery and the production of good quality preimplantation embryos. We also took the opportunity to obtain further information on follicular function by examining possible correlations between follicle size and indices of blood flow before HCG administration and follicular aspiration (attempted oocyte recovery).

Materials and methods

The aim was to study 100 follicles (≥ 14 mm mean maximum diameter) on two occasions, and subsequently oocyte recovery and preimplantation embryo quality during the treatment of infertility by IVF and embryo transfer. The study was approved by the Research Ethics Committee of King's College Hospital and the protocol has been described previously (Nargund *et al.*, 1996). The women were recruited from the Assisted Conception Units at King's College Hospital, London and the Thomson Medical Centre, Singapore. Identical methods, equipment and clinical report forms were used at both centres after the implementation of common training and quality control procedures

Treatment regimen for IVF and embryo transfer

All women received buserelin acetate (Suprefact; Hoechst UK Ltd, Hounslow, UK) from day 1 of menses (500 $\mu\text{g}/\text{day}$, s.c.) for 14 days, or until the ovaries and uterus appeared to be quiescent by pelvic ultrasonography. Human menopausal gonadotrophin (HMG, Pergonal i.m., Serono Laboratories Ltd., Welwyn Garden City, UK) and buserelin were then administered daily. The dose of HMG was determined according to the woman's age, previous response to treatment, and plasma concentration of follicle stimulating hormone (FSH; on days 2–4 of a previous menstrual cycle). Follicular and endometrial growth were monitored by pelvic ultrasonography. The dose of HMG was increased if necessary. Human chorionic gonadotrophin (HCG; Profasi 10 000 IU i.m., Serono Laboratories Ltd.) was administered when the mean diameter of the leading follicle reached 18 mm. Transvaginal ultrasound-directed follicle aspiration was performed 34–36 h after the injection of HCG (Waterstone *et al.*, 1992). Methods for the culture of oocytes, spermatozoa and embryos have been described (Bolton *et al.*, 1989). A maximum of three embryos was transferred 2 or 3 days after oocyte retrieval and IVF Progesterone pessaries (200 mg Cyclogest; Hoechst UK Ltd.) were given vaginally twice daily for 16 days from the day of follicle aspiration.

Inclusion criteria for study

All women recruited for the research study had a maximum of six follicles in either ovary before the administration of HCG. Their previous medical history indicated regular menstrual cycles (25–33 days), a baseline concentration of plasma FSH < 8 IU/l, and bilateral tubal occlusion. The current partner of each woman had normal spermograms with a sperm density $> 20 \times 10^6/\text{ml}$ semen, $> 60\%$ motility and $> 30\%$ normal forms on two separate occasions.

Colour Doppler imaging

The first examination with transvaginal colour Doppler imaging and pulsed Doppler spectral analysis was performed on the day of, but prior to, HCG administration (before HCG). The second was undertaken immediately before transvaginal ultrasound-directed follicle aspiration (before aspiration). All scans (in London and Singapore) were performed by the same operator (G.N.) and checked by a colleague. A consistent scanning plan was adopted for each scan in relation to the uterus and adjacent pelvic wall. During the first scan

the follicles were numbered 1 to 6 and photographed to aid subsequent identification. ATL-Ultramark 9, machines (Advanced Technology Laboratories, Bothell, WA, USA) with 5 MHz transvaginal probes were used for 96% of the scans. The spatial peak temporal average intensity for B-mode and colour Doppler imaging was < 80 mW/cm², which is well within the safety limits recommended by the Bioeffects Committee of the American Institute of Ultrasound in Medicine (Gill, 1982). The filter for spectral analysis was set out at 50 Hz. The sample volume range was 1.5–1.0 mm². All follicles were examined for the presence of colour signals indicative of vascularity. Care was taken to record only signals that could be confidently assigned to a given follicle. Flow velocity waveforms (FVWs) obtained from these vessels were used for spectral analysis. Indices of blood flow for each follicle were recorded from those vessels with the highest peak systolic velocity (PSV). A one-way analysis of variance of replicate data (three to five measurements) from a previous study of 18 women with preovulatory follicles or corpora lutea gave a coefficient of variation of 7.6% for PI and 4.4% for PSV (Collins *et al.*, 1991). A 16 mm double channel needle was used to puncture and aspirate each follicle. Residual fluid in the needle and tubing was displaced into the collecting tube by injecting 1.5 ml of medium into the follicle. This procedure allowed the source of each oocyte to be determined with confidence.

End-points

The maximum transverse (D_1), anteroposterior (D_2) and longitudinal (D_3) diameters of all follicles were measured (cm) and the volumes (ml) estimated according to the formula $\text{volume} = (0.523 \times D_1 \times D_2 \times D_3)$. Blood flow impedance was expressed as the pulsatility index (PI) which was calculated from curves fitted to FVWs over three cycles according to the formula $\text{PI} = (S - D)/M$, where S is the peak systolic shifted frequency, D is the maximum end diastolic frequency, and M is the time-averaged maximum frequency over the whole cardiac cycle. The angle of the probe was adjusted until a FVW with the maximum peak systolic shifted frequency was obtained, and the value was recorded. The PI was calculated from the same FVW. The absence of identifiable flow velocity waveforms around a given follicle (i.e. the velocity was below the sensitivity of the equipment, < 3 cm/s) was reported as no detectable blood flow.

The following additional data were recorded for each follicle: the recovery or non-recovery of an oocyte, the subsequent occurrence or non-occurrence of fertilization, the number of pronuclei (i.e. 0, 1, 2, 3 or > 3) on day 1 after oocyte recovery, the number of cells per preimplantation embryo and the morphological grade [from I (good) to IV (poor)] on day 2 or 3 according to the criteria of Bolton *et al.* (1989), except that the numbering system was reversed. The results obtained by ultrasonography were not revealed to the embryologists. The number of treatment cycles per patient, embryo transfers and clinical pregnancies (the presence of an active fetal heart by pelvic ultrasonography) were also recorded.

Statistical analysis

The variance of blood flow velocity between and within women was assessed using standard analysis of variance methods. Velocity was analysed as both a categorical variable (detectable/non-detectable) and when detectable as a continuous variable. A value ≥ 10 cm/s for the PSV [derived from a visual inspection of the data and the results from a previous study of natural ovarian cycles by Campbell *et al.* (1993)] was used for some analyses. Significance between two categorical variables was assessed by applying the χ^2 test, and the difference between groups of continuous variables was assessed with

Table I. Association between the detection of follicular blood flow on the day of, but prior to, human chorionic gonadotrophin (HCG) administration and oocyte recovery

Blood flow detected	Oocyte recovery		
	Yes <i>n</i> (%)	No <i>n</i> (%)	All <i>n</i> (%)
Yes	29 (31)	2 (2)	31 (33)
No	45 (48)	18 (19)	63 (67)
All	74 (79)	20 (21)	94 (100)

$P = 0.014$, χ^2 test.

Student's *t*-test. The Statistical Package for Social Sciences (SPSS) was used for all analyses.

Results

A total of 27 women (aged 29–43 years) were studied and 21 provided complete data for this analysis which involved 22 treatment cycles and 94 follicles: Five women had one or two study follicles; nine women had three or four study follicles and seven had five to eight. A maximum of five follicles were assessed in any one ovary. The mean maximum follicle diameter was 22 mm (range 18–31 mm). The oocyte recovery rate was 79%. A total of 40 preimplantation embryos were produced and 16 transfers (of between one and three embryos) were attempted. The clinical pregnancy rate was 18.2% per cycle, 25.0% per embryo transfer and 19.0% per patient. The variance of follicular PSV between women was not significantly different from the value within women. Consequently the values for PSV were analysed as independent variables.

Oocyte recovery

There was a significant association ($P < 0.05$; χ^2 test) between whether or not FVWs were detected (i.e. velocity < 3 cm/s) before HCG administration and whether or not an oocyte was recovered (Table I). The sensitivity of the test, based on the detection of follicular blood flow, for predicting oocyte recovery was $29/74 \times 100 = 39\%$. The positive predictive value of the test at this time during the treatment cycle was $29/31 \times 100 = 94\%$. The specificity of the test was $18/20 \times 100 = 90\%$ and the negative predictive value $18/63 \times 100 = 29\%$. Of 74 oocytes recovered, 29 (39%) originated from follicles with detectable blood flow.

Preimplantation embryo quality

A total of 18 oocytes from 31 follicles with detectable blood flow were fertilized (58%) and all produced grade I or II embryos. This finding was of borderline significance ($P = 0.0559$; χ^2 test) compared with the 22 oocytes recovered from 63 follicles with no detectable blood flow (35%) that went on to form an embryo; of these seven (32%) were grades III or IV. Furthermore, the PSV was significantly higher in follicles that contained a recoverable oocyte and subsequently resulted in a preimplantation embryo (Table II). There was no significant difference in the values for PI (Table II). The association

Table II. The pulsatility index (PI) and peak systolic blood velocity (PSV) on the day of, but prior to, human chorionic gonadotrophin (HCG) administration and the subsequent production of a preimplantation embryo after in-vitro fertilization

Index of follicular blood flow	Embryo produced				<i>P</i> value*
	Yes		No		
	<i>n</i>	Mean (SD)	<i>n</i>	Mean (SD)	
PI	18	0.71 (0.16)	13	0.71 (0.36)	> 0.05
PSV (cm/s)	18	12.7 (6.0)	13	8.5 (5.0)	$< 0.05^{**}$

*Student's *t*-test.

** $P = 0.032$ using logged data.

between follicular PSV with the non-production or morphological grade of embryo is shown in Table III. The probability of producing a grade I or II embryo was 75% if the PSV was ≥ 10 cm/s; 40% if the PSV was < 10 cm/s and 24% if blood flow was not detected. The value of measuring follicular PSV before the administration of HCG and before follicular aspiration (oocyte recovery) in this cohort of follicles is summarized in Table IV. It may be seen that 56/94 follicles (60%) had detectable blood flow before follicular aspiration (compared with 49% before HCG administration). The probability of a follicle with PSV ≥ 10 cm/s giving an oocyte which fertilized and produced a grade I or II embryo was similar at both times during the treatment cycle. However, the probability of producing a good embryo was much less if the PSV was < 10 cm/s before follicular aspiration.

Follicular volume

The volume was measured in 92 of the 94 follicles before the administration of HCG. There was no significant difference in the values between those follicles from which an oocyte and subsequently a preimplantation embryo were obtained (4.50 ± 2.64 ml, mean \pm SD, $n = 40$), and those where no oocyte or embryo were obtained (4.45 ± 2.54 ml, mean \pm SD, $n = 52$). Furthermore, the volumes were not significantly higher immediately before follicular aspiration (those that were associated with an embryo 7.28 ± 3.77 ml, mean \pm SD, $n = 39$, and those that were not 5.95 ± 2.76 ml, mean \pm SD, $n = 51$; $P = 0.057$).

Possible correlations

There was no significant correlation between the follicular PSV and volume before HCG administration ($r = 0.2$, $P = 0.28$) or between the factorial increases in volume and PSV immediately before follicular aspiration. There was, however, a highly significant correlation between follicular PSV before HCG administration and the corresponding value before follicular aspiration ($r = 0.78$, $P < 0.0001$). The factorial increase in PSV was significantly higher ($P < 0.05$) for follicles that were associated with the subsequent production of an embryo (1.81 ± 0.94 , mean \pm SD, $n = 18$) than for follicles that were not (1.21 ± 0.24 , mean \pm SD, $n = 13$).

Table III. Association between ultrasound derived indices of follicular blood flow on the day of, but prior to, human chorionic gonadotrophin (HCG) administration and the non-production or morphological grade of a preimplantation embryo

Index of perfollicular blood flow	Oocyte and/or embryo not produced		Oocyte and embryo produced			
			Grades III and IV		Grades I and II	
	n	(%)	n	(%)	n	(%)
FVW not detected	41	(65)	7	(11)	15	(24)
PSV <10 cm/s	9	(60)	0	(-)	6	(40)
PSV ≥10 cm/s	4	(25)	0	(-)	12	(75)
All	54		7		33	

FVW = flow velocity waveforms

Table IV. Value of measuring follicular peak systolic blood velocity (PSV) before the administration of human chorionic gonadotrophin (HCG) and before follicle aspiration (FA) for the production of a grade I or grade II preimplantation embryo

Index of perfollicular blood flow	Prediction of grade I or II preimplantation embryo			
	Before HCG		Before FA	
	Total no. follicles	Probability* (%)	Total no. follicles	Probability* (%)
FVW not detected	63	24	38	18
PSV <10 cm/s	15	40	23	9
PSV ≥10 cm/s	16	75	33	73
All	94		94	

*Probability of producing a grade I or II preimplantation embryo
FVW = flow velocity waveforms.

Discussion

There is now convincing evidence that mammalian ovulation has similarities to an inflammatory reaction and involves profound changes in localized blood flow (Espey, 1994). Previous studies by other investigators concerning the potential role of colour Doppler imaging in the overall assessment of follicular blood flow in patients undergoing IVF and embryo transfer have involved the use of either mean values for the PSV from many follicles (Balakier and Stronell, 1994) or the maximum PSV from serial monitoring (Tekay *et al.*, 1995). Under these conditions there was no difference in values between conception and non-conception cycles. We have taken a more detailed approach that involves the study of ultrasound-derived indices of blood flow and oocytes from less than six individual follicles per ovary at defined times in a selected cohort of women undergoing IVF and embryo transfer. The first analysis of these data showed that there was a significant relationship between the follicular PSV immediately before ultrasound-directed follicular aspiration, oocyte recovery and the subsequent production of high grade preimplantation embryos (Nargund *et al.*, 1996). To the best of our knowledge the current report is the first to relate ultrasound-derived indices of blood flow and size in individual follicles on the day of, but prior to, HCG administration, to the changes that occurred in the same variables immediately before follicular aspiration and the oocyte recovery rate and production of preimplantation embryos.

The results of this aspect of the study showed that there

was no direct relationship between follicular volume and the blood PSV or impedance as indicated by the PI, before the administration of HCG. There was, however, a significant relationship between the detection of follicular flow velocity waveforms within a given follicle and the recovery of an oocyte. Furthermore the PSV was significantly higher, in those follicles associated with the subsequent production of an embryo, whereas the follicular volumes were similar. The factorial increase in PSV was significantly higher immediately before follicular aspiration (attempted oocyte recovery) whereas the corresponding change in follicular volume was not significantly different. These findings are interesting and indicate that there was no bias towards seeking indices of higher blood flow in larger follicles. The findings are also consistent with the suggestion that early changes in follicular vascularity and blood flow may initiate important biochemical events which are essential for successful reproduction within the follicular environment. For example, there have been reports showing an association between either the oxygen concentration in follicular fluid (Gosden and Byatt-Smith, 1986; Fischer *et al.*, 1992) or the oxygen consumption or the ATP content of an oocyte (Magnusson *et al.*, 1986; Van Blerkum *et al.*, 1995) and the development of a human embryo.

In our study, only 33% of follicles had detectable flow velocity waveforms before the administration of HCG. This finding probably reflects the selection of patients with a relatively low response to gonadotrophin therapy, and the strict classification of follicles with a readily identifiable blood supply. The small number of follicles per ovary facilitated overcoming the technical difficulty of measuring indices of blood flow in the appropriate vessels, and relating each oocyte recovered to the follicle of origin. The experimental design also provided a balanced range of follicles in terms of size and PSV. The low proportion of follicles with detectable flow velocity waveforms contrasts with the findings from another study undertaken in our department of patients with 20 follicles, who were classified as being at risk of the ovarian hyperstimulation syndrome. An analysis of these data showed that the mean follicle vascularity index (i.e. the number of follicles with detectable blood flow/the total number of follicles×100) was 61% and the oocyte recovery rate was >75% (Oyesanya *et al.*, 1996).

The clinical usefulness of a test is dependent upon the reproducibility of the value for the end-point, both within and

between observers. Data on the reproducibility of measurements for follicle PSV are scarce and controversial. Velocity can only be measured in a given vessel if the angle of insonation is known. Unfortunately, this variable cannot be determined for small vessels. It is likely, however, that at least one vessel in the vascular bed will be located at an appropriate angle to give the PSV. Our initial work on the reproducibility of follicle PSV was based on replicate data (three to five sequential measurements by the same operator) from each of 13 women with a preovulatory follicle or corpus luteum. The mean within women coefficient of variation for the PSV was 4.4% over the range 5–30 cm/s (Collins *et al.*, 1991).

More recently, Sladkevicius and Valentin (1995) have reported on the intra- and interclass correlation coefficients (Scherjon *et al.*, 1993) for the time-averaged maximum peak systolic velocity (TAMXV) for data collected from 12 women during the late follicular and mid-luteal phases of the ovarian cycle. The value of 0.16 for the intraclass correlation coefficient during the preovulatory period over the range 3.4–12.0 cm/s, and the corresponding value of 0.10 for the interclass correlation coefficient over the range 4.7–21.0 cm/s raised doubts about the value of measuring follicle TAMXV. Moreover, Tekay and Joupilla (1996) raised similar doubts from their finding of an intraclass correlation coefficient of 0.63–0.68 for replicate measurements of the PSV (every 10–15 min, three occasions) in the stroma of the right and left ovaries of 10 women. We have recently obtained new data on the intra-observer reproducibility of PSV measurements from a study of 40 patients (15 with normal preovulatory ovaries, 15 with a presumed corpus luteum, eight with benign tumours and two with malignant tumours). For this study the on-screen display of results of the flow velocity waveform analysis from each scan was obscured from the operator, and a second examination of each ovary was performed ~5 min later (Tailor *et al.*, 1996). The intraclass correlation coefficient for the PSV was 0.992 with a coefficient of variability of 10.2% according to the method of Bland and Altman (1992). Accordingly, these results provide reassurance that it is possible for the observer to obtain reasonably reproducible results for the PSV within a given ovary. Furthermore, the lack of random, large changes in the follicle PSV at defined times over a 24 h period around the serum luteinizing hormone (LH) peak also implies that measurements of PSV are reproducible (Zaidi *et al.*, 1996).

The physiological significance of changes in follicle vascularity and blood flow is uncertain. A study of Rhesus monkeys has shown that enhanced vascularization is associated with the selection and maturation of the leading follicle (Zeleznik *et al.*, 1981). Other studies of rabbits (Kranzfelder and Maurer-Schultze, 1989), ewes (Murdoch *et al.*, 1983) or rats (Tanaka *et al.*, 1989) have shown increasing vascularization of developing follicles and the results also suggest that localized ovarian blood flow may play a crucial role in each ovulatory process (i.e. the release of mature oocytes from either ovary). Conversely, a decrease in the density of the vasculature may be associated with follicular atresia in the Rhesus monkey (Zeleznik *et al.*, 19981) and a decrease in blood PSV has been shown in humans during luteal regression (Bourne *et al.*, 1996).

The knowledge gained from our study of patients undergoing

IVF and embryo transfer may be useful for the design of new studies on the relationship between perifollicular blood flow, oocyte recovery and embryo quality. The potential value of colour Doppler imaging and pulsed Doppler spectral analysis prior to the administration of HCG, to identify follicles that yield significantly higher numbers of oocytes with either poor or good developmental potential, is a novel finding. At the moment, however, we can only suggest that the assessment of follicular blood flow by this minimally invasive method might help clinicians to make decisions regarding either the cessation of treatment during a given cycle, or the most appropriate time for the administration of HCG. Further in the treatment procedure the same information might be used to select oocytes for fertilization or embryos for transfer. There is an urgent need for complementary studies with a larger number of patients and follicles to provide more definitive information about all of these possibilities.

Acknowledgements

The authors are grateful to the staff at the Assisted Conception Units, King's College Hospital and the Thomson Medical Centre, Singapore, for their help and co-operation. The ultrasound machine at KCSMD was made available on extended loan by Advanced Technology Laboratories UK Ltd., Letchworth, UK.

References

- Balakier, H and Stronell, R.D (1994) Color Doppler assessment of folliculogenesis in *in vitro* fertilization patients. *Fertil. Steril.*, **62**, 1211–1216
- Bland, J.M. and Altman, D.G. (1992) Statistics as applied to Doppler studies. In Pearce J.M. (ed.), *Doppler Ultrasound in Perinatal Medicine*. Oxford University Press, Oxford, pp. 17–59
- Bolton, V., Hawes, S., Taylor, C.T. and Parsons, J.H (1989) Development of spare human embryos *in vitro*, an analysis of the correlations among gross morphology, cleavage rates and development to the blastocyst. *J. In Vitro Fertil. Embryo Transfer*, **6**, 30–35.
- Bourne, T.H., Jurkovic, D., Waterstone, J *et al.* (1991) Follicular blood flow during human ovulation. *Ultrasound Obstet Gynecol.*, **1**, 53–59.
- Bourne, T.H., Hagstrom, H-G., Hahlin, M *et al.* (1996) Ultrasound studies of vascular and morphological changes in the corpus luteum during the menstrual cycle. *Fertil. Steril.*, **65**, 753–758
- Campbell, S., Bourne, T.H., Waterstone, J. *et al.* (1993) Transvaginal color flow imaging of the periovulatory follicle. *Fertil. Steril.*, **60**, 433–438.
- Collins, W., Jurkovic, D., Bourne, T. *et al.* (1991) Ovarian morphology, endocrine function and follicular bloodflow during the peri-ovulatory period. *Hum. Reprod.*, **6**, 319–324.
- Espey, L.L. (1994) Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. *Biol. Reprod.*, **50**, 233–238.
- Fischer, B., Künzel, W., Kleinstein, J and Gips, H. (1992) Oxygen tension in follicular fluid falls with follicle maturation. *Eur. J. Obstet Gynecol. Reprod. Biol.*, **43**, 39–43
- Gill, R.W (1982) Accuracy calculations for ultrasonic pulsed Doppler blood flow measurement. *Aust. Phys. Sci. Med.*, **5**, 51–57.
- Gosden, R.G. and Byatt-Smith, J.G. (1986) Oxygen concentration gradient across the ovarian follicular epithelium: model predictions and implications. *Hum. Reprod.*, **1**, 65–68
- Kranzfelder, D. and Maurer-Schultze B (1989) Development of the perifollicular network. Autoradiographic and morphometric studies in the rabbit ovary. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **30**, 163–171
- Magnusson, C., Hillensjo, T., Hamberger, L. and Nilsson, L. (1986) Oxygen consumption by human oocytes and blastocysts grown *in vitro*. *Hum. Reprod.*, **1**, 183–184.
- Murdoch, W.J., Nix, K.J. and Dunn, T.G. (1983) Dynamics of ovarian blood supply to periovulatory follicles of the ewe. *Biol. Reprod.*, **28**, 1001–1006.
- Nargund, G., Bourne, T., Doyle, P. *et al.* (1996) Associations between

- ultrasound indices of follicular blood flow, oocyte recovery and preimplantation embryo quality *Hum. Reprod*, **11**, 109–113.
- Neumann, P.J., Gharib, S.D. and Weinstein, M.C. (1944) The cost of a successful delivery with in-vitro fertilisation *N Engl. J Med.*, **331**, 239–243
- Oyesanya, O.A., Parsons, J.H., Collins, W.P. and Campbell, S. (1996) Prediction of oocyte recovery rate by transvaginal ultrasonography and color Doppler imaging before human chorionic gonadotropin administration in *in vitro* fertilization cycles *Fertil. Steril*, **65**, 806–809
- Scherjon, S.A., Kok, J.H., Oosting, H. and Zondervan, H.A. (1993) Intra-observer and inter-observer reliability of the pulsatility index calculated from pulsed Doppler flow velocity waveforms in three fetal vessels *Br J Obstet Gynaecol.*, **100**, 134–138
- Sladkevicius, P. and Valentun, L. (1995) Reproducibility of Doppler measurements of blood flow velocity in the uterine and ovarian arteries in premenopausal women. *Ultrasound Med. Biol.*, **21**, 313–319
- Taylor, A., Jurkovic, D., Bourne, T.H. *et al.* (1996) A comparison of intratumoral indices of blood flow velocity and impedance for the diagnosis of ovarian cancer. *Ultrasound Med. Biol.*, **22**, in press
- Tan, S.L., Royston, P., Campbell, S. *et al.* (1992) Cumulative conception and live birth rates after *in vitro* fertilisation. *Lancet*, **339**, 1390–1394.
- Tanaka, N., Espey, L.L. and Okamura, H. (1989) Increase in ovarian blood volume during ovulation in the gonadotropin-primed immature rat. *Biol. Reprod.*, **40**, 762–768
- Tekay, A. and Jouppila, P. (1996) Intraobserver reproducibility of transvaginal Doppler measurements in uterine and intraovarian arteries in regularly menstruating women *Ultrasound Obstet Gynecol.*, **7**, 129–134
- Tekay, A., Martikainen, H. and Jouppila, P. (1995) Blood flow changes in uterine and ovarian vasculature and predictive value of transvaginal pulsed colour Doppler ultrasonography in an in-vitro fertilization programme. *Hum. Reprod*, **10**, 688–693
- Van Blerkum, J., Davis, P.W. and Lee, J. (1995) ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. *Hum. Reprod*, **10**, 415–424
- Waterstone, J. and Parsons, J.H. (1992) A prospective study to investigate the value of flushing follicles during transvaginal ultrasound directed follicle aspiration *Fertil. Steril*, **57**, 221–223
- Wittmaack, F.M., Kreger, D.O., Blasco, L. *et al.* (1994) Effect of follicular size on oocyte retrieval, fertilization, cleavage and embryo quality in *in vitro* fertilization cycles: a 6 year data collection *Fertil. Steril.*, **62**, 1205–1210
- Zaidi, J., Collins, W., Campbell, S. *et al.* (1996) Blood flow changes in the intra-ovarian arteries during the periovulatory period: relationship to the time of day *Ultrasound Obstet Gynecol.*, **7**, 135–140
- Zeleznik, A.J., Schuler, H.M. and Reichert, L.E. (1981) Gonadotropin-binding sites in the Rhesus monkey ovary: role of the vasculature in the selective distribution of human chorionic gonadotropin to the preovulatory follicle *Endocrinology*, **109**, 356–362

Received on May 16, 1996, accepted on September 23, 1996