The Effect of Intrauterine Infusion of Peripheral Blood Mononuclear Cells Culture on Subendometrial Blood Flow in Patients Undergoing ICSI Cycles

Wasan Hamad Jassim 1, Manal Taha Al-Obaidi 1, Haider F. Ghazi 2

1High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq. dr.wasan.hamad@gmail.com
2College of Medicine, Al Nahrain University, Baghdad, Iraq.

In recent years increasing evidence proposed that local immune cells at implantation site have largely contributed to embryo implantation. The intrauterine infusion of activated peripheral blood mononuclear cells culture 2 days before embryo transfer can enhance the implantation. One of the methods used to evaluate the endometrial receptivity is by assessing the sub endometrial blood flow. A total of 67 infertile women (30) women receives intrauterine non-invasive insemination of peripheral blood mononuclear cells (PBMC) culture 2 days before embryo transfer representing the PBMC test group, and (37) women without receiving any cell as Non-PBMC group. The cultured PBMC are administered into the uterine cavity of the patients. 2 days later, embryos are transferred into the uterine cavity. Endometrial thickness and sub-endometrial blood flow measurements are taken for all cases on trigger and embryo transfer days. On embryo transfer day there was no significant difference (p = 0.770) in mean endometrial thickness between the PBMC group and Non-PBMC group. There was a significant difference (p< 0.001) in the mean resistive index; the level being lower in the PBMC group. Moreover, there was a significant difference (p< 0.001) in the mean pulsatility index. Regarding all enrolled women, the pregnancy rate of 25.4 %, the rate was higher in the PBMC group in comparison with the Non-PBMC group, 43.3 % versus 10.8 %, respectively and the difference was significant (p = 0.002). The use of PBMC culture can improve sub-endometrial.

KEYWORDS
Endometrial Receptivity, ICSI, Fresh Embryo Transfer, Peripheral Blood Mononuclear Cells, Subendometrial Blood Flow

Received: 28-Sep-2021
Accepted: 06-Nov-2021
Published: 08-Nov-2021

How to cite:
Jassim WH; Al-Obaidi MT; Ghazi HF; The Effect of Intrauterine Infusion of Peripheral Blood Mononuclear Cells Culture on Subendometrial Blood Flow in Patients Undergoing ICSI Cycles; Iraqi Journal of Embryos and Infertility Researches (JEIR), (2021); 10(2): 53-72.
Doi: http://doi.org/10.28969/IJEIR.v10.i2.r5
1. Introduction

Having difficulty conceiving a child after 12 months of regular, unprotected sexual contact is a medical and social problem that can be defined as the inability to achieve a clinical pregnancy. Depending on the woman's age, medical and reproductive history, fertility interventions may be initiated in less than one year (Zegers-Hochschild, et al. [1]). Assisted reproductive technology (ART) is a difficult method to resort to, because it includes surgically removing an oocyte from a woman's ovaries and collecting sperms from her husband, mixing them in a special media in a laboratory, and returning them to the woman's uterus to conceive a child (Neri, et al. [2]).

Implantation, despite the medical and clinical progress made in fertility therapy, is a difficult process in which a foreign embryo has to be accepted by the mother's endometrium. Implantation failure is still a major human reproduction challenge. The underlying reason of implant failure is obviously multifaceted and cannot be linked to any certain defect. Uterine abnormalities, alterations in the hormone, immunology and thrombophilia are diverse maternal variables that cause failure of implantation (Maleki-Hajiagha, et al. [3]). According to the available literature, different maternal immune cell subsets contribute significantly to embryo implantation and are involved in all procedures necessary for the maintenance and completion of a successful pregnancy (Ghaebi, et al. [4]). It is widely acknowledged that the endometrium can only allow embryo implantation for a certain period of time, known as the implantation window (Blesa, et al. [5]). Based on the accumulation of data, it has been demonstrated that local immune cells at the implantation site actively contributed to successful implantation of the embryo. It has been discovered that stimulated peripheral blood mononuclear cells improve the rate of implantation (Fujiwara, et al. [6]). The use of high-resolution transvaginal probes made it possible to track endometrial changes during the whole menstrual cycle (Hannan, et al. [7]). Uterine receptivity is influenced by a variety of factors, the most important of which is uterine perfusion and endometrial perfusion. It has been discovered that there are differences in uterine perfusion between
infertile and fertile women. It has been speculated that poor uterine and endometrial perfusion may be the fundamental cause of failure. Blood flow resistance in the uterine artery and the endometrial area has been shown to be a predictive indication of implantation in ART cycles (Haouzi, et al. [8]). A large number of research have been undertaken on the hemodynamic alterations that occur in the utero-ovarian arteries during ART cycles. It is crucial to choose the most appropriate time for embryo transfer in order to maximize the success of ART. With the use of high-resolution transvaginal probes, because it is non-invasive and easily accessible, transvaginal sonography made it particularly suitable for serial follow up during the stimulated cycle (Lessey, BA [9]).

2. Materials and Methods

2.1. Patients' Selection

This is a prospective comparative clinical randomized study. It includes a sample of 67 patients assigned randomly to receive autologous Peripheral Blood Mononuclear Cells (PBMC) cultures by an intrauterine non-invasive insemination 2 days before embryo transfer representing the PBMC group and those patients without receiving any cell culture transfer prior to embryo transfer as non-PBMC group. PBMCs are isolated from the patients at the day of ovulation trigger and activated by incubation with human menopausal gonadotropin for three days. Thereafter, the cultured PBMC are infused into the uterine cavity of the patients to make adequate endometrial differentiation. Two days later, embryos are transferred (the patient should have at least one grade 1 embryo) into the uterine cavity. Measurement of endometrial thickness and sub-endometrial blood flow is done to the all cases at the day of ovum pickup and the day of embryo transfer. After the study received ethical approval, patients provided written informed consent.

2.2. Inclusion Criteria

Patients with age less than 40 years, BMI less than 30 Kg/m², absence of uterine pathology like hydrosalpinx uterine fibroids and endometriosis, and patients who doesn’t have at least one embryo grade 1 were excluded from the study.

2.3. Stimulation Protocol

All patients were enrolled in flexible antagonist protocol, which started at day 2 of the menstrual cycle, an ultrasound examination was accomplished in order to exclude those women with ovarian cyst and to assess the endometrial thickness. It involved ovarian stimulation with Gonadotropin (rFSH) (Gonal-F; Serono Laboratories, Saint Cloud, France) since the second day of the menstrual cycle followed by the administration of a GnRH antagonist (Cetrorelix acetate for injection 0.25 mg: Cetrotide®, Merk, Switzerland), in flexible method the Cetrorelix acetate injection with 0.25 mg according to the size of the largest follicles when they reach 13-14 mm. Transvaginal ultrasound was done on 5th day of stimulation and subsequent scan was done every 2-3 days as necessary. The follicles growth was tracked by serum E2 level and transvaginal ultrasound till the day of hCG administration (Hu, et al. [10]). Ovulation triggering was induced by the administration of recombinant hCG (rhCG 6500 IU, Ovitrelle®; Merck Serono) subcutaneously when two or more than two follicles have reached 18 mm (Trew, et al. [11]). All infertile patients received luteal support using progesterone treatment for 2 weeks in the form of 400 mg vaginal progesterone pessaries (Cyclogest; Actavis®, UK) once daily until a pregnancy test was achieved (Neri, et al. [12]).

2.4. Collection, Culture and Transfer of PBMC Culture

During the day of the hCG trigger, a sample of blood was drawn from patients undergoing ICSI cycles, and the blood was collected in citrated tubes in order to perform a density gradient separation (lymphocyte separation media) with a volume of 3 mL (lymphosep, bioweast; USA). With the use of cooling centrifuge (Hettich, universal 320R, Germany), we were able to separate the PBMC layer under the median plasma after centrifugation at 18–20 °C for 30–40 minutes at 400 g. It was necessary to transfer the PBMC layer to another tube, to which three volumes of phosphate-buffered saline (PBS) were added, and then centrifuge the mixture for 10 minutes at 18–20 °C and 60–100 g to obtain a
PBMC pellet. It was necessary to wash the lymphocyte pellet twice with PBS before it could be resuspended at 37 °C in RPMI 1640 complete culture medium ready for use (euroclone, Italy), cultured with 75 IU of HMG-Menogon R. (Ferring, Germany). 0.4 mL of clustered cells were infused into the endometrial cavity two days before embryo transfer after 72 hours of incubation in a CO₂ incubator (Memert, Germany) (Madkour, et al. [13]).

2.5. ICSI Protocol

Oocytes were retrieved by aseptic transvaginal ultrasound-guided oocyte aspiration, approximately 34–36 hours after rhCG administration under general anesthesia (Trew, et al. [11]). The patients were positioned in the dorsal lithotomy posture and their vaginas was thoroughly cleaned with saline irrigation. All follicles inside each ovary were aspirated using an ovum aspiration needle (Gynetics®, Belgium), and the follicular fluid was then provided straight to the embryologist, who then collect the cumulus-oocyte complexes that had been extracted from each follicle. All of the cumulus-oocyte complexes are then rinsed with flushing media to remove any remaining blood from the follicular aspirate, transferred into drops of Ferticult Flushing media, then washed with Gain medium, and overlaid with paraffin/mineral oil in an incubator set at 37 °C with 5% CO₂, and at 95% humidity. The intracytoplasmic sperm injection (ICSI) technique was achieved (4–6) hours after oocyte retrieval to all patients. In preparation for ICSI the cumulus corona cells are detached by a combined enzymatic and mechanical treatment to strip the oocytes from the cumulus cells. Each oocyte is carefully checked, observing the presence or absence of a germinal vesicle or the first polar body. Only those ova that have been extruded the first polar body (metaphase II) and morphologically normal were fit for microinjection (Oktay, et al. [14]). Husband sperms were collected either by masturbation into a clean, dry and sterile plastic dish after 3-5 days of abstinence, the sample was transported to the laboratory immediately and placed in an incubator at 37 °C for 30 minutes to allow liquefaction, or obtained surgically.
from testis, epididymis or vas deferens in azoospermic husband. Around 12-17 hours after ICSI technique, with the use of Nikon ICSI Microscope, fertilization was checked for the sign of normal fertilization which was defined as the presence of two pronuclei (2PN). First-day cleavage is checked 24 hours after fertilization, and the number and size of blastomeres were documented for each embryo. At 72 hours after microinjection, those embryos with good morphology were transferred into the uterine cavity (Neri, et al. [2]). Prior to embryo transfer, the developed embryos were graded regarding to the embryo grading system (Scott, et al. [15]). With this system, each embryo was graded as grade 1, 2 and 3.

2.6. Two-Dimensional Power Doppler Ultrasound of Endometrial and Subendometrial Zones

Transvaginal ultrasound scanning was performed to assess endometrial thickness, regularity, and echogenicity, as well as color Doppler indicators of subendometrial blood flow (PI and RI). The zones of vascular penetration had previously been identified as follows:

**Zone 1:** The zone of myometrium adjacent to the subendometrium

**Zone 2:** The outer hyperechogenic subendometrial zone

**Zone 3:** The inner hypoechogenic zone

**Zone 4:** The endometrium

The transvaginal scan was done using the 6 MHz vaginal probe of SonoAce-X6 Ultrasound Set (Medison, Seoul, South Korea). Ultrasound scans were achieved while the patient was in the dorsal lithotomy position. The endometrial thickness was measured, with the uterus in the sagittal plane, as the maximum thickness between the highly reflective interfaces of the endometrial-myometrial junction. The measurement involved both endometrial layers excluding the surrounding low amplitude echo layer, three measurements were taken and the average value was documented. After completion of the B-mode examination, a pulsed Doppler system was used for blood flow analysis.
Subendometrial vessels were visualized at the endometrial periphery, occasionally penetrating the hyperechogenic endometrial edge or even reaching the endometrial cavity (Elnaggar, et al. [16]). The blood flow velocity waveforms from the subendometrial vessels were obtained by placing the Doppler gate over the colored area at Zone 2 and activating the pulsed Doppler function. A recording was decided to be satisfactory when at least 3 successive uniform waveforms were obtained; each demonstrated the maximum Doppler shift. Three measurements for each parameter were taken and the average value was documented. Resistance Index (RI) and Pulsatility Index (PI) were calculated automatically by the ultrasound for both subendometrial vessels, according to the following formula:

\[
\text{RI} = \frac{\text{PSV} - \text{EDV}}{\text{PSV}}
\]

\[
\text{PI} = \frac{\text{PSV} - \text{EDV}}{\text{mv}}
\]

\[
\text{mv} = \frac{\text{PSV} + \text{EDV}}{2}
\]

PSV: Peak Systolic Velocity, EDV: End-Diastolic Velocity, mv: mean velocity.

3. Statistical Analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as number and percentage, whereas, quantitative (numeric) variables were first evaluated for normality distribution using Kolmogorov-Smirnov test, and then accordingly normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion), while those numeric variables that are not normally distributed were expressed as median (an index of central tendency) and inter-quartile range (an index of dispersion).

4. Results

The current study included a total of 67 infertile women who were categorized into a PBMC group (n = 30) and a Non-PBMC group (n = 37), based on intrauterine insemination of cultured PBMCs. The demographic
characteristics of those women are shown in Table 1. The mean age of all enrolled women was (29.97±5.33) years, that of PBMC group was (29.87±4.19) years and that of Non-PBMC group was (30.05±6.16) years; The mean body mass index (BMI) of all enrolled women was (26.72±2.83) Kg/m², that of PBMC group was (26.70±2.62) Kg/m² and that of Non-PBMC group was (26.73±3.02) Kg/m²; there was no significant difference in mean BMI between PBMC group and Non-PBMC group (p = 0.958). Regarding the type of infertility, there were 54 (80.6 %) women with primary infertility and 13 (19.4 %) women with secondary infertility and there was no significant difference in the frequency distribution of the infertile women according to the type of infertility between PBMC group and Non-PBMC group (p=0.911). The Oocyte and embryo characteristics of infertile women enrolled in this study are shown in Table 2. There was no significant difference in mean total oocyte count between PBMC and Non-PBMC group, (11.77±4.13) versus (12.03±7.91), respectively (p = 0.871). There was also no significant difference in mean MII oocyte count between PBMC and Non-PBMC group, (8.07±3.84) versus (7.08 ±5.23), respectively (p = 0.393). In addition, there was no significant difference in mean fertilized oocyte count between PBMC and Non-PBMC group, (6.33±3.13) versus (5.46±4.17), respectively (p=0.346). Furthermore, there was no significant difference in mean transferred embryo count between PBMC and Non-PBMC group, (2.33±0.84) versus (2.46±0.84), respectively (p=0.213). In all women, they have at least one grade 1 transferred embryo and have been transferred at day 3. At day of ovum pickup there was no significant difference between PBMC and Non-PBMC group in the mean endometrial thickness and in the mean resistive index. Moreover, no significant difference in mean pulsatility index and the p-values were (0.651, 0.395, 0.150) respectively, as shown in Table 3. At day of embryo transfer There was no significant difference in mean endometrial thickness between PBMC and Non-PBMC group (p=0.770). However, there was highly significant difference in mean RI (p< 0.001); the level being lower in PBMC group.
**Table (1):** Demographic characteristics of infertile women included in the present study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 67)</th>
<th>PBMC group (n = 30)</th>
<th>Non-PBMC group (n = 37)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>29.97±5.33</td>
<td>29.87±4.19</td>
<td>30.05±6.16</td>
<td>0.888 I</td>
</tr>
<tr>
<td>Range</td>
<td>18 -40</td>
<td>20 -38</td>
<td>18 -40</td>
<td>NS</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.72±2.83</td>
<td>26.70±2.62</td>
<td>26.73±3.02</td>
<td>0.958 I</td>
</tr>
<tr>
<td><strong>Type of infertility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary, n (%)</td>
<td>54 (80.6 %)</td>
<td>24 (80.0 %)</td>
<td>30 (81.1 %)</td>
<td>0.911 C</td>
</tr>
<tr>
<td>Secondary, n (%)</td>
<td>13 (19.4 %)</td>
<td>6 (20.0 %)</td>
<td>7 (18.9 %)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table (2):** Oocyte and embryo characteristics of infertile women enrolled in this study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 67)</th>
<th>PBMC group (n = 30)</th>
<th>Non-PBMC group (n = 37)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total oocyte count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>11.91±6.45</td>
<td>11.77±4.13</td>
<td>12.03±7.91</td>
<td>0.871 I</td>
</tr>
<tr>
<td>Range</td>
<td>2 -33</td>
<td>3 -18</td>
<td>2 -33</td>
<td>NS</td>
</tr>
<tr>
<td><strong>MII oocyte count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>7.52±4.65</td>
<td>8.07±3.84</td>
<td>7.08±5.23</td>
<td>0.393 I</td>
</tr>
<tr>
<td>Range</td>
<td>1 -21</td>
<td>3 -17</td>
<td>1 -21</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Fertilized MII count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.85±3.74</td>
<td>6.33±3.13</td>
<td>5.46±4.17</td>
<td>0.346 I</td>
</tr>
<tr>
<td>Range</td>
<td>1 -18</td>
<td>2 -15</td>
<td>1 -18</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Number of transferred embryos</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.39±0.73</td>
<td>2.33±0.84</td>
<td>2.46±0.84</td>
<td>0.213 I</td>
</tr>
<tr>
<td>Range</td>
<td>1 -4</td>
<td>1 -4</td>
<td>1 -4</td>
<td>NS</td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation; I: Independent samples t-test; HS: highly significant at p ≤ 0.01; NS: not significant at p> 0.05
**Table (3):** Ultrasound characteristics and biophysical profile of infertile women at the day of ovum pickup

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 67)</th>
<th>PBMC group (n = 30)</th>
<th>Non-PBMC group (n = 37)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endometrial thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>9.24±1.36</td>
<td>9.16±1.39</td>
<td>9.31±1.36</td>
<td>0.651 I NS</td>
</tr>
<tr>
<td>Range</td>
<td>6.4 -13.5</td>
<td>6.5 -13.5</td>
<td>6.4 -12</td>
<td></td>
</tr>
<tr>
<td><strong>RI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.53±0.10</td>
<td>0.52±0.08</td>
<td>0.54±0.12</td>
<td>0.395 I NS</td>
</tr>
<tr>
<td>Range</td>
<td>0.24 -0.8</td>
<td>0.33 -0.68</td>
<td>0.24 -0.8</td>
<td></td>
</tr>
<tr>
<td><strong>PI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.69±0.14</td>
<td>0.66±0.12</td>
<td>0.71±0.15</td>
<td>0.150 I NS</td>
</tr>
<tr>
<td>Range</td>
<td>0.35 -0.95</td>
<td>0.42 -0.93</td>
<td>0.35 -0.95</td>
<td></td>
</tr>
</tbody>
</table>

**Table (4):** Ultrasound characteristics and biophysical profile of infertile women at day of embryo transfer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 67)</th>
<th>PBMC group (n = 30)</th>
<th>Non-PBMC group (n = 37)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endometrial Thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>12.20±1.46</td>
<td>12.14±1.63</td>
<td>12.25±1.33</td>
<td>0.770 I NS</td>
</tr>
<tr>
<td>Range</td>
<td>9.4 -15.5</td>
<td>9.4 -15</td>
<td>9.5 -15.5</td>
<td></td>
</tr>
<tr>
<td><strong>RI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.49±0.11</td>
<td>0.43±0.08</td>
<td>0.53±0.11</td>
<td>&lt; 0.001 I HS</td>
</tr>
<tr>
<td>Range</td>
<td>0.27 -0.69</td>
<td>0.29 -0.55</td>
<td>0.27 -0.69</td>
<td></td>
</tr>
<tr>
<td><strong>PI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.62±0.14</td>
<td>0.55±0.10</td>
<td>0.69±0.13</td>
<td>&lt; 0.001 I HS</td>
</tr>
<tr>
<td>Range</td>
<td>0.31 -0.94</td>
<td>0.31 -0.71</td>
<td>0.4 -0.94</td>
<td></td>
</tr>
</tbody>
</table>
Figure (1): A comparison of mean resistive index (RI) between PBMC and Non-PBMC group at day of embryo transfer.

Figure (2): A comparison of mean pulsatility index (PI) between PBMC and Non-PBMC group at day of embryo transfer.
Moreover, there was a highly significant difference in the mean PI (p< 0.001); the level being lower in PBMC group, as shown in Table 4. The mean RI was compared between PBMC and Non-PBMC group at day of embryo transfer, as shown in Figure 1. Moreover, the mean PI between the groups at day of embryo transfer were compared, as shown in Figure 2. Regarding all enrolled infertile women, the pregnancy rate was 25.4 %. However, the rate was higher in the PBMC group in comparison with the Non-PBMC group, 43.3 % versus 10.8 %, respectively and difference was highly significant (p=0.002), as shown in Figure 3.

5. Discussion
To achieve successful implantation, the endometrium must be receptive, the embryo must be normal and functional, the dialogue between maternal and embryonic tissues must be coordinated. According to accumulating data, local immune cells at the implantation site have actively contributed to embryo implantation during the development of the embryo (Fujiwara, et al. [6]). According to another study, the infusion of mouse PBMC...
generated from unpregnant mice intrauterine prior to embryo implant had a positive effect on endometrial receptivity and embryonic implantation in mice with embryo implantation dysfunction (Yu, et al. [17], Yoshioka, et al. [18]). The endometrium, as a site of embryo implantation, provides optimal environments for early embryo development and implantation in response to ovarian estrogen and progesterone regulation. Endometrial receptivity is receiving more and more attention these days, because of the safety and simplicity of ultrasonic examination, an evaluation of endometrial thickness (EMT) is an important indicator for predicting pregnancy outcomes. However, the exact influence of EMT on pregnancy outcomes on the day of hCG administration remains controversial due to a lack of large-scale systematic studies on this topic (Ma, et al. [19]). In the current study, there is no significant difference between the PBMC and the non-PBMC group in EMT at the day of ovum pickup and at the day of embryo transfer. Argument on the predictive value measuring EMT before administering hCG for ovulation triggering in assisted reproduction techniques is continuing. A linear relationship between pregnancy rates and EMT has been demonstrated by some investigators (Eftekhar, et al. [20], Senturk, et al. [21]), Whereas others have hypothesized that pregnancy rates may even deteriorate once the EMT reaches 14 mm and that miscarriage rates may rise as a result (Ribeiro, et al. [22], Weissman, et al. [23]). The relationship between thin endometrium and poor clinical pregnancy rates has been shown by various research (Shaodi, et al. [24], von Wolff, et al. [25]), but the EMT threshold for successful IVF–ET pregnancy has yet to be determined. Studies have shown that when the EMT is less than 7 mm on the hCG day, the clinical pregnancy rate is dramatically lowered (Eftekhar, et al. [26]). The role of several ultrasound parameters in predicting pregnancy during stimulated IVF cycles has been studied extensively. Detection of low-velocity flow and visualization of small vessels are two applications where power Doppler imaging outperforms color Doppler imaging on a general basis. In the present study, the sub-
endometrial RI and PI were not significant in PBMC and in non-PBMC group at the day of ovum pickup. While the sub-endometrial RI and PI were significantly lower in PBMC than in non-PBMC group at the day of embryo transfer. After conducting their research, Abdel Kader, et al. came to the conclusion that unfavorable blood flow on the day of hCG in IVF/ICSI cycles indicates that pregnancy is unlikely, and that embryo transfer should be canceled, with all embryos being frozen for future transfer in order to increase the success rate (Abdel Kader, et al. [27], Sardana, et al. [28]). But in the present study with the use intrauterine infusion of PBMC culture, the PBMC group underwent significant changes in the sub-endometrial blood flow noticed at the day of embryo transfer after two days of PBMC culture being introduced to the endometrium, the subendometrial PI and RI were significantly lower in PBMC than in non-PBMC group at the day of embryo transfer. In contrast, Ibrahim, et al., stated that Doppler parameters are useful tools to assess endometrial receptivity in unexplained infertility patients undergoing ICSI, and they discovered that Uterine, Ovarian, sub-endometrial arteries PI and RI measured on the day of embryo transfer were higher than in fertile women, and that these parameters have value in judging. When uterine, ovarian, and subendometrial blood flow was assessed by measuring the PI and RI of these arteries, it was shown that individuals with unexplained infertility had lower flow than fertile women. In addition, the uterine arteries PI, ovarian arteries, and subendometrial arteries PI and RI differ in pregnancy outcome in unexplained infertility patients undergoing ICSI (Ibrahim, et al. [29]).

6. Conclusions
The use of PBMC culture can improve subendometrial blood flow by decreasing the RI and the PI in PBMC group, and improve the pregnancy rate in patients undergoing ICSI cycle.

Acknowledgment
We would like to acknowledge Al Nahrain University, Baghdad, Iraq.
**Funding**
This work was funded by the corresponding author.

**Author Contribution**
Jassim, WH, performed the study, examined and reviewed results, and manuscript writing with the help and supervision of Al-Obaidi, MT, and Ghazi, HF.

**Conflict of Interest**
The authors declare no conflict of interest.

**Ethical Clearance**
The study was approved by the Ethical Approval Committee.

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Authors at OrcID
Wasan H. Jassim
https://orcid.org/0000-0002-1705-7068

Manal T. Al-Obaidi
https://orcid.org/0000-0001-8722-3159

Haidar F. Ghazi
https://orcid.org/0000-0002-1282-2146

Peer Review Information
Double-Blind Peer Review in which both authors and reviewers does not know each other.

This work was reviewed by
Asst. Prof. Dr. Khulood Majid Saeed Alsaraf
Asst. Prof. Dr. Lubna Amer Al-Anbari

Editorial Policy
The editorial policy at IJEIR ensured that this article fit the standards of scientific publications.

This work was copyedited by
Dr. Taif Alawsi
Authors Biographies

Dr. Wasan Hamad Jassim

She received the M.B.CH.B. from Al-Mustansiryia University, Baghdad, Iraq, in 2010, the High diploma (equivalent to MSc.) in Clinical Infertility & ART from the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq, in 2018. She is a member in BLS (Basic Life Support), ACLS (Advanced Cardiac Life Support), ATLS (Advanced Trauma Life Support), PLS (Pediatric Life Support), Iraqi Medical association since 2010. Currently, she is an infertility Specialist working in the private sector, and a PhD student in Clinical Infertility & ART from the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.

Dr. Manal T. Al-Obaidi

She received the M.B.CH.B. from Al-Mustansiryia University, the High diploma (equivalent to MSc.) in Assisted Reproductive Technology, Ph.D. in reproductive physiology in 1997, 2003, and 2013 respectively. She occupied several academic positions including the head of the clinical reproductive physiology department and currently, she is the dean of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University. She published more than 33 articles both local and international.

Dr. Hayder F. Ghazi

He is an assistant professor at the College of Medicine, Al Nahrain University, Baghdad, Iraq. He published more than 60 articles both international and local publications.

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