

# Endometrial CD16+ Natural killer Cells and Sub-endometrial Doppler in Unexplained Infertility

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## **Abstract**

*This prospective case-control study was conducted to investigate the relation between endometrial natural killer CD16+ cells and unexplained infertility. 45 women with unexplained infertility and another 45 fertile women as their controls were recruited in the study. Transvaginal sonography and endometrial sampling were performed 5-9 days after ovulation (implantation window). Endometrial thickness and sub-endometrial blood flow indices were assessed, endometrial CD16+ were assessed using immunohistochemical staining. A significant higher incidence of CD16+ positive endometrium was found in infertile women than controls, as well as a highly significant difference between the 2 groups regarding endometrial thickness (being thinner in the infertility group) and both sub-endometrial Doppler parameters (RI and PI) ( $P < 0.001$ ). A positive correlation was found between endometrial positive CD16+ and both endometrial thickness and sub-endometrial RI ( $P = 0.035$  and  $P = 0.011$  respectively). This study suggests a significant association between endometrial CD16+ NK cells and unexplained infertility.*

## **Keywords**

*Unexplained infertility, Natural Killer cells, CD16, Sub-endometrial Doppler.*

## I. Introduction

Infertility affects 25% of couples in developing countries [1], and unexplained infertility is one of the most common diagnoses in these couples. It is defined as failure of conception after 1 year without any definable cause after a standard infertility work-up is normal [2]. Several conditions are probably caused by multiple factors that each on its own does not significantly affect fertility, but reduce pregnancy rate when combined together [3]. Poor endometrial receptivity, preventing implantation of blastocyst, may account as a cause of unexplained infertility [4].

Studies have suggested immunological testing as a part in the management of infertility [5], natural killer (NK) cells, being included the most abundant leukocytes in the deciduas, should be included [6]. CD16 NK cells are abundant in the endometrium during secretory phase, NK cells migration to the uterus together with endometrial differentiation from CD34+ stem cell precursors has been attributed as of CD16- origin [7].

Although many reproductive medicine centers consider offering the peripheral blood NK cell counts in infertile women as common practice, still, few clinical studies evaluated the correlation of endometrial and peripheral NK cell counts [8], and considering that peripheral blood NK cell count does not necessarily reflect increased NK activity [5], further studies are warranted to determine NK endometrial activity in infertility. A proper balance between anti-inflammatory and inflammatory cytokine expression and CD16+

over CD16- NK cell count ratio could be potential tools for immunological diagnosis of infertility [9].

This study is designed to investigate the relation between endometrial NK CD16+ cells and unexplained infertility.

## II. Patients and Methods

This prospective case-control study was conducted in Ain Shams University Maternity Hospitals, Cairo, Egypt from August 2015 till April 2016. Ethical approval was obtained from the Hospital local ethical committee, and a written informed consent was signed by all participants after explaining the aim, benefits and any potential risks before being included in the study.

Assuming that endometrial CD16+ expression ranges between 46.38+11.8 in infertile women and 37.9+11.7 in the fertile ones [10], a minimum sample size, calculated using Sata®IO software program, of 41 women in each group is enough to detect such difference at 0.05 alpha error and 0.90 power of the test. Thus, a total of 90 women were included in the study, 45 were diagnosed as women with primary unexplained infertility (group 1) and the other 45 were normal fertile women as control group (group 2) recruited from the family planning clinic. Diagnosis of unexplained infertility was established with normal husband's semen analysis [11], ovulatory cycles confirmed by mid-luteal progesterone  $\geq 10$  ng/ml, normal hormonal (LH, FSH, TSH and prolactin) levels, patent fallopian tubes diagnosed by hysterosalpingogram (HSG) and/or laparoscopy.

Women with extremes of body mass index (BMI) (<18.5 and >30 Kg/m<sup>2</sup>), extremes of reproductive age (<18 and >35 years), endometriosis evidenced by laparoscopy ± histopathology, tubal factor infertility, ovulatory dysfunction, anatomical uterine pathology, male factor infertility, medical disorders that may affect fertility (e.g., diabetes mellitus, systemic lupus erythromatosis, hypo or hyperthyroidism), Polycystic ovarian syndrome, and women who are current or past smokers who stopped smoking only within 6 months before starting the study were excluded.

After confirmation of the inclusion and exclusion criteria, all included women were asked to avoid sexual intercourse in the current cycle or to use condoms. Trans-vaginal sonography was performed 5-9 days after ovulation (implantation window) using Medison X6 ultrasound machine (serial number: A9A510300001836) with 7.9 MHz endocavitary transducer with pulsed color Doppler (serial number: C3-7EP). Endometrial thickness was measured at the maximum thickness between the highly reflective interfaces of the endometrial-myometrial junction. The blood flow velocity waveforms from the sub-endometrial vessels were obtained where resistance index (RI) and pulsatility index (PI) were calculated.

Endometrial samples were obtained during implantation window using Wallach Endocell® (Endometrial Cell Sampler REF/908014A) from (Wallach Surgical Devices, 95 Corporate Dr., Trumbull, CT06611 USA, [www.wallachsurgical.com](http://www.wallachsurgical.com)). Immunohistochemical staining for CD16+ (Vector Labs VP-C333) were performed with

the labeled streptavidinbiotin- peroxidase technique. Sections were stained with hematoxylin, dehydrated, and evaluated using light microscope. The results of CD16+ positive were examined visually in 10 × 400 magnification microscopic fields and represented qualitatively (positive or negative).

Statistical analysis was done using Microsoft Excel (version 2007) and SPSS for Windows version 15.0. Normality of numerical data distribution was tested using D'Agostino Pearson test. Normally distributed data were presented as mean and standard deviation. Qualitative data were presented as number and percentage. For normally distributed numerical data, the independent t-test was used to compare means. Pearson chi-square test was used to compare categorical variables. Pearson's correlation coefficient (for metric variables) and Spearman's correlation coefficient (for rank variables) were used to estimate association between variables.

### III. Results

There was statistical difference between the 2 groups regarding age and BMI; the mean age was 29.31±4.3 years in group 1 and 28.62±4.1 years in group 2 (P=0.439) and mean BMI was 25.16±2.13 vs. 24.62±2.05 (P=0.226) in groups 1 and 2 respectively. A significant higher incidence of endometrial CD16+ positive women was encountered in group1 than controls (P<0.001) as well as a highly significant difference between the 2 groups regarding endometrial thickness (being thinner

in the infertility group) and both sub-endometrial Doppler parameters (RI and PI) (Table 1). A positive correlation was found between endometrial positive CD16+ and both endometrial thickness and sub-endometrial RI, while no correlation could be found with sub-endometrial PI, age or BMI (Table 2).

#### IV. Discussion

Unexplained infertility is commonly diagnosed in fertility clinics with lacking specific treatment secondary to absence of accurate diagnosis of a specific cause [12]. It is suggested that elevation of NK cells may affect the reproductive performance of women, thus, testing NK cells serum levels is currently used to guide initiation of therapies in infertile patients [13]. New therapies are designed to modulate the function and number of these cells, however, still more studies are needed to characterize endometrial NK cells in different reproductive pathologies such as implantation failure. Most data are based on abnormal serum NK cell counts in women with recurrent miscarriage [14].

This study suggests a significant association between endometrial CD16+ NK cells and unexplained infertility, also a significant correlation was found with poor peri-implantation endometrial thickness. Considerable controversy is present about value of endometrial thickness in prediction of endometrial receptivity. Other studies agreed

with the results of this study reporting significantly lower endometrial thickness in infertile women [15], yet, no consensus is reached regarding the minimum endometrial thickness needed for successful pregnancy. Higher pregnancy rates were observed with endometrium of at least 10 mm thick [16], pregnancy did not occur with endometrial thickness less than 7mm [17]. However, other studies found endometrial thickness of 6 mm acceptable for implantation [18], even some reported successful pregnancies with endometrium as thin as 4 mm [19]. The suggested correlation between endometrial NK cells and endometrial thickness might have possible explanation for this variation in pregnancy incidence in relation to endometrial thickness; further studies should be warranted to discuss this relation.

Concerning the question; could the increased endometrial NK cells affect endometrial blood flow? This study failed to answer this question; it found a positive correlation between endometrial positive CD16+ NK cells and sub-endometrial RI but no correlation with PI. Studies have showed that decreased endometrial and sub-endometrial flow is associated with increased rates of implantation failures [15, 20]. Finding a possible relation between endometrial NK cell count and blood flow might introduce other treatment modalities that increase both implantation and

pregnancy rates especially in patients with unexplained infertility.

Although others agreed with this study in the increased CD16+ NK cell counts in females with unexplained infertility [21], yet, there is still deficient data about the normal values of endometrial CD16+ NK cell count, NK sub-populations, and NK cell markers. Few studies used flow cytometry which provides better analysis of NK cell populations, most studies, as this one, relied on histological evaluation which has limited value in differentiating different subsets of NK cells [5].

## V. Conclusion

Immunological cause of unexplained infertility seems to be a possible etiology and further evaluation of NK cells role in endometrial receptivity and blood flow is still needed with analysis of different endometrial NK cell populations and its implication on implantation and pregnancy rates.

## VI. Acknowledgement

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**Table (1).** Comparison of tested outcomes between cases of unexplained infertility and controls

	<b>Group 1 (n=45)</b>	<b>Group 2 (n=45)</b>	<b>P value</b>	<b>Mean diff./ O.R.</b>	<b>95% C.I.</b>
<b>CD16 +ve (number of cases)</b>	26 (57.8%)	9 (20%)	< 0.001	0.183	0.071to 0.468
<b>Endometrial thickness (mm)</b>	5.51±1.93	10.18±1.96	< 0.001	-4.67	-5.48 to -3.85
<b>Sub-end. RI</b>	0.69±0.82	0.54±0.86	< 0.001	0.153	0.118 to 0.188
<b>Sub-end. PI</b>	1.13±0.18	1.30±0.14	< 0.001	-0.174	-0.242 to -0.107

**Table (2).** Correlation between endometrial CD16+ and other variables

	<b>CD16 +ve (n=35)</b>	<b>CD16 -ve (n=55)</b>	<b>P value</b>	<b>Mean diff.</b>	<b>95% C.I.</b>
<b>Endometrial thickness (mm)</b>	7.0±3.0	8.38±2.97	0.035*	1.38	0.10 to 2.66
<b>Sub-end. RI</b>	0.65±0.10	0.59±0.12	0.011*	-0.062	-0.109 to -0.015
<b>Sub-end. PI</b>	1.17±0.20	1.24±0.17	0.059	0.074	-0.003 to 0.152
<b>Age (years)</b>	29.2±4.03	28.8±4.33	0.714	-0.335	-2.146 to 1.476
<b>BMI (Kg/m<sup>2</sup>)</b>	25.27±2.07	24.65±2.09	0.168	-0.627	-1.523 to 0.269

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