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Endometrial and Subendometrial Vessels in Cases of Unexplained Infertility (Case Control Study)

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Abstract

Background: Infertility defined as failure to conceive after 12 months of unprotected regular intercourse. Unexplained infertility describes couples with infertility in whom standard investigations including semen analysis, tests of ovulation, and tubal patency have no gross abnormality.

Aim of Study: To evaluate the endometrial and subendometrial blood flow in mid-luteal phase after measuring serum progesterone level in unexplained infertility patients and compare those parameters with fertile women in order to reveal the role of the uterine perfusion in unexplained infertility.

Patients & Methods: In this study, we collected 100 participants presented to the outpatient clinic in Kasr El-Aini maternity hospital, we classify them into two groups: Group A: 50 women presented with unexplained infertility either primary or secondary (the case group). Group B: 50 women who had no history of infertility and at least have one child who came for any gynecological procedure (the control group) Primary outcome measures (VI) (FI) & (VFI) of endometrial and subendmetrial areas using (VOCAL) in mid luteal phase after measurement of serum progesterone level in both groups.

Results: As regards endometrial vascularity index (VI), it was higher in control group (0.64±0.05) than in case group (0.51±0.09), Sub endometrial (VI) also was higher in control group (2.34±0.13) than in case group (1.92±0.22), endometrial flow index (FI), it was higher in control group (29.26± 1.98) than in case group (24.47±4.19), sub endometrial flow index (FI), it was higher in control group (36.29±3.10) than in case group (30.52±2.52), endometrial vascular flow index (VFI), it was higher in control Group (0.36±0.05) than in case group (0.25±0.04) & subendometrial vascular flow index (VFI), it was higher in control group (1.13±0.25) than in case group (0.83±0.07) all of that are statistically significant. As regards to serum progesterone level it was higher in control group (12.87±2.47) than in case group (12.66±3.17) which was statistically insignificant.

Conclusion: From our study we found that impairment of uterine perfusion could have a role in cases of unexplained infertility.

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Key Words: *Unexplained infertility – Endometrial blood flow by VOCAL – Subendometrial blood flow by VOCAL).*

Introduction

INFERTILITY is considered when failure to conceive after one year of unprotected regular intercourse. 17% of the couples may complain of infertility, with 40% the female pathology is the cause [1].

Infertility is classified into primary, in women who have never get pregnant, and secondary, in women who have at least one pregnancy. This review will deal with unexplained infertility which represents 10% to 20% of infertility cases [2].

Unexplained infertility is a term used to describe couples with no abnormality revealed with the standered investigations.

Whole investigations including semen analysis, tests of ovulation, and tubal patency have failed to detect any gross abnormality [3].

Uterine perfusion one of the factors that regulate endometrial receptivity [4].

Abnormal uterine perfusion may be a contributing factor to the pathology of infertility especially in couples with unexplained infertility. Pulsed Doppler is an excellent tool for examining the female reproductive system. It is a non-invasive method to assess the uterine perfusion [5].

It has been proved that 3-D ultrasound is a highly reproducible technique. With 3-D ultrasound, a volume of a region of interest can be acquired and stored. 3-D ultrasound, allows for a whole assessment of relevant vessels and quantitative

assessment of vessel density and perfusion within a specified area. A whole evaluation is then possible for endometrial and subendometrial vascularization [6].

Transvaginal ultrasonography with colour Doppler imaging and pulsed Doppler spectral analysis have been used to evaluate the endometrial and subendometrial vascularization through detecting vascularization index (VI), flow index (FI), and vascularization flow index (VFI).

The aim of this study is to evaluate the mid-luteal phase serum progesterone level with evaluation of endometrial and subendometrial blood flow in unexplained infertility patients and compare those parameters with fertile females in order to reveal the possible role of the uterine perfusion in the infertility etiopathogenesis.

Patients and Methods

The study was performed during the period of two years from (June 2013) till (2015) we had 100 participants presented to the Outpatient Clinic in Kasr El-Aini Maternity Hospital; we classified them into two groups:

- *Group A:* 50 participants presented to infertility clinic with unexplained infertility either primary or secondary (the case group).
- *Group B:* 50 participants presented to gynecological clinic without any history of infertility and at least had one child who came for gynecological procedure (the control group).

At the time of initial recruitment, the purpose of the study was adequately explained to each participant. Consent was taken complete history was taken and serum progesterone level was sampled at mid luteal phase then transvaginal power Doppler ultrasonography was performed to all patients in luteal phase (day 21-22) of cycle to detect blood flow in endometrial and subendometrial area presented by the indices vascularization index (VI), flow index (FI), and vascular flow index (VFI). The indices between the two groups were compared.

Inclusion criteria for the infertility group:

- Age between 18-40 years old.
- Infertility either primary or secondary and the duration of infertility.
- *Investigations were done:*
  - Semen analysis to exclude male factor.
  - Hormonal profile (FSH, LH, E2 & prolactin).
  - HSG 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.

Inclusion Criteria for the control group:

- Age between 18-40 years old.
- Parous women in mid luteal phase.
- No hormonal contraception.
- No IUCD in place.

Exclusion criteria for both groups:

- Infertility due to ovarian factor.
- Infertility due to tubal disease.
- Infertility due to uterine or cervical cause.
- Infertility due to male factor.
- Pregnant woman.
- Patient who refuse to participate in the study.

Steps and methods:

Patients to be recruited gave an informed consent and were subjected to:

1- *Complete history taking:*
   - Personal history.
   - Menstrual history.
   - Obstetric history.
   - Past medical and surgical history.
   - Drug history.

2- *General, abdominal and pelvic examinations:*
   - *General examination:*
     - *General examination:* Body decuibetis, signs of hirsiutism and signs of thyroid disease.
     - Breast examination for swelling or nipple discharge.
   - *Abdominal examination:* Excludes any pelvi abdominal mass.
   - Complete pelvic examination.

3- *Investigations: (infertility group):*
   - Semen analysis to exclude male factor of infertility.
   - Hormonal profile at 3rd day of the cycle (FSH, LH, Estradiol and prolactin).
   - HSG to confirm the patency of the fallopian tubes, and to exclude any structural abnormality of the uterus.

• *Serum progesterone level at 21st - 22nd day of the cycle:*

- The sample is collected from patient.

- Centrifugation is done to obtain at least 1-ml of serum.

- Serum progesterone level is a radioimmunoassay performed with in-house reagents. The radioimmuno-assay technique is based on the competitive binding of the serum progesterone and a radio labeled progesterone preparation. They compete for binding to an antibody specific for progesterone. The antibody-bound radio labeled progesterone is separated and the quantity is determined by counting in a gamma spectrometer. Results for the unknown are read from a curve prepared by plotting results for a set of known standards. Sera with pre-determined concentrations are included in every extraction procedure and assayed for quality control purposes).

4- *Type of ultrasound:* We use the scanner (Voluson 730 PRO) with 7.5 MHZ endocavitary transducer with pulsed colour Doppler and 3D facility in examination of our participants.

Technique of transvaginal ultrasound:

1- Empty the urinary bladder and prepare the patient for ultrasound examination.

2- The examination table: For the scanning procedure an in-line transducer probe (a probe that has an end firing scan head with its shaft and handle on the same axis), so a flat ultrasound examination table was appropriate. The elevated thighs enabled free movement of the probe in the horizontal plane by the operator.

3- The equipment and the transducer: All the patients were subjected to the following:

• The vaginal probe was covered with a condom containing a small amount of gel. The gel ensured good contact between the transducer and the overlying condom. Care was taken to avoid trapping of any air bubbles, which might have created unwanted artifacts on the screen. Cross infection was prevented by the use of probe cover and disinfectants.

• Transvaginal probe will be inserted into the vagina and placed in the anterior fornix. Both internal cervical os and the external one are identified, and uterine examination will be performed to rule out any uterine anomaly that might interfere with pregnancy such as uterine septum, bicornuate uterus, and uterine myomas and to rule out any abnormalities in the secretory endometrium.

• The probe will be moved 90 degree to have transverse section in the uterus then moved laterally to get the right ovary in the ovarian fossa which will be identified then the left one will be identified by the same way.

• A longitudinal view of the uterus was then obtained then power Doppler mode was activated, subsequently the region of interest was defined by a movable sector on the screen which was placed over the endometrium and subendometrial area (where 5mm of the endometrial borders were included), virtual organ computer aided analysis (VOCAL) was used to calculate the endometrial volume. The histogram facility was used to generate the vascularization index (VI) characterized vessel density, the flow index (FI) described the intensity of blood flow, and the vascular flow index (VFI) assessed both vascularity and perfusion as shown in Figs. (1,2).

Statistically analysis:

Quantitative variables are expressed as mean ± standard deviation (±S.D) and comparisons were performed using the *t*-test for independent samples if the samples were normally distributed or by 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.

Data were statistically described in terms of mean ± standard deviation (±S.D), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student t test for independent samples. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. p-values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows.

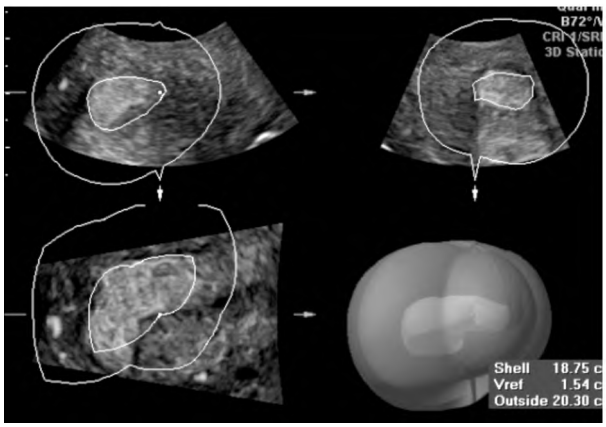

Fig. (1): Obtaining endometrial and subendometrial vascularity volume by 3D power Doppler.

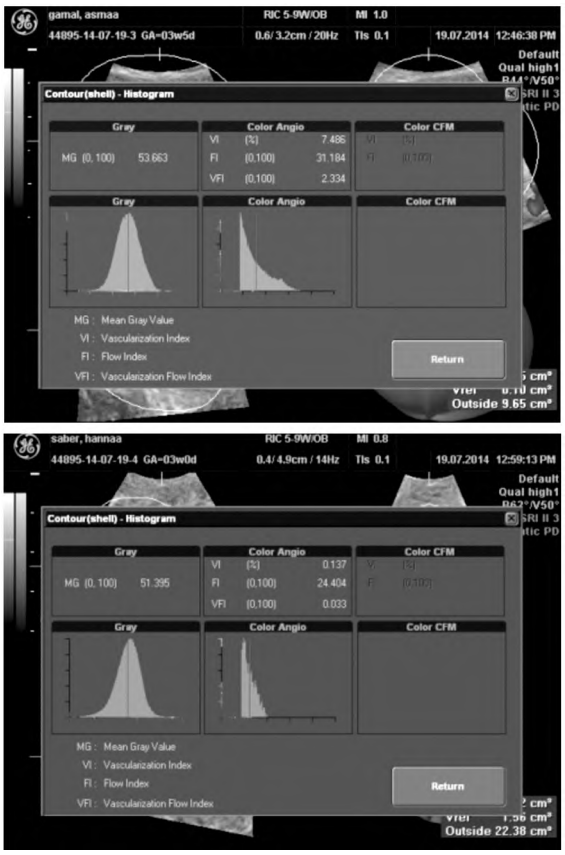

Fig. (2): Calculation of vascular indices by 3D power Doppler using VOCAL method.

Results

From our study we got the following results: The age in control group (27.72±5.86) was higher than case group (26.56±5.32) but with no statistical significance (*p*-value 0.302). Gravidity was higher in control group (2.30± 1.22) than in case group (1.22± 1.40) which was statistically significant, (*p*-value 0.001). Parity was higher in control group (2.04± 1.14) than in case group (0.92 ±1.26) which was statistically significant, (*p*-value 0.001).

As regards endometrial vascularity index (VI), it was higher in control group (0.64±0.05) than in case group (0.51 ±0.09) which was statistically significant (*p*-value 0.001). Subendometrial (VI) also was higher in control group (2.34±0.13) than in case group (1.92±0.22) which was statistically significant (*p*-value 0.001).

As regards endometrial flow index (FI), it was higher in control group (29.26± 1.98) than in case group (24.47±4.19) which was statistically significant (*p*-value 0.001). As regards sub endometrial flow index (FI), it was higher in control group (36.29±3.10) than in case group (30.52 ±2.52) which was statistically significant (*p*-value 0.001).

As regards endometrial vascular flow index (VFI), it was higher in control group (0.36±0.05) than in case group (0.25 ±0.04) which was statistically significant (*p*-value 0.001). As regards subendometrial vascular flow index (VFI), it was higher in control Group (1.13 ±0.25) than in case group (0.83 ±0.07) which was statistically significant (*p*-value 0.001).

As regards to serum progesterone level it was higher in control group (12.87±2.47) than in case group (12.66±3.17) which was statistically insignificant (*p*-value 0.718).

Table (1): Comparison in age, gravidity and parity between case and control group.

	Control group (n=50)	Case group (n=50)	*p*-value
Age:			
Mean ± S.D	27.72±5.86	26.56±5.32	0.302 (NS)
Gravidity:			
Mean ± S.D	2.30± 1.22	1.22± 1.40	0.001
Parity:			
Mean ± S.D	2.04± 1.14	0.92± 1.26	0.001

Table (2): Comparison of CS incidence between the case and control group.

	Control (n=50)	Cases (n=50)	*p*-value
0	30 (60%)	37 (74%)	0.137 (NS)
1	20 (40%)	13 (26%)	

Table (3): Type of infertility in infertility group (primary or secondary).

	Number	Percent (%)
Primary	23	46
Secondary	27	54

Table (4): Comparison in vascularization index (VI) between case and control group.

	Control (n=50)	Cases (n=50)	*p*-value
End VI	0.64±0.05	0.51±0.09	0.001
Sub end VI	2.34±0.13	1.92±0.22	0.001

Table (5): Comparison in flow index (FI) between case and control group.

	Control (n=50)	Cases (n=50)	*p*-value
End FI	29.26±1.98	24.47±4.19	0.001
Sub end FI	36.29±3.10	30.52±2.52	0.001

Table (6): Comparison in (VFI) between case and control group.

	Control (n=50)	Cases (n=50)	*p*-value
End VFI	0.36±0.05	0.25±0.04	0.001
Sub end VFI	1.13±0.25	0.83±0.07	0.001

Table (7): Comparison in serum progesterone level between case and control group.

	Control (n=50)	Cases (n=50)	*p*-value
Serum progesterone	12.87±2.47	12.66±3.17	0.718 (NS)

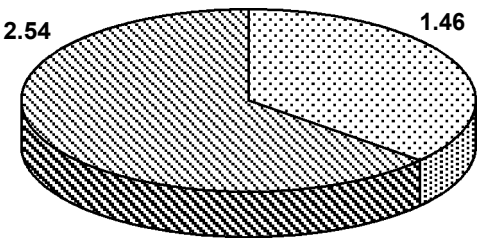

Fig. (3): Type of infertility in infertility group (primary or secondary).

Discussion

The main objective of this study was to determine whether women with unexplained infertility had an abnormality in vascularity of endometrial and subendometrial areas than women who had at least one live child and no history of infertility.

Kim et al., [7] reported the highest pregnancy rate in the group with lower resistance to blood flow in the uterine spiral arteries. This finding revealing the decrease in peripheral impedance in the uterine vasculature reflected by a low uterine artery PI was considered to be a consequence of increased blood flow and a sign of high tissue perfusion, and this might be an important prerequisite for successful pregnancy either natural or after IUI.

Some studies mentioned that a poor uterine perfusion might be one of the causes of unexplained infertility, Revel [8] however, few studies correlate unexplained infertility and Doppler flowmetry of the arteries responsible for uterine perfusion.

Inadequate blood flow would thus prevent implantation, although optimal uterine perfusion does not always mean pregnancy. In addition to this, high uterine resistance is observed in less than 10% of non-conception cycles, which suggests that this parameter is responsible for failure in implantation in very few cases Cacciatore et al., [9].

A study done by Ziegler et al., [10] has suggested that low levels of progesterone might cause the uterine vascular impedance to increase. Therefore, using the secretory endometrium as an inclusion criterion in the present study was intended to determine (however indirectly) whether hormonal action was adequate in our group of women with unexplained infertility, and to ensure that the examination occurred during the luteal phase.

It has been proposed to consider the endometrial and subendometrial area as a whole when the uterine perfusion is assessed by colour Doppler, since there is no difference between the endometrial and subendometrial blood flow with respect to the possibility of achieving pregnancy Chien LW et al., [11].

Compared the difference in uterine and spiral artery impedance to blood flow among women with unexplained and tubal infertility during spontaneous and gonadotropin-stimulated cycles in mid luteal phase and found that there were no differences in the uterine artery pulsatility index or peak systolic velocity during the spontaneous or the in-vitro fertilization cycle between the two groups [2].

Raine-Fenning et al., [12] observed that endometrial and sub-endometrial vascularity are significantly reduced in women with unexplained infertility but There were no differences in endometrial thickness or volume between the groups or in the plasma concentrations of estradiol or progesterone. These findings are in accordance with the results of our study.

Other studies showed that endometrial and subendometrial vascularity indices increased throughout the follicular phase, decreased to a

nadir 2 days after follicular rupture and then increased again during the luteal phase. Endometrial and subendometrial volume increased rapidly during the follicular phase and then remained almost unchanged during the luteal phase **[13]**.

All the studies show to demonstrate some changes in the endometrium and subendometrium using 3D ultrasound and they all proved that changes do occur and that 3D ultrasound is a reliable method to do this, more so than 2D ultrasound. Although, their main objective was to link these results to uterine receptivity in order to create the perfect environment for embryos to implant.

In conclusion, the presence of good uterine and endometrial blood flow is an important prerequisite for successful implantation and continuation of pregnancy. Those patients may have abnormalities in the uterine and endometrial blood flow. Despite these findings we could not find any cut off values that could predict the main cause of infertility in this group of which may be attributed to small sample size so larger prospective studies are needed aiming to confirm such results and reaching values that can accurately predict such cases.

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الأوعية الدموية فى أوعية بطانة الرحم والجزء من تحت بطانة الرحم فى حالات عدم الأنجاب الغير معروف السبب

يظل عدم الأنجاب غير المسبب مشكلة محبطة للأطباء ومؤرقة للزوجين. قد تم أعداد هذه الدراسة فى العيادة الخارجية فى مستشفى النساء والتوليد بقصر العينى حيث تم تصنيف المرضى إلى مجموعتين: مجموعة (أ) وتحتوى على ٥٠ مريضة والتى لديها تاريخ مرضى لعدم الأنجاب غير المسبب. مجموعة (ب) التى تحتوى ٥٠ مريضة لم يكن لديها أى تاريخ مرضى لعدم الأنجاب غير ولديها طفل واحد على الأقل. وقد تم عمل سونار ثنائى الأبعاد ودوبلر وكذلك ثلاثى الأبعاد لكل منهما. قد تم إعلام المرضى محل الدراسة وأخذ موافقة كل منهن وتم أخذ تاريخ مرضى كامل بالأضافة إلى فحص شامل عام وللبطن والحوض وقد تم إستخدام سونار ثلاثى الأبعاد لبطانة الرحم، وتم معرفة مؤشر تدفق الأوعية الدموية، وقد حصلنا على النتائج الأتية:

- فيما يتعلق بمؤشر الأوعية الدموية فى جدار الرحم وكان أعلى فى المجموعة (ب) (٠.٠٠٥±٠.٠٦٤) مما كانت عليه فى المجموعة (أ) (٠.٠٠٩±٠.٠٥١)، وكان لهذا قيمة إحصائية بينما مؤشر الأوعية الدموية فى بطانة الرحم كان أعلى فى المجموعة (ب) (٠.١٣±٢.٣٤) مما كانت عليه فى المجموعة (أ) (٠.٢٢±١.٩٢) مما كان له قيمة إحصائية.

- وفيما يتعلق مؤشر التدفق فى جدار الرحم كان أعلى فى المجموعة (ب) (١.٩٨±٢٩.٢٦) من المجموعة (أ) (٤.١٩±٢٤.٤٧) وكان له قيمة إحصائية بينما مؤشر التدفق فى بطانة الرحم كان أعلى فى المجموعة (ب) (٣.١٠±٦٣.٢٩) عنه فى المجموعة (أ) (٢.٥٢±٣٠.٥٢) وكان لهذا قيمة إحصائية.

- وفيما يتعلق بمؤشر تدفق الأوعية الدموية فى جدار الرحم كان أعلى فى المجموعة (ب) (٠.٠٠٥±٠.٠٣٦) من المجموعة (أ) (٠.٠٠٤±٠.٠٢٥) وكان لهذا قيمة إحصائية أيضاً بينما مؤشر تدفق الأوعية الدموية فى بطانة الرحم كان أعلى فى المجموعة (ب) (٠.٠٢٥±٠.١٣) منه فى المجموعة (أ) (٠.٠٠٧±٠.٠٨٣) وكان لهذا قيمة إحصائية أيضاً.

إذاً من رداستنا هذه تبين أنه من الممكن إفادة الحالات التى لديها التاريخ المرضى لعدم الأنجاب غير مسبب بتحسين تدفق الدم للرحم.