

Assessment of Sub-Endometrial Blood Flow and Endometrial Leukemia Inhibitory Factor as a Marker for Endometrial Receptivity in Women with Unexplained Infertility

Hazem A. El-Zenneni¹, Rowaa A. Moustafa¹, Magda N. Nasr Eldeen²,
Amr Ahmed Riad¹, Ibrahim S. El-Shazly¹

Departments of Obstetrics & Gynecology ¹ and Pathology ², Faculty of Medicine - Ain Shams University
*Corresponding author: Ibrahim S. El-Shazly, Tel: 01200520500, Email: dr.ibrahim.elshazly@gmail.com

ABSTRACT

Background: Infertility is customarily defined as the inability to conceive after 1 year of regular unprotected intercourse. The infertility evaluation is typically initiated after 1 year of trying to conceive, but in couples with advanced female age (> 35 years), most practitioners initiate diagnostic evaluation after an inability to conceive for 6 months. **Aim of the Work:** To assess endometrial receptivity in women with unexplained infertility using sub-endometrial vascular flow resistant index and endometrial leukemia inhibitory factor (LIF). **Patients and Methods:** This clinical controlled trial was conducted at Ain Shams University Maternity Hospital during the period from August 2014 to September 2017 on 140 patients divided into two equal groups: **Group I (study group):** women with unexplained infertility defined as inability to conceive in spite of regular marital life for at least 12 months. **Group II (control group):** matched women with infertility due to tubal factor, recruited from outpatient gynecology or infertility clinic at Ain Shams University Maternity Hospital. **Results:** A cutoff value for LIF staining score of ≤ 1 predicted unexplained infertility with a sensitivity of 73.33% and specificity of 70.67%; whereas a cutoff of > 0.71 for subendometrial blood flow RI predicted unexplained infertility with a sensitivity of 70.67% and specificity of 86.67%. A cutoff of ≤ 10 mm for endometrial thickness had the highest sensitivity of 100%, but lacked specificity (only 16%). **Conclusion:** Leukemia inhibitory factor may be a predictor for unreceptive endometrium in cases of unexplained infertility. Subendometrial blood flow RI and endometrial thickness may be used rather than LIF IHC (due to its invasive nature) for the prediction of endometrial factor status in cases of unexplained infertility because of the statistically significant negative correlation between the above parameters. **Recommendations:** Further studies are needed to evaluate the predictive value of the multiple logistic model including (endometrial thickness, subendometrial blood flow color Doppler and LIF IHC score and even other proposed cytokines as VEGF, IL6 and integrins) in unexplained infertility. **Keywords:** sub-endometrial blood flow, endometrial leukemia, inhibitory factor, endometrial receptivity, unexplained infertility.

INTRODUCTION

Unexplained infertility refers to the absence of a definable cause for a couple's failure to achieve pregnancy after 12 months of attempting conception despite a thorough evaluation, or after six months in women 35 and older ⁽¹⁾.

Unexplained infertility affects 15% of couples. Infertility rates are influenced by a woman's age. Although the rate is approximately 10% at 30 years of age, it can be as high as 40% by the time a woman reaches 40 years of age ⁽²⁾.

Diagnosis of unexplained infertility includes a semen analysis, assessment of ovulation, a hysterosalpingogram, and if indicated, tests for ovarian reserve and laparoscopy. When the results of a standard infertility evaluation are normal, practitioners assign a diagnosis of unexplained infertility. Although estimates vary, the likelihood that all such test results for an infertile couple are normal

(i.e., that the couple has unexplained infertility) is approximately 15% to 30% ⁽³⁾.

In the absence of a correctable abnormality, the therapy for unexplained infertility is, by default, empiric. Proposed treatment regimens include intrauterine insemination (IUI), ovulation induction with oral or injectable medications, combination of IUI with ovulation induction, and assisted reproductive technologies (ART) ⁽³⁾.

Embryo implantation represents the most critical step of the reproductive process in many species. It consists of a unique biological phenomenon, by which the blastocyst becomes intimately connected to the maternal endometrial surface ⁽⁴⁾.

The endometrium is normally a non-receptive environment for an embryo, except during implantation window. Implantation window is a period during which the endometrium is optimally receptive to implanting blastocyst. Implantation of the human embryo may occur only during a regulated "implantation

window" on days 6-10 postovulation, and surrounded by refractory endometrial status⁽⁵⁾.

The use of digitally analyzed power angiography, a new non-invasive technology to assess blood flow and vascular characteristics, could provide information regarding the local angiogenic processes occurring in the endometrium. Local angiogenesis is essential for implantation and gestation⁽⁶⁾.

Endometrial blood flow reflects properly the uterine receptivity because the endometrium is the site where embryonic implantation takes place⁽⁷⁾. Many endometrial derived cytokines and growth factors play an important role in the initial process of successful implantation in human. Any failure in the production or regulation of these cytokines or growth factors may be a cause of unexplained infertility, among these cytokines, is leukemia inhibitory factor (LIF)⁽⁸⁾.

Maternal LIF affects trophoblast growth and development and is essential for implantation and has been described as a marker of the embryo implantation process. LIF is expressed on endometrium of uterus. Low levels of LIF are found in the proliferative phase and maximal expression is found during the mid-secretory phase which occurs between days 5 and 10 following the luteinising hormone (LH) surge⁽⁹⁾.

AIM OF THE WORK

To assess endometrial receptivity in women with unexplained infertility using sub-endometrial vascular flow resistant index and endometrial leukemia inhibitory factor (LIF).

PATIENTS AND METHODS

Study Design

- Clinical controlled trial

Study Setting

- The study was accomplished at Ain Shams University Maternity Hospital during the period from August 2014 to September 2017 in the following departments:
 - Gynecology and infertility outpatient clinics
 - Main Theaters
 - Histopathological (early cancer detection unit).
 - Ultrasound unit (fetal care unit).

Study Population

The study will include 2 groups of women:

- **Group I (study group):** women with unexplained infertility defined as inability to conceive in spite of regular marital life for at least 12 months with the **following:**

Inclusion criteria

1. Age: 18 – 35 years old.

2. Normal husband's semen analysis, (*WHO criteria 2010*):
 - a) Semen volume: 1.5 ml or more.
 - b) pH: 7.2 or more.
 - c) Sperm concentration: 15 million spermatozoa per ml or more.
 - d) Total sperm number: 39 million spermatozoa per ejaculate or more.
 - e) Total motility (percentage of progressive motility and non-progressive motility): 40% or more motile or 32% or more with progressive motility.
 - f) Vitality: 58% or more live spermatozoa.
 - g) Sperm morphology (percentage of normal forms): 4% or more.
3. Monitoring ovulation by ultrasound folliculometry plus serum progesterone ≥ 3 ng/ml
4. Patent Fallopian tubes as evident either by hysterosalpingogram (HSG) and/or laparoscopy.
5. Normal transvaginalultrasound scan.

Exclusion criteria

1. Male factor of infertility
 2. Any other cause of infertility
 3. Any subtle endometriotic features in diagnostic laparoscopy
 4. Any previously known medical co-morbidity especially primary or secondary vasculopathy (immunological disease, diabetes mellitus)
 5. Using any method of induction of ovulation in the past 3 cycles
- Women of group I will be recruited from infertile women attending outpatient gynecology or infertility clinics at Ain Shams University Maternity Hospital.
 - **Group II (control group):** matched women with infertility due to tubal factor, recruited from outpatient gynecology or infertility clinic at Ain Shams University Maternity Hospital.

Inclusion criteria

1. Age: 18 – 35 years old.
2. Normal husband's semen analysis, (*WHO criteria 2010*):
 - a. Semen volume: 1.5 ml or more.
 - b. pH: 7.2 or more.
 - c. Sperm concentration: 15 million spermatozoa per ml or more.
 - d. Total sperm number: 39 million spermatozoa per ejaculate or more.
 - e. Total motility (percentage of progressive motility and non-progressive motility): 40%

- or more; 32% or more with progressive motility.
- f. Vitality: 58% or more live spermatozoa.
- g. Sperm morphology (percentage of normal forms): 4% or more.

3. Monitoring of ovulation by ultrasound folliculometry plus serum progesterone ≥ 3 ng/ml .
4. Abnormal Fallopian tubes as evident either by hysterosalpingogram (HSG) and/or laparoscopy.
5. Normal transvaginal ultrasound scan apart from the morphology of the fallopian tubes

Exclusion criteria

1. Male factor of infertility
2. Any other cause of infertility
3. Any subtle endometriotic features in diagnostic laparoscopy
4. Any medical co-morbidity especially primary or secondary vasculopathy (immunological disease, diabetes mellitus)
5. Using any method of induction of ovulation in the past 3 cycles

Sample Size Justification

Sample size was calculated using Pass sample size program version 13 by adjusting the power of the test to 80%, confidence is 95 % and percent of error accepted to 5%. The least accepted sample size was 140 patients divided into two groups.

METHODOLOGY

All included women (either of the study or control groups) will be subjected to the following:

- o History taking with particular emphasis on past medical history, menstrual history and infertility workup.

- o General, abdominal and local examination.
- o 2D Power-Doppler ultra-sonography is to be performed for measuring in the mid secretory phase of the cycle after ultrasound documentation of ovulation by 6 days:
 - Endometrial thickness and pattern.
 - The endometrium should be measured in the long-axis or sagittal plane. The measurement is of the thickest echogenic area from one basal endometrial interface across the endometrial canal to the other basal endometrium. Care should be taken not to include the hypo-echoic myometrium in this measurement.
 - Sub-endometrial vascular flow resistance index.
 - The blood-flow velocity waveforms from the sub-endometrial vessels were obtained by placing the Doppler gate over the color area and activating the pulsed Doppler function. A recording was considered satisfactory when at least five consecutive waveforms were obtained, each demonstrating the maximum Doppler shift. The resistance index (RI=peak systolic velocities - peak diastolic velocities/peak systolic velocities) was calculated on three consecutive uniform waveforms.
 - Both parameters will be measured 3 times and an average value will be calculated for each of them, 2D Power-Doppler ultrasonography will be performed by the same sonographer in the ultrasound unit in Ain Shams Maternity Hospital (Xono-ace R5 Madison Korea).

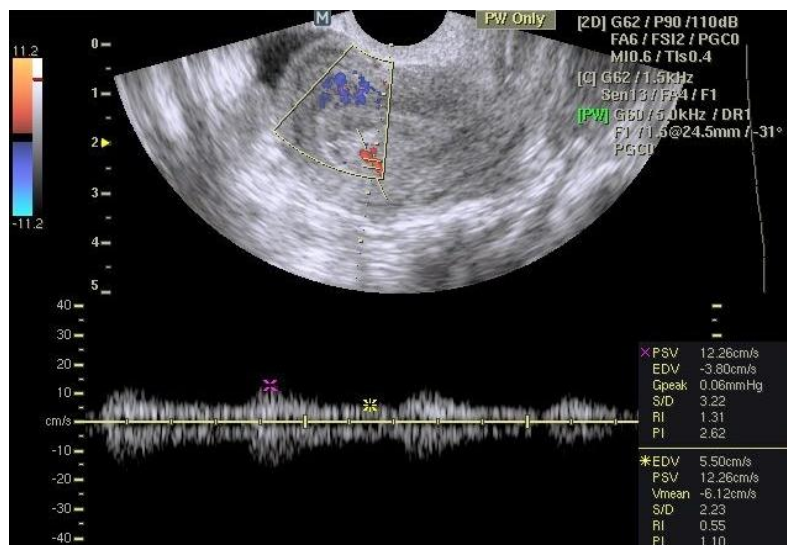


Figure (1): Sub-endometrial blood flow by color Doppler study showing a good vascularity (Tubal infertility control group).

- Endometrial sample is to be taken by traditional dilatational curettage biopsy in the mid secretory phase of the cycle after documentation of ovulation by 6 days.

Histopathological samples processing

- Endometrial samples will be fixed in 10% paraformaldehyde solution in a sterile container labeled with the patient's study number. Samples will be refrigerated at -4c till the time of histopathological examination.
- The biopsy samples will be embedded in paraffin and cut into 2 μ m sections. For each specimen, two paraffin sections will then be prepared for routine H&E staining. Two other paraffin sections will be cut on positively

charged slides for IHC study using the primary antibody (Anti-LIF Picoband™ AntibodyCatalog Number: PB9036 *ready-to use for immunohistochemical staining* of LIF from BOSTER BIOLOGICAL TECHNOLOGY3942 B Valley Ave, Pleasanton, CA 94566,USA).

- Briefly, no staining is scored as 0; 1–10% of positive cells stained scored as 1; 11–50% as 2; 51–80% as 3; and 81–100% as 4. Staining intensity is rated on a scale of 0–3, with 0 = negative; 1 = weak; 2 = moderate, and 3 = strong. The raw data were converted by multiplying the quantity and staining intensity scores⁽⁹⁾

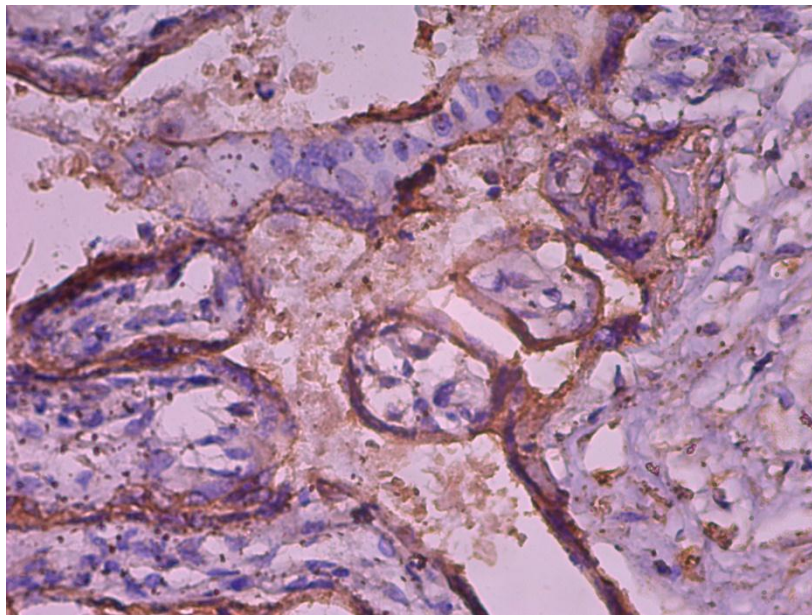


Figure (2): Positive control staining (placenta) magnification X400

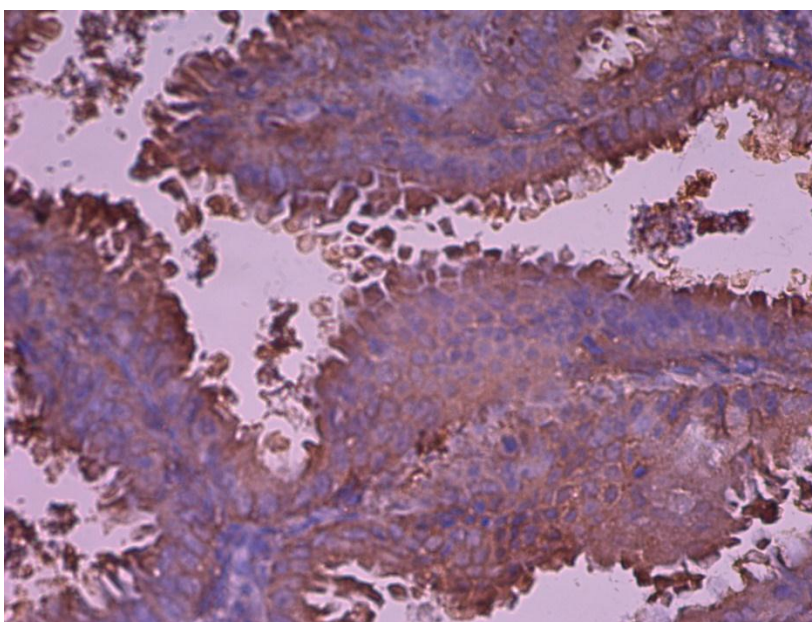


Figure (3): Tubal infertility staining grade +3 magnification X400

Elimination of Bias

- All the histological samples were inspected by the same observer as well as the sonographic study
- o **Ethics:**
 - The study was approved by the **Ethics Committee** of the Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University.
 - **Informed written consent** was taken from all participants before recruitment in the study, and after explaining the purpose and procedures of the study.

Statistical Methods

Statistical analysis was done on a personal computer using IBM® SPSS® Statistics for Windows version 20 (IBM® Corp, Armonk, NY).

Univariate analysis was performed to compare women with tubal infertility with those with unexplained infertility. The Mann-Whitney U test was used to compare skewed numerical data and the chi-square test for trend (Cochran-Armitage test) to compare ordinal categorical data. Exact probability was calculated if the number of observations was too few to apply the Cochran-Armitage test.

Bivariate correlation was tested non-parametrically using Spearman's rank correlation. The correlation coefficient (ρ) was interpreted as follows:

- o < 0.2 = no correlation
- o $0.2 - 0.39$ = mild correlation
- o $0.4 - 0.69$ = moderate correlation
- o $0.7 - 1.0$ = strong correlation

Variables associated with a P value of < 0.25 by univariate analysis were included in multiple logistic regression analysis. This permissive inclusion criterion of a $P < 0.25$ has been previously recommended in order to avoid missing pertinent variables should a conventional level of significance of $P < 0.05$ is applied. On the other hand, more permissive criteria are believed to be associated with variables of dubious significance⁽¹⁰⁾. For predictors derived from common variables, only one variable was included in the model. Factors expected to be associated with the outcome variable were also included on substantive basis.

The 'enter/simultaneous' method was used to force all selected covariates into the model to avoid automatic exclusion of key variables from the model. A receiver operating characteristic (ROC) curve was plotted using MedCalc® version 12.3.0.0 (MedCalc® Software, Mariakerke, Belgium) to test

the value of the regression model for prediction of the outcome of interest (i.e., unexplained infertility). To test the predictive value of individual quantitative variables, a series of ROC curves were plotted and the area under the curve (AUC) estimated. The AUC was interpreted as follows:

- o < 0.6 = non-predictive
- o $0.6 - 0.69$ = poor predictive value
- o $0.7 - 0.79$ = fair predictive value
- o $0.8 - 0.89$ = good predictive value
- o $0.9 - 1.0$ = excellent predictive value

The best cut-off criterion on the ROC curve was defined as that associated with the highest Youden's index (J statistic), where $J = \text{sensitivity} + \text{specificity} - 1$.

All P values are two-tailed. $P < 0.05$ was considered statistically significant.

Comparison of ROC curves

The DeLong method⁽¹¹⁾ was used for calculation of the Standard Error (SE) for the AUC and of the SE for the difference between any pair of AUCs. The 95% CI for the AUC is calculated based on binomial exact probability which was used to estimate statistical significance for the difference between the AUCs of any pair of ROC curves.

Results

The current study was conducted in the infertility clinic of Ain Shams University Maternity Hospital. A total of 140 women were included in the study.

The process of recruitment and handling the study population during the course of the study is shown in the flow diagram according to the CONSORT (CONsolidated Standards of Reporting Trials) 2010 guidelines.

Descriptive Analysis of the Study Groups

The aim of this section is to analyze the basic characteristics of the study groups for the possibility of presence of any confounding factors that might affect the study results and interpretation.

Basic demographic and clinical characteristics of the study groups

Patients of the tubal factor group had significantly a higher order of parity, longer duration of infertility and higher proportion of patients with secondary infertility reflecting the nature of tubal infertility as a major cause of secondary infertility. No statistically significant differences were found between the mean ages of both groups.

Table (1): Comparison between study groups regarding demographic and clinical characteristics

	Unexplained Infertility group	Tubal Factor group	P
Age (Yrs)			
Range	24.0 – 35.0	24.0 – 35.0	0.46 ^a
Mean±SD	30.21 ± 3.74	29.85 ± 3.49	
Parity			
Median (IQR)	0 (0 – 0)	1 (0 – 2)	<0.001 ^a
Duration of infertility (Yrs)			
Range	1.0 – 3.0	1.0 – 5.0	0.009 ^a
Mean±SD	1.64 ± 0.79	1.96 ± 0.81	
Type of infertility			
Primary (%)	67 (95.7%)	23 (32.8%)	<0.001 ^b
Secondary (%)	3 (4.3%)	47 (67.2%)	

^aAnalysis using Mann-Whitney test.
^bAnalysis using Fisher’s exact test.

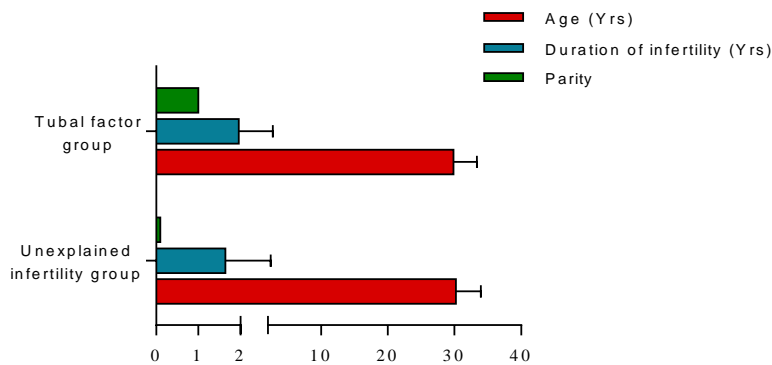


Figure (4): Grouped bar graph for the demographic data of the study population.

Analysis of LIF immunohistochemical staining and U/S parameters in both study groups

LIF staining intensity score was significantly lower in the unexplained infertility group compared to the tubal factor group. Also, subendometrial blood flow RI and endometrial thickness were significantly lower in the unexplained infertility group than the tubal factor group.

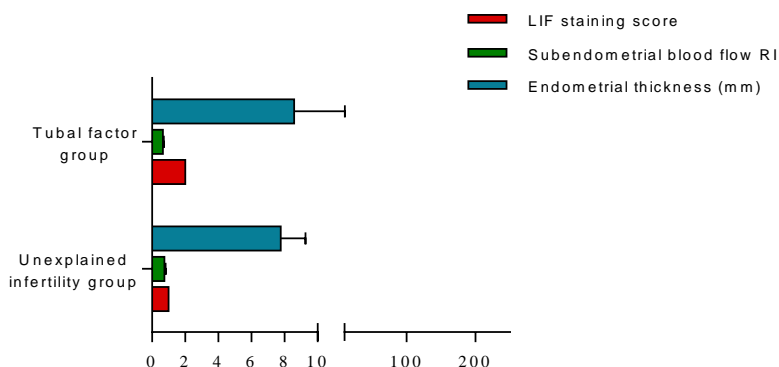


Figure (5): Grouped bar graph for the LIF immunohistochemical staining scores and U/S parameters in both study groups.

Table (2): Comparison between study groups regarding LIF immuno-histochemical staining and U/S parameters

	Unexplained Infertility group	Tubal Factor group	P
LIF staining intensity score	1 (1 – 2)	2 (1 – 2)	<0.001^a
Median (IQR)	0 – 3	1 – 3	
Range			
Subendometrial blood flow RI	0.52 – 0.89	0.53 – 0.75	<0.001^a
Range	0.74 ± 0.085	0.66 ± 0.052	
Mean±SD			
Endometrial thickness	4.0 – 10.0	6.0 – 13.0	0.04^a
Range	7.78 ± 1.49	8.58 ± 1.86	
Mean±SD			

^aAnalysis using Mann-Whitney test.

Diagnostic Performance of LIF Immunohistochemical Staining and U/S Parameters in Prediction of Unexplained Infertility

Diagnostic performance of LIF immunohistochemical staining and U/S parameters in prediction of unexplained infertility is shown in table 1. All the studied parameters were statistically significant predictors of unexplained infertility. A cutoff value for LIF staining score of ≤1 predicted unexplained infertility with a sensitivity of 73.33% and specificity of 70.67%; whereas a cutoff of > 0.71 for subendometrial blood flow RI predicted unexplained infertility with a sensitivity of 70.67% and specificity of 86.67%. A cutoff of ≤10 mm for endometrial thickness had the highest sensitivity of 100%, but lacked specificity (only 16%).

Table (3): Receiver-operating characteristic (ROC) curve analysis for prediction of complete cure using calculated scores.

Predictor			
Variable	LIF staining score	Subendometrial blood flow RI	Endometrial thickness
AUROC	0.765	0.821	0.590
95% CI	0.68-0.83	0.75-0.87	0.50-0.66
p-value (AUC=0.5)	< 0.001	< 0.001	0.048
Cut-off value	≤ 1.0	> 0.71	≤ 10
Validity	Sensitivity (%)	73.33%	100%
	Specificity (%)	70.67%	16.0%
	PPV (%)	71.4%	84.1%
	NPV (%)	72.6%	74.7%
	Positive likelihood ratio	2.50	5.30
	Negative likelihood ratio	0.38	0.34

Multivariable Logistic Regression Analysis For Prediction Of Unexplained Infertility Using Other Independent Variables

Multivariable logistic regression analysis for the prediction of unexplained infertility using other independent variables showed that LIF staining score, subendometrial flow RI and endometrial thickness remained significant after adjustment for age, duration of infertility and other variables.

Assessment of regression model fitness showed that it could correctly predict 79.33% of cases.

Table (4): Multivariable logistic regression analysis for prediction of unexplained infertility using independent variables.

Variable	Coefficient	Std error	Odds ratio (95% CI)	P
Age	0.038	0.061	1.03 (0.92 – 1.17)	0.53
Duration of infertility	0.29	0.280	0.74 (0.42 – 1.29)	0.29
LIF staining score				
Score: 0	20.30	15.32	10.92 (3.71 – 12.45)	<0.001
Score: 1	2.68	0.45	1.98 (0.80 – 4.87)	<0.001
Score: 2	0.39	0.93	1.48 (0.23 – 9.35)	0.02
Subendometrial blood flow RI	5.42	1.95	8.81 (3.22 – 11.21)	0.007
Endometrial thickness	-0.26	0.13	0.76 (0.58 – 0.99)	0.04
Constant	10.14			

Table (5): Classification table for the regression model at a predicted probability of 0.5.

<i>Predicted</i>			
Observed	Tubal factor	Unexplained infertility	Percent correct
Tubal factor	61	14	81.33%
Unexplained infertility	17	58	77.33%
Percent of cases correctly classified			79.33%

Table (6): Assessment of regression model fit

Overall model fit	
Null model-2 Log Likelihood	207.94
Full model-2 Log Likelihood	135.55
Chi-square	72.38
DF	7
Significance level	<0.001
Hosmer&Lemeshow test	
Chi-square	9.20
DF	8
Significance level	0.32

Assessment of Performance of Subendometrial Flow RI and Endometrial thickness in Prediction of Immunohistochemical LIF status of the Patient

Being an invasive procedure, this might affect the clinical value of the immuno-histochemical study of LIF status of individual patients in everyday practice. Statistical analysis was done to evaluate the performance of subendometrial blood flow RI and endometrial thickness as screening tools for prediction of LIF status of the patient, allowing segregation of the portion of the patients with high likelihood to benefit from invasive assessment of LIF immunohistochemical status.

Statistically significant negative correlation was found between LIF staining score and the subendometrial blood flow RI; whereas no statistically significant correlation was found with the endometrial thickness. However, care should be taken that the sample size justified for analysis of the primary outcome of the study might limit the power drawn from inferences regarding this secondary outcome.

Table (7):Correlation between LIF staining score with the observed U/S parameters.

	r (95% CI)	p
LIF staining score		
Subendometrial blood flow RI	-0.60 (-0.70 – -0.49)	<0.001
Endometrial thickness	0.11 (-0.04 – 0.27)	0.15

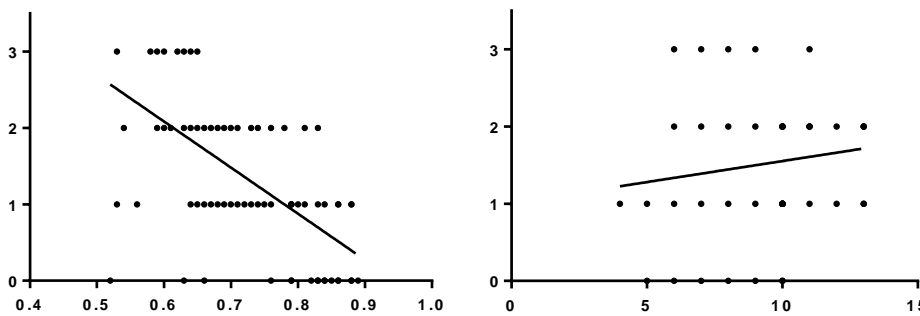


Figure (6): Scatter diagram for the relation between LIF staining score (y-axis) and subendometrial blood flow RI (left) and endometrial thickness (right).

DISCUSSION

This study has focused on one of the cytokines; LIF that is likely to be involved in endometrial - trophoblastic talk to make the uterus receptive to implantation. Our objective was to relate vascular imaging data to molecular events in order to determine whether a combination of the two can provide useful information about mechanisms preventing uterine receptivity.

Maternal LIF affects trophoblast growth and development and is essential for implantation and has been described as a marker of the embryo implantation process⁽⁹⁾.

In consistency with our study some investigators have shown that the expression of LIF and LIF-R was significantly lower in the epithelial cells of women with unexplained infertility compared with the fertile controls⁽¹⁰⁾.

In agreement with our study the work of Wu *et al.*, who found that moderate expression of LIF in the proliferative phase and higher expression of LIF in the secretory phase were found in fertile women. However, lower expression of LIF was found in unexplained infertile women with multiple implantation failure compared to fertile women. Data suggested that the initial lower expression of LIF in proliferative phase may be one of the causes for multiple failure of implantation^(9,11).

On the contrary of the current study, Quaas and Dokras⁽¹²⁾ found that women with unexplained infertility had lower IHC expression of LIF in the secretory endometrium. However, it was statistically non significant when compared to

control group of fertile women. This can be attributed to the inclusion of some patients with tubal factor infertility and smaller sample size of both the study and the control groups⁽¹³⁾.

Also Consistent with our study the work by Margioulas-Siarkou C *et al.* In a prospective observational case-control study, infertile women were divided according to infertility cause in women with tubal factor, poor ovarian reserve and unexplained infertility with a control group of fertile women. LIF expression in patients with unexplained infertility was significantly compared with controls (P=0.006). No significant difference was observed between patients with tubal factor, poor ovarian reserve and endometriosis compared with control group (P=0.78, P=0.44 and P=0.56 respectively). The study indicated impaired LIF expression levels only in women with unexplained infertility⁽¹⁴⁾.

In our study there were statistically significant differences between both groups concerning endometrial thickness Regression analysis of the endometrial thickness as a predictor of endometrial cause of unexplained infertility showed a significant value in the detection of case group, with the optimum criterion ≤ 10 (best cut off value) [sensitivity 100% and specificity 16.0%].

There has been considerable controversy concerning the value of endometrial thickness in the prediction of endometrial receptivity. Many groups report significantly lower endometrial thickness in infertile women several other groups report significantly higher mean endometrial thickness in

conception compared with non-conception cycles. According to *Oliveira et al.* ^(15,16), higher pregnancy rates were observed in subjects where endometrial thickness reached at least 10 mm.

In another prospective observational study by *Singh et al.* ⁽¹⁷⁾, a total of 101 infertile women were recruited from our IVF-ET program from January to December, 2009. Women with tubal factor, male factor and unexplained infertility were included in the study to evaluate the role of endometrial thickness, pattern and sub-endometrial blood flows measured by 2D power Doppler ultrasound to predict pregnancy during in-vitro fertilization (IVF) treatment and concluded that with a thin endometrium (≤ 7 mm) and no-triple-line endometrial pattern coexisting in an in-vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) candidate, cryopreservation should be recommended. With a thin endometrium and a good texture (triple-line), other prognostic factors, such as embryo quality, should be taken into account. The endometrial vascularity has a useful predictive value on the implantation rate in IVF cycles irrespective of the morphological appearance of the endometrium ⁽¹⁶⁾.

No consensus has been reached with regard to the minimum endometrial thickness required for successful pregnancy. Pregnancies did not occur when the endometrial thickness was less than 7 mm; ⁽¹⁷⁾ however; other studies found that a minimum endometrial thickness of 6 mm is acceptable for implantation ⁽¹⁸⁾. Interestingly, Sundström reported a successful pregnancy with an endometrial thickness as little as 4 mm ⁽¹⁹⁾.

In our study there were statistically significant differences between both groups concerning sub endometrial colored 2-D Doppler resistance index (RI). Regression analysis of sub endometrial colored 2-D Doppler resistance index (RI) as a predictor of endometrial cause of unexplained infertility showed that the Resistance index is an independent variable with a significant value in the detection of case group, with the optimum criterion > 0.71 (best cut off value) [sensitivity 70.67% and specificity 86.67%].

Many studies have been conducted to evaluate the role of various ultrasound parameters in predicting pregnancy during stimulated IVF cycles ⁽²⁰⁾, but little information exists in the literature with regard to their role in women with unexplained infertility. **In our observational study** in assessing endometrial receptivity we compared endometrial thickness and sub-endometrial blood flow assessed by 2-D colored Doppler in the 2 study arms, to find out if there is a statistically significant difference between the two groups in any parameter and what the cut of values are. We found a statistically significant

difference between fertile and infertile groups and as mentioned before.

As previously mentioned in agreement with Yang et al ⁽²⁰⁾ For an embryo to implant, the quality of the endometrium as well as the (sub-)endometrial perfusion and vascularization may be more important factors than the global flow throughout the uterus ⁽²¹⁾.

Edmond et al. ⁽²²⁾ using conventional color Doppler found that in natural unstimulated cycles, arterial blood flow was detected in more than 80% of fertile patients in the sub-endometrial area and in different phases of these cycles and in more than 95% of fertile women at the mid luteal phase.

Pitfalls and recommendations:

1. In order to assess endometrial factor of infertility during the luteal phase endometrial dating by any mean should be done in order to define endometrial asynchronization.
2. Sonographic assessment of the endometrium either by endometrial thickness or sub-endometrial color Doppler is cheap, available and reliable study for endometrial receptivity during patient assessment and follows up.
3. Our study parameters and results can be used in further randomized double blinded placebo controlled trials to evaluate the role of different drugs (estrogen, LMWH, corticosteroids, nitric oxide donors and others) on endometrial angiogenesis and its impact on pregnancy rate.
4. Further studies are needed to evaluate the predictive value of the multiple logistic model including (endometrial thickness, subendometrial blood flow color Doppler and LIF IHC score and even other proposed cytokines as VEGF, IL6 and integrins) in unexplained infertility.

CONCLUSIONS

- Leukemia inhibitory factor may be a predictor for unreceptive endometrium in cases of unexplained infertility.
- Different ultrasonic parameters have a significant role in predicting unreceptive endometrium in cases of unexplained infertility as endometrial thickness and sub-endometrial color Doppler.
- Subendometrial blood flow RI and endometrial thickness may be used rather than LIF IHC (due to its invasive nature) for the prediction of endometrial factor status in cases of unexplained infertility because of the statistically significant negative correlation between the above parameters.

REFERENCES

- 1) **ASRM (2008):** Optimizing natural fertility. *Fertil Steril.*, 80: 1-6.
- 2) **Ray A, Shah A, Gudi A and Homburg R (2012):** Unexplained Infertility: An Update and Review of Practice. *Reprod Biomed Online*, 24: 591-602.
- 3) **ASRM. (2015):** Diagnostic evaluation of the infertile female: a committee opinion. *Fertile Steril.*, 103:e44-50.
- 4) **Aplin JD (2000):** The cell biological basis of human implantation Baillieres. *Best Pract Res Clinical Obs. Gyn.*, 14: 757- 764.
- 5) **Aboubakr ME and Gamal I (2004):** Endometrial receptivity. *Middle East Fertility Society Journal*, 9 (1): 10-24
- 6) **Nardo LG (2005):** Vascular endothelial growth factor expression in the endometrium during the menstrual cycle, implantation window and early pregnancy. *Curr. Opin. Obstet. Gynecol.*, 17 (4): 419-423.
- 7) **Merce LT (2002):** Ultrasound markers of implantation. *Ultrasound Rev ObstetGynecol.*, 2:110-23.
- 8) **Sharkey AM and Smith SK (2003):** The endometrium as a cause of implantation failure. *Best Pract Res ClinObstetGynaecol.*, 17:289–307.
- 9) **Wu M, Yin Y, Zhao M, Hu L and Chen Q (2013):** The low expression of leukemia inhibitory factor in endometrium: possible relevant to unexplained infertility with multiple implantation failures. *Cytokine*, 62(2):334-9.
- 10) **Mickey RM and Greenland S (1989):** The impact of confounder selection criteria on effect estimation. *Am J Epidemiol.*, 129(1):125-37.
- 11) **DeLong ER, DeLong DM, Clarke-Pearson DL (1988):** Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*, 44(3):837-45.
- 12) **Quaas A, and Dokras A (2008):** Diagnosis and Treatment of Unexplained Infertility. *Rev Obstet Gynecol. Spring*, 1(2): 69–76.
- 13) **Margioula-Siarkou C, Prapas Y, Petousis S, Miliias S, Ravanos K, Kalogiannidis I (2016):** LIF and LIF-R expression in the endometrium of fertile and infertile women: A prospective observational case-control study. *Molecular Medicine Reports*, 13: 4721-4728.
- 14) **Franasiak J, Holoch K, Yuan L, Schammel D, Young S, and Lessey B (2014):** Prospective assessment of midsecretory endometrial leukemia inhibitor factor expression versus anβ3 testing in women with unexplained infertility *Fertility and Sterility*. <https://www.ncbi.nlm.nih.gov>
- 15) **Margioula-Siarkou C, Prapas Y, Petousis S, Miliias S, Ravanos K, Dagklis T, Kalogiannidis I, Mavromatidis G, Haitoglou C, Prapas N, Rouso D (2017):** LIF endometrial expression is impaired in women with unexplained infertility while LIF-R expression in all infertility subgroups. *Cytokine*, 96:166-72.
- 16) **Oliveira JB, Baruffi RL, Mauri AL, Petersen CG, Borges MC, Franco JG (1997):** Endometrial ultrasonography as a predictor of pregnancy in an in-vitro fertilization programme after ovarian stimulation and gonadotrophin-releasing hormone and gonadotrophins. *Human Reproduction*, 12(11):2515-8.
- 17) **Singh N, Bahadur A, Mittal S, Malhotra N and Bhatt A (2011):** Predictive value of endometrial thickness, pattern and sub-endometrial blood flows on the day of hCG by 2D doppler in *in-vitro* fertilization cycles *J Hum Reprod Sci.*, 4(1): 29–33.
- 18) **Coulam CB, Bustillo M, Soenksen DM, Britten S (1994):** Ultrasonographic predictors of implantation after assisted reproduction. *Fertility and Sterility*, 62(5):1004-10.
- 19) **Sundström P (1998):** Establishment of a successful pregnancy following in-vitro fertilization with an endometrial thickness of no more than 4 mm. *Human reproduction (Oxford, England)*, 13(6):1550-2.
- 20) **Yang JH, Wu MY, Chen CD, Jiang MC, Ho HN, Yang YS (1999):** Association of endometrial blood flow as determined by a modified colour Doppler technique with subsequent outcome of in-vitro fertilization. *Human Reproduction*, 14(6):1606-10.
- 21) **Schild RL, Holthaus S, d'Alquen J, Fimmers R, Dorn C, van Der Ven H, Hansmann M (2000):** Quantitative assessment of subendometrial blood flow by three-dimensional-ultrasound is an important predictive factor of implantation in an in-vitro fertilization programme. *Human Reproduction*, 15(1):89-94.
- 22) **Edmond CO, Edi-Osagie MD, Mourad W *et al.* (2004):** Characterizing the endometrium and tubal factor infertility: A multiparametric investigation. *Fertile Steril.*, 82:1479-1389.