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## **EFFECT OF ANTIPHOSPHOLIPID IN IRAQI WOMEN UNDERGOING ICSI TECHNIQUE**

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### **Abstract**

*This study was conducted on 84 Iraqi women samples under the ICSI program. The sample was divided into two parts: intravenous blood section for WBC measurement and serum section for antiphospholipid (IgG & IgM) measurement, while hCG was used to indicate the success of ICSI and the presence or absence of pregnancy. The results showed a significant difference ( $p < 0.05$ ) between the pregnancy failure group and the abortion group compared with the control group (pregnancy continuation) about the level of Antiphospholipid (IgG-IgM) and WBC. The current study found a positive relationship between the level of Antiphospholipid (IgG-IgM) and WBC of these two factors, concluded that both criteria may indicate one for the other in women who suffer from miscarriage and pregnancy failure after ICSI.*

### **Keywords**

Miscarriage, ICSI Technique, Antiphospholipid (IgG-IgM), WBC

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## **1. Introduction**

Infertility is a widespread disease worldwide and it means "the inability of the spouses to achieve pregnancy within one year of marriage" and the estimated rate of infertility in the world is around 15-20% (Poongothai et al.,2009). Many factors can be affected on couple's marriage such as hormonal disorders, vitamin D, and other biomarkers to occur infertility case in one of the couples or both (Albaldawy & Alsalami, 2017; Hammood et al.,2019; Al-Shimerty & AL-Sallami,2020)

In vitro fertilization, intracytoplasmic sperm injection (ICSI), and intrauterine insemination (IUI) he is the main methods of assisted reproductive technology (ART). Found several Various studies in recent years have indicated that occur risk factor for implantation failure of women after In vitro fertilization - ICSI which may be immunological parameters or biochemical parameters may be affected on In vitro fertilization - intracytoplasmic sperm injection results (Poongothai et al.,2009).

White blood cells (WBCs) are considered an important part of the immune system in the body, It should be noted that the increase in the number of white blood cells or their lack of a normal limit indicates the presence of a health problem that afflicts the patient, the cause of white blood cells rise for many reasons, the most important of which are summarized below For pregnancy and childbirth or spontaneous miscarriage because increase Infection in Urinary tract with or immune system problem(Glenn & Armstrong,2019).

Anti-Phospholipid antibody (APA) syndrome is one of the autoimmune diseases that leads to blood clotting in both arteries and veins. "This is due to clogged blood vessels in the placenta that are responsible for delivering food and oxygen from mother to fetus during pregnancy (Erkan et al., 2017).

## **2. Materials and Methods**

This study was conducted in the laboratories of the Department of Biology, College of Science, University of Kufa, and in the Laboratory of the Fertility Center in Sadr City Medical City in Najaf Governorate / Najaf Health Directorate / Ministry of Health / Iraq.

Took about five milliliters of intravenous blood samples were drawn in the morning from Iraqi women undergoing the intracytoplasmic sperm injection (ICSI) technique. From three parts, the first part after 14 days from an injection, the second part after trimester spontaneous miscarriage while the third part for women who be continuously pregnant. Four milliliters of the blood then were left in a gel tube at room temperature for 10 minutes to complete the blood clotting, then centrifuged at 3000 rpm for 5 minutes, then the serum was separated for measurements of  $\beta$ -hCG level and Antiphospholipid (IgG-IgM) level by ELISA method and one milliliter of the blood was put in

EDTA-Na<sub>2</sub> treated collection tubes for measurements of the number of white blood cells count by GENEX HEMATOLOGY ANALYZER.

### 3. Statistical Analysis

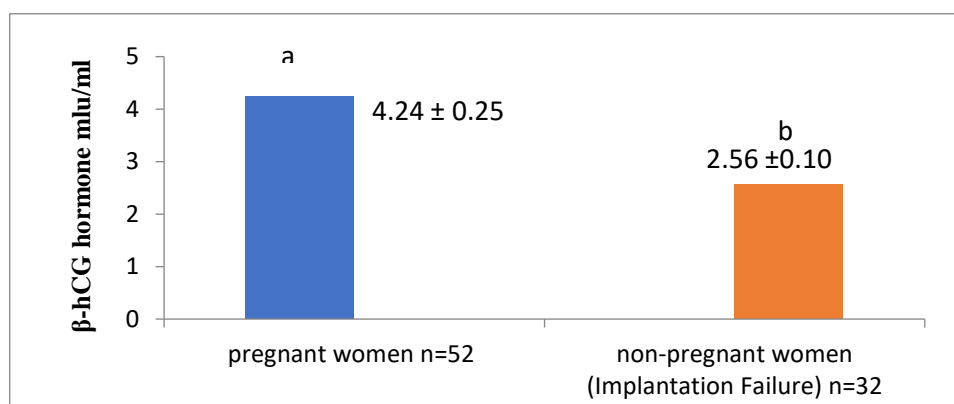
The popular statistical system (Graph Pad prism ver. 5) was adopted, and one-way analysis of variance table - ANOVA method (by Tukey's multi-comparative test) was used to compare the groups divided into the measured parameters. The results are expressed as (Mean  $\pm$  Stander Error). Descriptive statistics and correlation coefficients were performed using mega stat (V10.12 version) for excel 2010 (Motulsky,2003).

### 4. Results

The results of the current study, which was conducted on Iraqi women who underwent intracytoplasmic sperm injection technique, showed that there are significant differences between the studied groups:

#### 4.1 Results of $\beta$ - Human Chorionic Gonadotropin Hormone Test

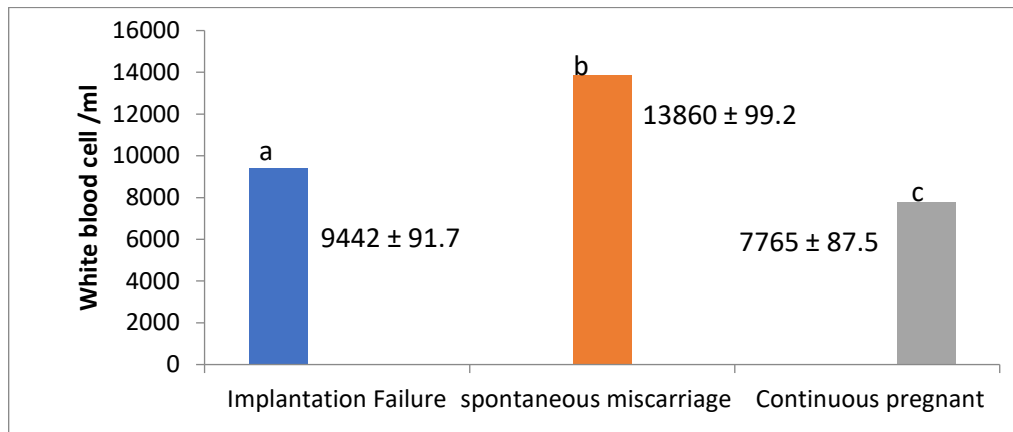
This test showed found a significant difference ( $p < 0.05$ ) between pregnant women which were 52 ( $4.24 \pm 0.25$ ) and non-pregnant women (Implantation Failure) ( $2.56 \pm 0.10$ ) which was 32 from women who undergoing intracytoplasmic sperm injection technique as shown in figure 1. The results of the current research showed a significant increase at the level of significance ( $p < 0.05$ ) in the level of the hormone  $\beta$ -hCG and that the increase in the level of this hormone is evidence of the presence of high success rates for pregnancy in women who performed operations IVF, where the success rate at the beginning of the matter reached 61.9%, after which it decreased to 33.3% after the first three months due to the occurrence of a spontaneous miscarriage of pregnant women.



**Figure 1:**  $\beta$ - Human chorionic gonadotropin hormone test which differs between pregnant women and non-pregnant (Implantation Failure). Different letters mean significant differences at ( $p < 0.05$ ) (Source: Self)

#### 4.2 Results of White Blood Cell Count

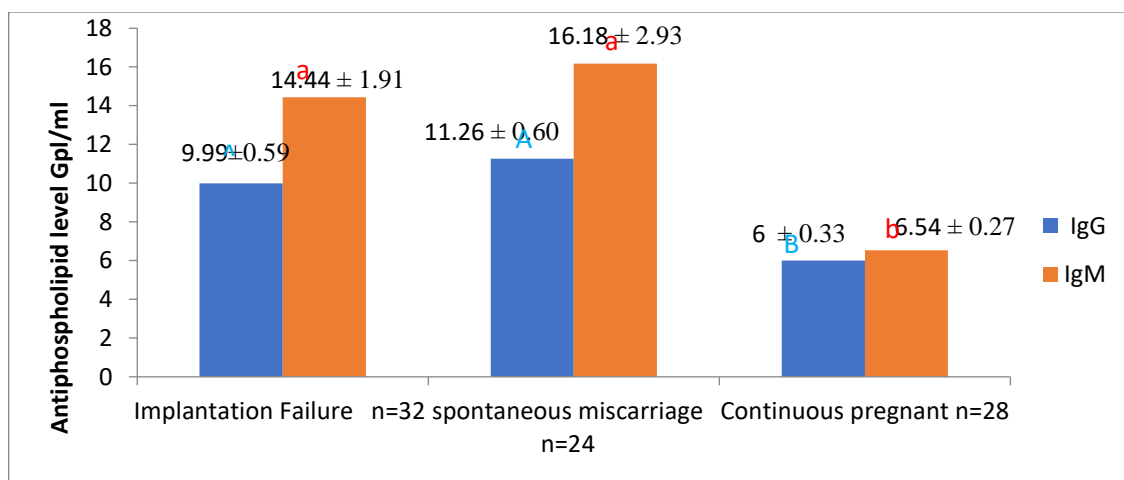
This test showed found a significant difference ( $p < 0.05$ ) between Continuous pregnant women (control group) which was 28 women and non-pregnant women groups which were 56 from women which undergoing intracytoplasmic sperm injection technique (Implantation Failure (32) and found a non-significant difference with spontaneous miscarriage (24)) figure 2.



**Figure 2:** White blood cell count test which differs between Continuous pregnant women (control group) and non-pregnant women groups which undergoing ICSI technique (Implantation Failure (32) and spontaneous miscarriage (24)). Different letters mean significant differences at ( $p < 0.05$ ) (Source: Self)

#### 4.3 Results of Antiphospholipid (IgG-IgM) Test

The results of Figure 3 showed that there was no significant difference between the two groups of spontaneous miscarriage and implantation failure, and the appearance of a clear significant difference when compared with the pregnancy continuity group in the level of Antiphospholipid effect.

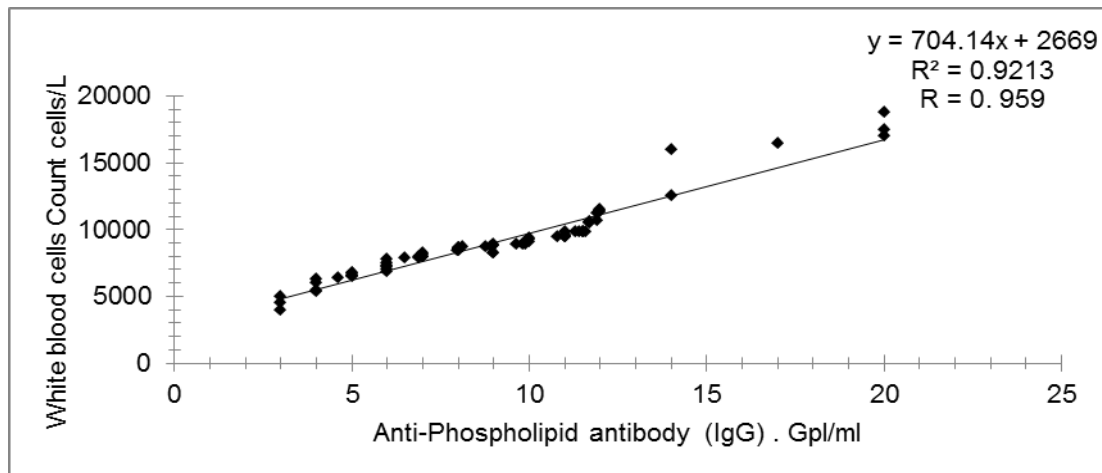


**Figure 3:** Antiphospholipid (IgG & IgM) test which differ between Continuous pregnant women (control group n=28) and non-pregnant women groups (Implantation Failure n=32) &

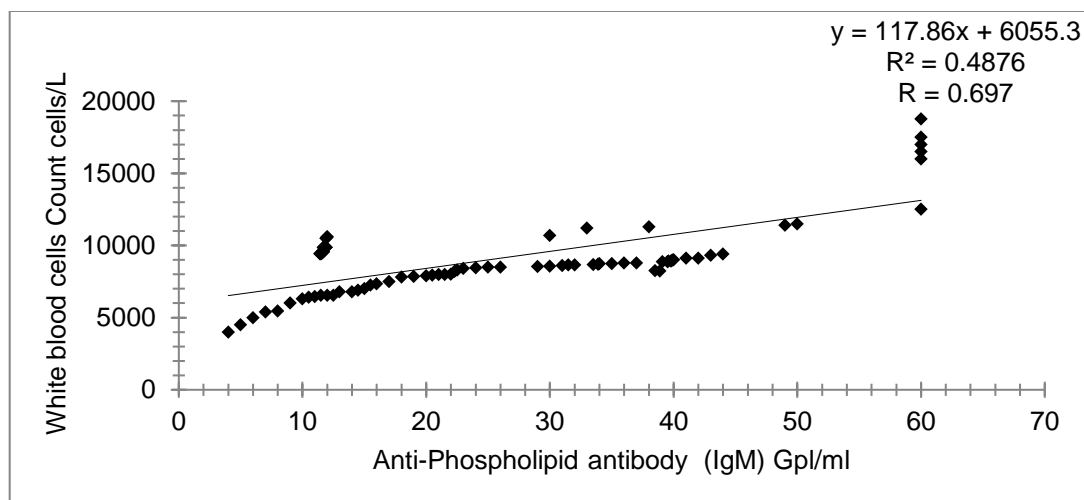
spontaneous miscarriage n=24) which undergoing ICSI technique. Different letters mean significant differences at ( $p < 0.05$ ) (Source: Self)

#### 4.4 The Correlation Between Antiphospholipid (IgG & IgM) Test with White Blood Cells Count

The study showed the presence of a positive correlation between, Antiphospholipid (IgG & IgM) test with White blood cell count in Figures 4 & 5.



**Figure 4:** The correlation between the Antiphospholipid (IgG) test with WBC in Iraqi women undergoing ICSI technique (Source: Self)



**Figure 5:** The correlation between the Antiphospholipid (IgM) test with WBC in Iraqi women undergoing ICSI technique (Source: Self)

## 5. Discussion

The results of the current research showed a significant increase in the level of significance ( $p < 0.05$ ) in the level of the  $\beta$ - Human chorionic gonadotropin hormone, and the reason for this is that this hormone is evidence of the presence of pregnancy in women who underwent ICSI operations maybe because it is excreted mainly from the placenta during the formation of the fetus and this study

is consistent with what reached (Gold et al.,2000) in this regard and that the increase in the level of this hormone is evidence of high success rates of pregnancy for women who conducted ICSI operations, where the success rate initially reached 61.9%, after which it decreased to 33.3% after the first three months due to the occurrence of spontaneous abortions for women. Pregnant women for various immunological and physiological reasons, as this study indicated, and these results are consistent with what was reached (Humaidan et al.,2005; Harun et al.,200; Theilen et al., 2017).

The results of the current research showed a significant increase ( $p<0.05$ ) of antiphospholipid antibody (IgG-IgM) in an implantation failure group and spontaneous miscarriage group compared with the continuous pregnancy group (control group) while the differences were not significant between the implantation failure group and the spontaneous miscarriage group. The reason for the anti-phospholipid antibody (IgG-IgM) concentration increase in the miscarriage women and the pregnancy failure group may be because coagulation syndrome is a blood clotting disorder that causes blood to clot in the capillaries, which may be because of the lack of blood flow and into the uterus, This leads to a weak endometrium, and thus, reduces fetal consistency in the uterus, which increases the possibility of miscarriage or pregnancy failure, while in the case of continuing the pregnancy (control group). The anti-phospholipid antibody (IgG-IgM) level is low, which means that no thrombus in the capillaries, and thus the blood flow is normal and therefore so that the fetus is a strong adhesion due to the strength of the inner lining of the uterus, which leads to the lack of miscarriage, and these results are consistent with other researches (Gao et al ., 2019 ) and (Mansour et al ., 2020) they showed that the increase in the level of anti-phospholipids antibody (IgG-IgM) in aborted women during the first trimester of pregnancy, compared with non-pregnant women, was about 15 %.

The results of the current research showed a significant increase at the level of significance ( $p<0.05$ ) between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continuing pregnancy) with respect to white blood cells count while The differences were significant between the (pregnancy failure) group and the (spontaneous miscarriage) group, and this study is consistent with the findings of (Dimitris et al., 2016 and Sharef et al ., 2020) and this result may be explained that the reason for this is that pregnant women have a low white blood cells count level compared to aborted women This may be because white blood cells count is evidence of the presence of infections in the genital and urinary tract as a result of the abortion process, where these results are consistent with what was reached Funasaka et al., (2018) , Rudnicka et al., (2020), Hammood, & Al-Sallami (2020) and Al-Shimerty &AL-Sallami, (2021) where its explained the presence of a significant increase in white blood cells women abortifacients and

pregnancy after a period when the failure of vaccination compared to non-spontaneous miscarriage women.

The current study also showed a positive correlation between the level of anti-phospholipid antibody (IgG-IgM) and WBC and perhaps the reason for this is that the increase in the level of white blood cells during pregnancy or spontaneous miscarriage in women, which is often accompanied by an increase in urinary and genital tract infections which leads to an increase in the level of anti-phospholipid antibody (IgG-IgM) (Rudnicka et al., 2020) The increase in inflammation and its indicators may cause the development of other diseases such as osteoporosis (Al-Masaoodi et al., 2019).

## **6. Conclusion**

With the strong positive correlation between white blood cells and antiphospholipid (IgG-IgM) antibodies, the current research concludes that both parameters may be indicative of one another in women who experience miscarriage and pregnancy failure after ICSI. That is, women who undergo ICSI must continue the simple and main tests, which are white blood cells with antiphospholipids, which can avoid abortion and perpetuate the continuation of pregnancy and the birth of a healthy child.

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