

# Ovarian reserve testing and uterine blood flow assessment using two-dimensional and three-dimensional Doppler in patients with unexplained recurrent miscarriage

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## Background and aim

The aim of this study was to evaluate ovarian reserve, uterine artery blood flow, and endometrial and subendometrial blood flow in women with unexplained recurrent miscarriage compared with normal fertile women.

## Patients and methods

The study design was a case–control one. The study was conducted in the Department of Obstetrics and Gynaecology, Benha University, Benha, Egypt. Women were divided into two groups: those with a history of unexplained recurrent miscarriage (the study group,  $n=50$ ), and those who had no history of miscarriage and had at least one child born at term (the control group,  $n=50$ ). At days 3–4 of the cycle, ovarian reserve testing was performed for all women by measuring follicle-stimulating hormone, luteinizing hormone and oestradiol levels. Thereafter, two-dimensional (2D) and 3D transvaginal ultrasonography and power Doppler were performed to detect antral follicle count, ovarian volume, and ovarian stromal Vascularization Index (VI), Flow Index (FI) and Vascularization Flow Index (VFI). Between days 18 and 23 of the cycle, transvaginal ultrasonography was performed for all women using 2D, pulsed Doppler and 3D-power Doppler mode to detect endometrial thickness, Uterine Artery Pulsatility Index, endometrial volume and endometrial and subendometrial blood flow presented by the indices VI, FI and VFI. The indices between the two groups were compared.

## Results

Uterine Artery Pulsatility Index was significantly higher in the study group ( $P=0.001$ ), whereas endometrial VI ( $P\leq 0.001$ ), FI ( $P=0.01$ ) and VFI ( $P\leq 0.001$ ) and subendometrial VI, FI and VFI ( $P\leq 0.001$ ) were significantly lower in the study group. The ovarian reserve tests, endometrial thickness and endometrial volume, however, were not significantly different between the two groups.

## Conclusion

The presence of good uterine and endometrial blood flow is an important prerequisite for successful implantation and continuation of pregnancy as shown by higher uterine artery blood flow resistance and lower endometrial and subendometrial blood flow in recurrent unexplained miscarriage cases. We could not find any cutoff values that could predict the occurrence of miscarriage, and hence larger prospective studies are needed to confirm such results and reaching values that can accurately predict the occurrence of miscarriage.

## Keywords:

ovarian reserve, recurrent miscarriage, subendometrial flow, uterine artery Doppler, uterine receptivity

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## Introduction

Recurrent pregnancy loss (RPL) is a loss of three or more spontaneous and consecutive pregnancies at less than 20 weeks of gestation and foetal weight less than 500 g [1]. RPL affects 0.5–3% of women in the reproductive age group [2,3]. The causes of RPL are classified as genetic, anatomic, endocrinal, immunological, microbiological, and environmental [4].

Despite thorough investigation according to various clinic protocols, the underlying cause remains unexplained in about 70% of cases [1].

The process of female reproductive ageing is attributed to a decrease in both oocyte quantity and quality, eventually resulting in menopause [5]. The main acknowledged manifestation of decreased quality is the occurrence of chromosomal abnormalities in the oocyte, leading to aneuploidy in the conceptus, which

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has been established as the reason of pregnancy loss in 35–75% of all cases [6].

The quality of a woman's oocytes cannot be assessed clinically, but the quantity of the remaining follicle pool can be estimated using so-called ovarian reserve tests. Various studies have suggested that the quantitative ovarian reserve is predictive for the chance of miscarriage. An elevated level of basal follicle-stimulating hormone (FSH) and oestradiol (E2), low level of anti-Mullerian hormone and low antral follicle count (AFC) have been described to be related to increased miscarriage rates [7–9]. Moreover, a high incidence of decreased ovarian reserve has been found among women with unexplained RPL [10,11].

Although female fertility declines with age, it is difficult to predict the pace of reproductive decline in an individual woman [12]. Decreased ovarian reserve is distinct from menopause or premature ovarian failure [13].

Although ovarian reserve diminishes with age, young women with RPL may have diminished ovarian reserve [11]. It was demonstrated that serum FSH increases several years before elevations in serum luteinizing hormone (LH), and, as a result, the first intimation of a diminished ovarian reserve may be an elevated FSH/LH ratio [14]. Basal FSH, E2 levels and FSH/LH ratio may indirectly reflect the poor quality and quantity of the oocytes [7]. A variety of ultrasound parameters, including ovarian volume [15], antral follicle number [16] and ovarian stromal blood flow [17], have been suggested as independent predictors of ovarian reserve. The above studies are challenged by a number of comparable studies that do not demonstrate such a relation [18,19].

Several investigators have postulated the existence of a correlation between the degree of endometrial development evaluated using ultrasound and the probability of embryo implantation [20,21]. The hemodynamic study of the uterus and ovaries during the menstrual cycle of fertile [22] and infertile [23] women has been facilitated by the introduction of Doppler scanner. Among potential uterine predictors for implantation measurable using ultrasound are endometrial thickness and volume, endometrial pattern, and blood flow in the uterine and subendometrial arteries [24].

Uterine receptivity is likely to be regulated by a number of factors, including uterine perfusion, and is of great importance in achieving a normal pregnancy [25,26].

In fact, during the normal menstrual cycle, the impedance to uterine artery blood flow diminishes progressively during the luteal phase, reaching the lowest values in the period that temporally coincides with the implantation window [22,27,28].

Many published reports show that high blood flow resistance is associated with reduced conception and that women with lower Pulsatility Index (PI) values have the highest possibility of becoming pregnant [29–33]. However, this is not a universally held opinion, as many investigators have not been able to document an association between abnormal uterine perfusion and pregnancy complications [34].

Power Doppler ultrasound has the advantage of being sensitive to low flow, and thus overcomes the angle dependence and aliasing of standard colour Doppler. The implementation of three-dimensional (3D) display permits the physician to see the dimensions on the screen interactively, rather than mentally assembling the sectional images. Thus, the 3D-power Doppler system may enable physicians to study the region of interest in a more detailed manner [35].

With the advent of 3D-power Doppler sonography, quantitative assessment of vessel density and perfusion within the subendometrial area has become possible, which may give an idea about the interaction between overall blood supply in the subendometrial area and pregnancy rate and complications. The 3D ultrasound with power Doppler provides a unique tool to examine the blood supply towards the whole endometrium and the subendometrial region [36–42].

The aim of the present study was to evaluate the potential role of ovarian reserve, uterine artery blood flow and endometrial and subendometrial blood flow in women with unexplained RPL compared with normal fertile women.

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### Patients and methods

The study was conducted in the Obstetrics and Gynaecology Department in Benha University Hospital during the period from October 2013 to December 2016. The study was approved by the Research Ethical Committee. All participants signed an informed consent form. The participants were divided into two groups: those with a history of recurrent unexplained miscarriage (study group,  $n=50$ ), and those who had no history of miscarriages

and had at least one child born at term (control group,  $n=50$ ).

The inclusion criteria for the study group were as follows: not being pregnant, having had regular menstrual cycles in the 3 months preceding enrolment; using neither hormonal contraception nor an intrauterine device; having a history of three or more unexplained miscarriages in the first trimester of pregnancy fathered by the same partner; and having no living child.

Autoimmune and endocrine disorders were ruled out in women with unexplained RPL, and thorough uterine evaluation revealed nothing abnormal. In this group titres were less than 20 G phospholipids (GPL) for IgG anticardiolipin antibody and less than 20 M phospholipids (MPL) for IgM anticardiolipin antibody; values for activated partial thromboplastin time were normal, and no antinuclear antibodies were detected. The endocrine evaluation consisted of measuring thyroid-stimulating hormone, free thyroxin and progesterone level on day 21 of the menstrual cycle, and the results were normal. Results of glucose tolerance test were also normal. Hysterosalpingography revealed no congenital anomalies, masses or adhesions within the uterine cavity and patent fallopian tubes, and transvaginal ultrasonography revealed adequate secretory endometrium within the luteal phase. Both parents had normal karyotyping.

The inclusion criteria for the control group were as follows: not being pregnant, having had regular menstrual cycles in the 3 months preceding enrolment; using neither hormonal contraception nor an intrauterine device, having normal obstetric history with no miscarriages, and at least one child born at term.

Exclusion criteria for both groups were having uterine alterations on transvaginal ultrasonography, systemic diseases that may affect haemodynamic indices, and a history of oophorectomy or tubal ligation.

After full history taking and examination, all women were evaluated on days 3–4 for FSH, LH and E2 levels. Hormone analysis of serum FSH, LH and E2 levels was performed using commercial chemiluminescent enzyme immunoassay (Immulate; Diagnostic Product Corporation, 5700 West 96th Street Los Angeles, CA, United States). Thereafter, ultrasound assessment of the number of antral follicles (follicles measuring 2–10 mm) was carried out. A single acquisition was obtained for each ovary and the average was calculated. Finally, the 3D mode and power Doppler mode was

activated; subsequently, the application of a region of interest over the ovary was adjusted. An automated mechanical sweep of this region through 90° was undertaken using the slow sweep mode, and the resultant multiplanar display was examined to ensure that the entire ovary had been captured. Ovarian volume and ovarian stromal vascularity were detected using Virtual Organ Computer-aided Analysis (VOCAL; GE Healthcare, GE Kretz, Zipf, Austria). During the analysis and calculation, the manual mode of the VOCAL Contour Editor was used to cover the whole 3D volume of the ovary with a 15° rotation step. A single acquisition was obtained for each ovary and the average was calculated. Quantification of power Doppler information within the resultant 3D ovarian model was performed using 'histogram facility'. Three indices of vascularity were generated: the Vascularization Index (VI), the Flow Index (FI) and the Vascularization Flow Index (VFI).

Between days 18 and 23, an initial 2D ultrasound assessment was carried out to detect endometrial thickness, and then pulsed colour Doppler assessment of the right uterine artery, identified at the lateral border of the uterine isthmus and repeated for the left side, and the average PI of the bilateral uterine arteries were calculated. Finally, 3D mode and power Doppler mode were activated; the resulting truncated sector covering the endometrial cavity in a longitudinal plane of the uterus was adjusted and moved, and the sweep angle was set to 90° to ensure that a complete uterine volume encompassing the entire subendometrium was obtained. The patient and the 3D transvaginal probe remained as still as possible during the volume acquisition. The multiplanar display resulting from the acquisition was examined to ensure that a complete volume of the uterine corpus had been captured. Endometrial volume and three power Doppler indices were calculated using VOCAL software (GE Healthcare). During the analysis and calculation, the manual mode of the VOCAL Contour Editor was used to cover the whole 3D volume of the endometrium with a 15° rotation step. The flow indices that were calculated were as follows: VI, FI and VFI.

Following the assessment of the endometrium itself, the subendometrium was examined through the application of 'shell imaging', which allows the user to generate a variable contour that parallels the originally defined surface contour. In the present study, the sub-endometrial region was considered to be within 5 mm of the originally defined myometrial–endometrial

contour. The VI, FI and VFI of the subendometrial region were obtained accordingly.

All examinations were performed with the scanner (Voluson 730 Pro V; GE Medical Systems, Milwaukee, Wisconsin, USA) with 7.5 MHz endocavitary transducer with pulsed colour Doppler and 3D facility.

### Statistical analysis

Quantitative variables were expressed as mean±SD and comparisons were made using the *t*-test for independent samples if the samples were normally distributed or using the Mann–Whitney test otherwise. The significance level was 0.05 and *P*-values less than 0.05 were considered significant. Binary logistic regression analysis model has been performed to predict a categorical variable from a set of predictor variables.

## Results

The mean age, menstrual cycle length and BMI were 28.4±3.5 years, 30.3±8.4 days and 24.5±1.2 kg/m<sup>2</sup> in the study group and 28.6±3.4 years, 31.1±9.7 days and 24.7±1.1 kg/m<sup>2</sup> in the control group, respectively, but the difference was not statistically significant (*P*=0.6, 0.7 and 0.3).

The mean gestational age at losses in the study group was 7 (5.2–11.8) weeks.

There were no statistically significant differences as regards the basal FSH level, basal LH level, FSH/LH ratio and basal E2 level between the two groups (*P*=0.09, 0.6, 0.4 and 0.3, respectively). Similarly,

there was no significant difference between the two groups as regards the AFC, ovarian volume and ovarian stromal VI, FI and VFI (*P*=0.1, 0.6, 0.1, 0.2 and 0.1, respectively) (Table 1).

There were no statistically significant differences as regards the endometrial thickness and endometrial volume between the two groups (*P*=0.8 and 0.2) (Table 2).

There were highly statistically significant differences between the two groups.

Uterine Artery PI was significantly higher in the study group (*P*=0.001), whereas endometrial VI (<0.001), FI (0.01) and VFI (<0.001) were significantly lower in the study group. Similarly, subendometrial VI (<0.001), FI (<0.001) and VFI (<0.001) were significantly lower in the study group (Table 3).

## Discussion

This study investigated the potential role of ovarian reserve in women with a history of unexplained RPL. There is insufficient evidence to recommend that any ovarian reserve test now available should be used as a sole criterion [12]. Because no single measure of ovarian reserve has a 100% sensitivity and specificity, biochemical and imaging measures should be combined to improve test characteristics [43]. We found that the levels of FSH, LH, FSH/LH ratio, E2 and AFC, ovarian volume and ovarian stromal VI, FI and VFI at days 3–4 were not significantly different between women with unexplained RPL and their healthy counterparts. This finding is in agreement with

**Table 1 Comparison of antral follicle count, ovarian volume and ovarian stromal Vascularization Index, Flow Index and Vascularization Flow Index in the two groups (n=100)**

	Study group (M±SD)	Control group (M±SD)	<i>P</i> -value
FSH (IU/l)	7.03±1.1	7.7±1.4	0.09
LH (IU/l)	5.02±1.4	5.2±1	0.6
FSH/LH ratio	1.4±0.5	1.3±0.3	0.4
E2 (pg/ml)	54.8±30.8	45.5±24.2	0.3
AFC (n)	6.5±2.8	7.5±3.2	0.1
Ovarian volume (cm <sup>3</sup> )	5.7±2.4	5.6±2.3	0.6
Ovarian VI (%)	3.3±1.1	3.4±0.9	0.1
Ovarian FI (0–100)	35.5±3.5	34.6±2.9	0.2
Ovarian VFI (0–100)	1.3±0.5	1.5±0.3	0.1

AFC, antral follicle count; E2, oestradiol; FI, Flow Index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; VFI, Vascularization Flow Index; VI, Vascularization Index.

**Table 2 Comparison of endometrial thickness and volume in the two groups (n=100)**

	Study group (M±SD)	Control group (M±SD)	<i>P</i> -value
Endometrial thickness (mm)	9.8±1.2	9.6±1.6	0.8
Endometrial volume (ml)	4.52±0.5	4.47±0.8	0.2

**Table 3 Comparison of Uterine Artery Pulsatility Index and endometrial and subendometrial Vascularization Index, Flow Index and Vascularization Flow Index in the two groups (n=100)**

	Study group (M±SD)	Control group (M±SD)	P-value
UAPI	2.6±0.3	1.6±0.2	0.001
Endometrial VI (%)	0.1±0.02	0.3±0.01	<0.001
Endometrial FI (0–100)	20.6±10.5	26.7±12.6	0.01
Endometrial VFI (0–100)	0.02±0.01	0.05±0.01	<0.001
Subendometrial VI (%)	1.1±0.6	1.7±0.8	<0.001
Subendometrial FI (0–100)	24.2±18.2	31.9±19.1	<0.001
Subendometrial VFI (0–100)	0.1±0.09	0.3±0.1	<0.001

FI, Flow Index; VFI, Vascularization Flow Index; UAPI, Uterine Artery Pulsatility Index; VI, Vascularization Index.

previous studies conducted on patients with RPL [19,44,45].

Despite that, there are studies that reported a significantly decreased ovarian reserve in women with RPL than in healthy controls [10,11].

Elevated uterine arterial impedance is associated with RPL [32,33]. Moreover, on the basis of studies from in-vitro fertilization-embryo transfer programs, impedance of blood flow through the uterine arteries is an indicator of the probability of subsequent pregnancy outcome [30]. In the present study uterine artery PI was measured in the luteal phase of spontaneous cycles. It was found that uterine artery PI was significantly higher in the recurrent miscarriage group compared with the control group. This finding is in agreement with previous studies [32,33].

The study showed no statistically significant differences in the mid-luteal endometrial thickness and endometrial volume between the two groups, a finding that is in agreement with previous studies [33,45–47].

Although the relationship between the 3D-power Doppler angiography indices to true flow is still under investigation, they have been accepted as representative of vascularity and flow intensity in the region of interest [48,49].

Several studies have been conducted to evaluate the role of endometrial and subendometrial vascularity measured using 3D Doppler ultrasound in the prediction of live birth following in-vitro fertilization cycles [50,51]. On the basis of these findings the authors postulated that a better endometrial and subendometrial vascularity can lead to better placental development during pregnancy, which is associated with a low risk for miscarriage and a higher chance of live birth following assisted reproductive technology (ART) [50–52].

This study demonstrated that patients with unexplained recurrent miscarriage had significantly reduced endometrial–subendometrial VI, FI and VFI compared with the healthy fertile women. This result suggests that endometrial–subendometrial vascularity is affected adversely in patients with unexplained recurrent miscarriage during the peri-implantation period and may negatively influence early pregnancy maintenance. Furthermore, it was found that 3D-power Doppler angiography could be useful in developing therapy strategies and evaluating management effects for those patients with unexplained recurrent miscarriage. Similar results were reported by other previous studies [45,47,50,53,54].

In conclusion, this study observed that ovarian reserve test results were not significantly lower in women with unexplained RPL compared with healthy controls. However, the presence of good uterine artery and endometrial and subendometrial blood flow is an important prerequisite for successful implantation as shown by higher uterine artery blood flow resistance and lower endometrial and subendometrial blood flow in those patients with unexplained RPL. Despite these findings we could not find any cutoff values that could predict the occurrence of miscarriage. That may be attributed to small sample size and short period of the study, and hence larger prospective studies are needed aiming to confirm such results and reaching values that can accurately predict such cases.

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Nil.

#### Conflicts of interest

There are no conflicts of interest.

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