Title: The effect of melatonin on ultrasound markers of follicular development: a double-blind placebo-controlled randomised trial

Running title: Melatonin and US for IVF

Authors and Affiliations

Dr Shavi FERNANDO^{1,2,5}
shavif@hotmail.com

Prof Euan Morrison WALLACE^{1,2}
Euan.wallace@monash.edu

Prof Luk ROMBAUTS^{1,4,5}
Luk.rombauts@monash.edu

Ms Nikki WHITE³
Nix789@hotmail.com

Ms Jennifer HONG³
Jennifer.hong@monashultrasound.com.au

A/Prof Beverley VOLLENHOVEN^{1,4,5}
Beverley.vollenhoven@monash.edu

Dr Nicholas LOLATGIS⁴
lolatgis@bigpond.net.au

Dr Nicole HOPE⁴
nicolejhope@hotmail.com

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Dr Melissa WONG⁴
wongliow@gmail.com

Dr Mark LAWRENCE^{4,5}
lawrence@baysidewomenshealth.com.au

Dr Anthony LAWRENCE^{4,5}
tanyaveto@bigpond.com

Dr Chris RUSSELL⁴
c.russell@exemail.com.au

Dr Kenneth LEONG⁴
docleong@yahoo.com

Dr Philip THOMAS^{4,5}
philip@philipthomas.com.au

A/Prof Fabricio DA SILVA COSTA ^{1,3,5}
fabriciouece@hotmail.com

¹The Ritchie Centre, Department of Obstetrics and Gynecology, Monash University, and

²Hudson Institute of Medical Research Clayton, Australia; ³Monash Ultrasound for Women, Clayton, Australia; ⁴Monash IVF, Clayton, Australia; ⁵Monash Women's, Monash Health, Clayton, Australia

Corresponding author

Dr Shavi Fernando
shavif@hotmail.com
<https://orcid.org/0000-0001-9160-0084>

The Ritchie Centre

Department of Obstetrics and Gynecology, Monash University. Level 5, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria, Australia 3168

Ph: +613 95946666

F: +613 85457299

Conflict of interest disclosure statement

SF, EW, BV, NL, NH, MW, ML, AL, PT, KL have nothing to declare.

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Unblinded sentence from Materials and Methods: These participants were recruited from Monash IVF centres in Melbourne, Australia between September 2014 and September 2016.

DR. SHAVI VIDHURA FERNANDO (Orcid ID : 0000-0001-9160-0084)

PROF. BEVERLEY VOLLENHOVEN (Orcid ID : 0000-0002-8756-3994)

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Abstract

Background

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Melatonin is a potent oxygen scavenger and is capable of altering blood flow in various vascular beds.

Aims

We aimed to determine the effect of melatonin on ovarian vascular indices during ovarian stimulation for IVF.

Materials and Methods

A pilot double-blind placebo-controlled randomised trial. Sixty-nine women (mean age 35.8 years $\pm$ 4.3) undergoing their first cycle of IVF were randomised to receive either placebo, 2mg, 4mg or 8mg of melatonin, twice a day. Each participant underwent a transvaginal ultrasound at day 6-10 assessing follicular number and size. The vascularisation index (VI), flow index (FI) and vascularisation-flow index (VFI) were measured. These indices were then correlated with embryological outcomes. Informed consent was obtained from participants. This trial was registered with the ANZCTR (ACTRN12613001317785).

Results

The number of follicles did not differ between groups ($p=0.4$). There were no differences in the VI ($p=0.4$), FI ($p=0.1$) or VFI ($p=0.3$) in the right ovary or the FI ($p=0.3$) or VFI ($p=0.3$) in the left ovary between groups. When comparing placebo to any dose of melatonin, there was no differences in any measured parameter. While there was correlation between the number of follicles on ultrasound and all measured embryological outcomes, there was no correlation between ovarian vascular indices and these important clinical outcomes.

Conclusions

Melatonin does not appear to change ovarian vascular indices during ovarian stimulation. In addition, such vascular indices cannot predict the number or quality of oocytes or embryos obtained in an IVF cycle. These findings require confirmation in future larger studies.

Introduction

Traditionally the ovarian response to hormonal stimulation prior to assisted reproductive technology (ART) has been measured biochemically, by serial oestradiol concentrations, together with standard two-dimensional (2D) ultrasound determination of follicular number and size.¹ Information obtained from these follicular phase ultrasounds are used to make decisions regarding whether to continue the cycle and also when the ideal time is to trigger follicular maturation. Unfortunately, while providing useful insights into ovarian response and follicular number, traditional 2D ultrasound measurements do not provide insights to either follicular or oocyte quality. Such insights would be likely to assist in the prediction of the ultimate success of the assisted reproductive procedure. As ultrasound technology has improved, the usefulness of newer, non-invasive measurement techniques, such as 3-dimensional (3D) power Doppler (PD), have begun to be evaluated.²

Promising results have indicated that follicular power Doppler studies are positively correlated with ovarian response and other markers of ovarian reserve, but not necessarily with clinical pregnancy after IVF/ET,³ and not in women with polycystic ovarian syndrome.

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Others have focussed on the measurement, by 3D PD, of vascularity index (VI), flow index (FI) and vascularisation flow index (VFI),⁵ VI estimates the number of vessels in the measured volume, FI estimates the average flow rates through these vessels by measuring the intensity of the colour output and VFI is an aggregate measure of the two.^{5,6} However, the utility of these measures in predicting ART success is still under-investigated and uncertain.

There has been growing interest in the use of the potent oxygen scavenger, melatonin, as a means of improving IVF success rates. One proposed mechanism of action is that due to its variable effect on human vasculature,^{7,8} melatonin may have a beneficial effect on blood flow to the ovaries. Considering this possibility, a subset of patients recruited for the 'Melatonin in Assisted Reproductive Technologies (MIART) trial'⁹ were used to determine whether the administration of different doses of melatonin can change the blood flow to the ovaries during gonadotrophin stimulation and to assess whether these indices are predictive of oocyte and embryological outcomes in this cohort.

Materials and Methods

A sample of 69 participants was obtained by offering all randomised participants of the MIART trial⁹ inclusion in the ultrasound arm of the study. These participants were recruited from (XXX) between September 2014 and September 2016. Inclusion criteria are outlined in Table 1. These participants were offered additional measurements at their routine Day 6-10 follicular ultrasound scan. Of 160 participants, 84 declined participation in this arm, because of an inability to attend the trial ultrasound scan. Seventy-six were consented and randomised using the minimisation method by the factors age, body mass index (BMI), parity and smoking status.¹⁰ After randomisation, 7 patients withdrew from the MIART trial before commencing the trial medication.

Therefore, 69 participants were available for per protocol analysis: placebo (n=21), 2mg (n=13), 4mg (n=17) and 8mg (n=18) of melatonin twice per day (BD). Of these, 5 patients did not have reliable study scans, but were included in the analysis (Figure 1). All patients took the allocated trial medication twice per day (between 0800 and 1000 and between 2000 and 2200) from Day 2 of their cycle until the night before egg collection.

This study was double-blinded, with allocation known only by the prescribing pharmacy and an independent party. Routine 2D ultrasound with a 6-12MHz transvaginal transducer was used to take grayscale measurements of follicular development at Day 6-10, together with endometrial appearance and thickness, ovarian volume and follicular number >11mm, which were counted manually. In addition, 3D power Doppler was used to measure ovarian VI, FI and VFI.

All measurements were taken by one of two highly trained sonographers using a transvaginal probe on a General Electric Voluson E8 Expert, Software EC250 copyright 2013 General Electric (GE Healthcare Austria GmbH & Co OG, 2013) using Vocal II software (GE healthcare, Austria, 2004) and analysed by a specialist gynaecological sonologist. All women were examined in the lithotomy position after emptying their bladder.

Ovarian volume was determined using the methods described by Makled et al,¹¹ and Jokubkiene et al¹² using Virtual Organ Computer-aided Analysis (VOCAL). In the 3D ultrasound mode with power Doppler, a longitudinal section of the ovary was obtained, and a 3D power Doppler ultrasound data set was obtained. The setting "Quality mid 2" was chosen as the sweep speed for the 3D ultrasound. After recording of each volume, the resultant multiplanar display was examined to ensure that a complete volume of the ovary had been

captured. Volumes were transferred to a server for storage in a database for later analysis. Ovarian vascular indices (VI, FI and VFI) were then determined offline using the 4D-view™ software, version 9.1 (GE Medical Systems, Zipf, Austria) with the virtual organ computer-aided analysis (VOCAL™) software. All calculations were performed on images of the ovary using the midsagittal view as the reference image and recording in the longitudinal, transverse and coronal plane. Based on the findings of Jokubkiene and associates,¹² we chose a rotation step of 30 degrees for all images. The outline of each ovary was traced manually in all planes, and VI, FI and VFI were calculated. Inter- and intra-observer reliability in 3D power Doppler measurements has been previously reported,¹³⁻¹⁵ however, we chose to re-evaluate this in our study.

Tests for intra-observer variation were performed on a subset of 14 (21.7%) participants chosen at random from the sample with the same specialist taking two measurements from the same ovaries in two separate sittings. Separate tests for inter-observer error were also performed for the same participants with another specialist sonographer blinded to the findings of the other sonologist. Each sonologist had over 10 years of experience in scanning.

Embryological outcomes were determined by skilled blinded embryologists. Oocytes were scored for maturity. Embryos were scored as 'good', being a grade of 'A' or 'B'; or 'poor', representing grades of 'C' or 'D'. Clinical pregnancy was defined as the presence of a live intrauterine pregnancy at 7-week ultrasound.

All statistical analyses were performed using SPSS v22 (IBM, Armonk, New York) per protocol with $p < 0.05$ considered to be statistically significant. Baseline demographics were compared for similarity using ANOVA for normally distributed continuous data and Chi square with Fishers exact test where required for categorical data. Vascular indices were compared between groups using Kruskal-Wallis for non-parametric data and ANOVA for normally distributed data. Dose-effects were analysed using chi-square for trend (categorical variables) and Spearman's rank correlation for continuous outcomes. Intraclass correlation coefficients were determined for the intra-observer (One-way random, consistency, single measures) and inter-observer (Two-way mixed, consistency, single measures) analysis. Spearman correlation analysis was performed to assess the relationship between vascular indices and the total number of oocytes, MII oocytes and embryos.

Human research ethics approval for this study was obtained from the Monash Health Human and Research Ethics Committee (Project number: 13402B). All participants provided written informed consent. This trial was prospectively registered with the ANZCTR (ACTRN12613001317785).

Results

Sixty-nine participants were available for per protocol analysis. There was no significant difference in baseline demographics between the groups (Table 2). Fifteen (21.7%) women were at least 40 years or older, and these were evenly distributed across the groups. Eighty-four (52.5%) women who were offered inclusion in this study, declined or were unable to attend for the trial ultrasound scan. There were no differences in baseline characteristics between the women who were and were not included in this study (data not shown).

To assess the reliability and reproducibility of ovarian vascular indices, intra and inter-observer measurements were performed on a random sample of 14 participants. We found moderate to good agreement for all indices for intra-observer analyses (Intraclass correlation (ICC) of 91-97%, $p < 0.001$) (Supplementary Table 1) and a wider range for inter-observer analysis (ICC 84-97%, $p < 0.001$) (Supplementary Table 2).¹⁶

The left sided VI was statistically higher in the 4mg BD group compared with the 8mg BD group (Median (IQR): 6.3 (5.3-10.4) vs 3.6 (1.4-6.0), $p < 0.01$). There were no other statistically significant differences in VI, FI or VFI in either laterality or between any other groups (Table 3).

There were no statistically significant differences in 2D measures of stimulation response, including the total number of follicles, follicles > 11 mm, endometrial thickness or ovarian volume between groups. When combining all melatonin groups and comparing with placebo, there were no significant differences in these parameters (Table 3).

The embryological outcome data of all patients were pooled, and a Spearman correlation analysis was performed assessing oocyte and embryo outcomes. As expected, the total number of follicles on ultrasound was positively correlated with the number of oocytes (Spearman's $\rho = 0.68$, $p < 0.001$, $N = 64$), number of MII oocytes (Spearman's $\rho = 0.28$,

$p < 0.05$, $N = 64$) and number of embryos (Spearman's $\rho = 0.62$, $p < 0.001$, $N = 64$). However, there was no correlation between any ovarian vascular index and total number of oocytes, MII oocytes, fertilised oocytes, embryos or embryos utilised (Table 4).

There were 4 miscarriages in this cohort (6.3%), two in the placebo group and two in the 2mg BD melatonin group. There were 6 clinical pregnancies (9.4%), three in the placebo group, one in the 2mg BD melatonin group and two in the 4mg BD melatonin group.⁹ All clinical pregnancies resulted in live births.⁹

Discussion

In this pilot randomised double-blinded trial, we have shown that increasing oral melatonin doses, at least in the range 2mg-8mg twice daily, have no apparent effect on ovarian blood flow. In addition, we have shown that ovarian vascular indices do not correlate with the number of oocytes or the embryo utilization rate, which is consistent with previous literature.^{17, 18}

Melatonin is a potent oxygen scavenger and has been shown to have effects on the vascular system in both animals and humans. This effect is mediated through stimulation of the melatonin receptors, MT1 and MT2 on the smooth muscle cells of blood vessels.

Interestingly, the effects of melatonin on the vasculature appears to be dose-mediated, however higher doses do not necessarily equate to a greater effect.¹⁹ Vascular smooth muscle MT1 activation has been shown to result in vasoconstriction through its effects on noradrenaline pathways²⁰, and the stimulation of MT2 has been shown to result in vasodilation.^{19, 21} Vasodilation can also occur without melatonin receptors being involved.²²

This direct action of melatonin on vasculature together with the difference in effect that occurs as a result of the activation of melatonin receptors might, at least in part, account for the variable effect on the human vasculature that has also been demonstrated.⁷ It is, therefore, conceivable that different vascular beds contain a variable distribution of MT1 and MT2 receptors which might account for the variation in response to melatonin.

Ovarian blood flow changes during the menstrual cycle, with improvements in flow being observed from the day before ovulation.²³ Links have previously been drawn between high ovarian follicular blood flow and improved follicular development,²⁴ with some suggestion that pregnancy rates are improved with well-perfused follicles.^{25, 26} Very high ovarian peak

systolic velocity (PSV) and very low PSV are associated with better and worse success rates respectively.²⁷

Some investigators have found that VI, FI and VFI are not able to predict the difference between 'poor ovarian responders' and 'normal' responders to ovarian stimulation.²⁸ Engels et al performed a study of 79 intrauterine insemination (IUI) cycles measuring these ovarian blood flow indices. They found a significantly lower sub follicular VI and VFI in women who became pregnant in that cycle.⁶ They argue that while this seems counterintuitive, it is in agreement with another study which investigated unstimulated cycles,²⁹ and may indicate an inversely proportional relationship between follicular blood flow and follicular response, whereby a reduction in vascularisation might improve the response to ovarian stimulation.⁶ We did not find this in our study, where ovarian vascular indices did not correlate with the number of follicles.

While our study was not powered to detect the effects of blood flow differences in relation to pregnancy rates, we did show that ovarian indices did not change with melatonin dose. This suggests that while melatonin may have an influence on the vascular system in other body systems, this does not apply to stimulated ovaries, even when using doses four times the currently accepted 'standard' dose.³⁰ In addition, we have shown that these ovarian vascular indices are not predictive of oocyte or embryo outcomes in our cohort.

While we did not find any change in blood flow or vascularisation in the ovaries, the study lacks power to detect very small differences in ovarian vascular indices. However, the clinical significance of such small differences is uncertain. Furthermore, the sample of patients used to compare reliability of ovarian vascular indices was limited to 14, and only two observers were tested. This may result in inaccuracies in reporting of reproducibility of measurements and cannot account for larger variations in investigators.

In addition, just over half of the women who were recruited for the main study were not able to attend for a trial ultrasound scan, and therefore could not be included in this parallel study. While this could result in selection bias, there were no differences between this patient group and those that were included in the study.

In this pilot double-blind placebo controlled randomised study, we have shown that melatonin does not appear to affect ovarian flow indices. In addition, we have shown that

these indices do not correlate with oocyte number or the embryo utilization rate. This suggests that the ovarian vascular indices of VI, FI and VFI do not, in their current state, provide useful clinical information predicting the success of an IVF cycle. However, as this study was small and lacked power, these findings require further investigation in large randomised controlled trials.

Figure legends

Figure 1: Recruitment flowchart

BD: twice per day; IVF: in-vitro fertilization; MIART: Melatonin in Assisted Reproductive Technology trial; mg: milligrams

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Table 1: Eligibility criteria

IVF: in vitro fertilization; ICSI: intra-cytoplasmic sperm injection; GnRH: gonadotrophin releasing hormone;
OCP: oral contraceptive pill

Inclusion criteria	Undergoing first cycle of IVF or ICSI Aged between 18 and 45 Body mass index (BMI) between 18 and 35 Undergoing a GnRH antagonist cycle (without OCP or Provera scheduling)
Exclusion criteria	Current untreated pelvic pathology – moderate to severe endometriosis, submucosal uterine fibroids/polyps assessed by the treating specialist to affect fertility, pelvic inflammatory disease, uterine malformations, Asherman's syndrome and hydrosalpinx. Currently enrolled in another interventional clinical trial Concurrent use of other adjuvant therapies (e.g. Chinese herbs, Coenzyme-Q10, acupuncture) Current pregnancy Malignancy or another contraindication to IVF Autoimmune disorders Undergoing preimplantation genetic diagnosis (PGD) Hypersensitivity to melatonin or its metabolites Concurrent use of any of the following medications Fluvoxamine (e.g. Luvox, Movox, Voxam) Cimetidine (e.g. Magicul, Tagamet) Quinolones and other CYP1A2 inhibitors (Ciprofloxacin, Avalox) Carbamazepine (e.g. Tegretol), rifampicin (eg.Rifadin) and other CYP1A2 inducers Zolpidem (e.g. Stilnox), zopiclone (e.g. Imovane) and other non-benzodiazepine hypnotics Inability to comply with trial protocol

Table 2: Baseline characteristics

BMI: body mass index; ART: assisted reproductive technology; IVF: in vitro fertilization;
ICSI: intra-cytoplasmic sperm injection

	Placebo BD N=21	Melatonin 2mg BD N=13	Melatonin 4mg BD N=17	Melatonin 8mg BD N=18	Any melatonin N=48
Age Mean(SD)	34.5 (4.5)	35.2 (4.5)	36.9 (3.7)	36.7 (4.6)	36.4 (4.2)
BMI Mean (SD)	24.0 (4.7)	24.5 (4.6)	25.9 (4.4)	24.0 (4.3)	24.8 (4.4)
Parity N (%)					
0	18 (85.7)	11 (84.6)	13 (76.5)	16 (88.9)	40 (83.3)
≥1	3 (14.3)	2 (15.4)	4 (23.5)	2 (11.1)	8 (16.7)
Type of ART					
IVF, n (%)	8 (38.1)	7 (53.8)	7 (41.2)	9 (50.0)	23 (47.9)
ICSI, n (%)	13 (61.9)	6 (46.2)	10 (58.8)	8 (44.4)	24 (50.0)
Both IVF and ICSI	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)	1 (2.1)
Smoker	2 (9.5)	0 (0.0)	0 (0.0)	1 (5.6)	1 (2.1)
Aetiology					
Endometriosis	3 (14.3)	1 (7.7)	1 (5.9)	3 (16.7)	5 (10.4)
PCOS	1 (4.8)	0 (0.0)	1 (5.9)	1 (5.6)	2 (4.2)
Tubal	3 (14.3)	3 (23.1)	2 (11.8)	3 (16.7)	8 (16.7)
Male factor	4 (19.0)	4 (30.8)	5 (29.4)	5 (27.8)	14 (29.2)
Idiopathic	11 (52.4)	6 (46.2)	10 (58.8)	8 (44.4)	24 (50.0)
Day of scan					
6-7	0 (0.0)	0 (0.0)	2 (11.8)	2 (11.1)	4 (8.5)
8-10	21 (100.0)	13 (100.0)	15 (88.2)	16 (88.9)	44 (91.7)

Table 3: Ultrasound parameters including vascular indices

*Kruskal Wallis; [†]ANOVA – these variables are normally distributed

[‡]Fishers exact; [§] Spearman correlation; ^{||}Mann-Whitney U; VI: vascularity index; FI: flow index; VFI: vascularity flow index; ET: endometrial thickness

	Placebo BD N=21	Melatonin 2mg BD N=13	Melatonin 4mg BD N=17	Melatonin 8mg BD N=18	P value between groups	P value for trend[§]	Any Melatonin N=43	P value
Left VI (Median (IQR))	4.8 (3.6- 8.3)	4.5 (3.4- 5.8)	6.3 (5.3- 10.4)	3.6 (1.4-6.0)	0.03 [*]	0.2	4.6 (2.8- 7.6)	0.5
Left FI	33.8 (32.2- 36.6)	36.7 (32.0- 40.4)	34.5 (33.1- 37.1)	33.5 (29.1- 36.3)	0.3 [*]	0.6	34.5 (31.6- 37.9)	0.6
Left VFI	1.7 (1.2- 3.1)	1.6 (1.2- 2.4)	2.1 (1.8- 3.9)	1.8 (0.6-2.6)	0.3 [*]	0.7	1.8 (1.0- 2.8)	0.9
Right VI	5.6 (3.2- 7.8)	4.5 (3.1- 6.3)	4.2 (2.9- 5.4)	5.3 (2.8-6.9)	0.4 [*]	0.3	5.0 (3.0- 6.1)	0.1
Right FI	34.6 (31.6- 36.9)	34.8 (30.5- 38.5)	32.1 (29.1- 34.9)	32.7 (30.1- 34.4)	0.1 [*]	0.04	32.7 (30.3- 35.6)	0.1
Right VFI	2.0 (1.1- 2.9)	1.7 (1.1- 3.9)	1.3 (0.9- 1.9)	1.9 (1.0-2.3)	0.3 [*]	0.4	1.7 (1.0- 2.3)	0.3
Median total follicles (Left plus Right)>11mm (IQR)	6.0 (4.0- 10.5)	6.0 (1.5- 9.0)	5.0 (2.0- 8.0)	6.0 (1.0-8.0)	0.9 [*]	0.4	6.0 (2.0- 8.0)	0.4
Median follicles on left >11mm	3.0 (2.0- 3.0)	2.0 (1.0- 4.0)	2.0 (0.5- 4.0)	2.0 (0.5-4.0)	0.6 [*]	0.2	2.0 (1.0- 4.0)	0.2

(IQR)								
11-14mm	2.0 (1.0-3.0)	2.0 (0.0-3.0)	1.0 (0.0-2.5)	1.0 (0.0-2.5)	0.6 [*]	0.2	1.0 (0.0-3.0)	0.2
15-17mm	1.0 (0.0-1.0)	0.0 (0.0-1.0)	0.0 (0.0-1.5)	0.0 (0.0-1.0)	0.8 [*]	0.5	0.0 (0.0-1.0)	0.4
>18mm	0.0 (0.0-0.5)	0.0 (0.0-1.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.5 [*]	0.3	0.0 (0.0-0.0)	0.6
Median follicles on right >11mm (IQR)	4.0 (1.0-6.0)	2.0 (0.5-5.5)	2.0 (2.0-4.5)	2.0 (1.0-5.5)	0.9 [*]	0.5	2.0 (1.0-5.0)	0.6
11-14mm	2.0 (1.0-4.0)	1.0 (0.0-3.5)	2.0 (1.0-3.0)	1.0 (0.0-2.5)	0.4 [*]	0.2	1.0 (0.0-3.0)	0.2
15-17mm	0.0 (0.0-1.0)	1.0 (0.0-2.0)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.5 [*]	0.9	0.0 (0.0-1.0)	0.6
>18mm	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.9 [*]	0.8	0.0 (0.0-1.0)	0.7
ET Mean (SD) †	9.1 (2.8)	8.9 (2.1)	8.6 (2.6)	8.9 (2.6)	0.9	0.8	8.8 (2.4)	0.6
Endometrial appearance[‡]								
Non-specific, N(%)	2 (9.5)	1 (7.7)	1 (5.9)	1 (5.6)	1.0	0.7	3 (6.3)	1.0
Proliferative, N(%)	19 (90.5)	12 (92.3)	16 (94.1)	17 (94.4)			45 (93.8)	
Median left ovarian volume (IQR)	19.3 (10.4-21.6)	18.2 (9.3-24.0)	10.4 (6.9-16.6)	11.7 (7.6-18.4)	0.3 [*]	0.1	12.0 (7.7-22.2)	0.3
Median right ovarian	17.8 (11.4-	20.4 (9.5-27.2)	11.1 (6.4-27.7)	13.1 (8.7-23.7)	0.3 [*]	0.1	14.4 (8.1-23.7)	0.5

volume (IQR)	29.4)							
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Table 4: Correlation between ovarian vascular indices and oocyte and embryo outcomes: all groups combined

*Spearman correlation coefficient, p value; IQR: inter-quartile range; RVI: right vascularity index; RFI: right flow index; RVFI: right vascularity-flow index; LVI: left vascularity index; LFI: left flow index; LVFI: left vascularity-flow index

	Total follicles	Total oocytes retrieved	MII oocytes	Fertilised oocytes	Total embryos	Poor quality embryos	Good quality embryos	Utilised embryos
Median (IQR)	5.5 (2.0-8.8)	8.0 (3.0-14.0)	5.0 (2.0-7.0)	3.0 (0.0-6.0)	3.0 (0.25-6.0)	0.5 (0.0-2.0)	2.0 (0.0-3.8)	1.0 (0.0-2.8)
RVI (rho, p)*	0.02, 0.86	0.09, 0.46	0.01, 0.99	-0.08, 0.56	-0.09, 0.50	-0.02, 0.89	-0.02, 0.87	-0.12, 0.36
RFI (rho, p)*	-0.05, 0.69	0.03, 0.84	0.03, 0.79	0.09, 0.49	0.07, 0.59	0.15, 0.23	0.06, 0.62	0.06, 0.65
RVFI (rho, p)*	-0.16, 0.22	-0.06, 0.67	-0.04, 0.76	-0.19, 0.14	-0.18, 0.16	-0.02, 0.87	-0.14, 0.27	-0.24, 0.06
LVI (rho, p)*	0.12, 0.35	0.15, 0.24	-0.06, 0.66	0.11, 0.41	0.08, 0.56	0.05, 0.71	0.07, 0.57	-0.04, 0.79
LFI (rho, p)*	0.04, 0.74	-0.02, 0.88	0.04, 0.74	0.06, 0.66	0.04, 0.75	0.06, 0.64	0.01, 0.95	-0.04, 0.74
LVFI (rho, p)*	0.04, 0.79	0.03, 0.83	0.04, 0.79	-0.02, 0.91	-0.06, 0.66	-0.04, 0.77	-0.05, 0.67	-0.17, 0.19
Total follicles (rho, p)*	-	0.68, <0.001	0.28, 0.03	0.63, <0.001	0.76, <0.001	0.42, <0.001	0.54, <0.001	0.51, <0.001

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Author/s:

Fernando, S;Wallace, EM;Rombauts, L;White, N;Hong, J;Vollenhoven, B;Lolatgis, N;Hope, N;Wong, M;Lawrence, M;Lawrence, A;Russell, C;Leong, K;Thomas, P;da Silva Costa, F

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