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Original Research Paper

Study of hematological parameters in natural pregnancy and pregnancy with IVF/ICSI techniques:

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Abstract: The present study included 90 women (36 naturally pregnant, 34 conceived via ICSI/IVF, and 20 non-pregnant controls) to evaluate hematological parameters (CBC) and inflammatory indices, including (NLR and PLR). The study was conducted in Najaf Governorate (Al-Zahraa Hospital and Fertility Center) between September 2025 and January 2026. **Results:** Both pregnancy groups exhibited significantly elevated White Blood Cell (WBC) ($p = 0.001$*), lymphocyte counts ($p = 0.009$), and granulocyte counts ($p = 0.002$*) compared to controls. In contrast, Red Blood Cell (RBC) were significantly reduced ($p = 0.001$*). Hemoglobin (HGB) and Hematocrit (HCT) levels were also significantly decreased ($p = 0.001$*) in both groups, with a more pronounced decline observed in the natural pregnancy group. Mean Corpuscular Volume (MCV) was significantly increased ($p = 0.001$*) in the IVF/ICSI group relative to both natural pregnancy and control groups. Mean Corpuscular Hemoglobin Concentration (MCHC) showed a significant reduction ($p = 0.001$*) in the IVF/ICSI group compared to natural pregnancies, although values remained higher than those of controls. Mean Corpuscular Hemoglobin (MCH) was significantly elevated ($p = 0.009$) in the IVF/ICSI group. Furthermore, both pregnancy groups demonstrated significantly higher NLR values ($p = 0.001$*) compared to controls, while PLR was significantly increased ($p = 0.028$*) exclusively in the IVF/ICSI group. **Conclusion:** In conclusion, IVF/ICSI pregnancies exhibit more pronounced hematological alterations than natural pregnancies, reflecting the impact of assisted reproduction.

Keywords: IVF/ICSI, PLR, NLR, CBC

1. Introduction

The pregnancy period is defined by the American College of Obstetricians and Gynecologists as beginning with the implantation of the embryo in the uterine wall [1]. The pregnancy stage is a period of physical changes meant to support the growth of the fetus and prepare the body for childbirth and breast-feeding [2].

In vitro fertilization (IVF) was developed to overcome infertility caused by blocked fallopian tubes. However, IVF surpasses natural fertility and all other treatments in terms of the chances of achieving a live birth in a single cycle and is therefore used to treat infertility from almost any cause [3]. Controlled ovarian stimulation, oocyte harvesting, external fertilization, and the subsequent transfer of embryos into the uterus are all part of IVF [4].

The immune response of pregnant women undergoes several alterations throughout the pregnancy period to fight off possible infections while preventing any attacks from the maternal immune system on the fetus, which is genetically distinct from her. Pregnancies are highly dependent on the ability of the maternal immune system to be adaptable to different changes that occur during the pregnancy. There is a shift in the maternal immunity profile throughout pregnancy; initially, there is a predominance of the pro-inflammatory profile followed by the anti-inflammatory one [6].

White blood cells (WBCs), red blood cells (RBCs), hemoglobin (HGB) concentration, hematocrit (HCT),

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and thrombocytes are among the hematological characteristics that are linked to pregnancy. "Physiologic anemia of pregnancy" is the term used to describe the change in these measures [7]. Physiologic anemia decreases blood viscosity, enhancing vascular permeability and the circulation of freshly generated uteroplacental components. Consequently, the developing newborn receives adequate oxygen and nutrition [8].

The Platelet to Lymphocyte Ratio (PLR) has been found to be an important indicator for any alteration in the number of platelets (PLT) and lymphocytes (LYM). This is increase due to acute thrombosis and inflammation. The ratio between PLR/PLT and LYM is termed as PLR [9]. A high neutrophil-to-lymphocyte ratio indicates physiological stress and an inflammatory state, as the NLR reflects the balance between increased neutrophils (activation of innate immunity) and decreased lymphocytes (adaptive immunity) [10].

2. Methodology

This study was conducted between September 2025 and January 2026. Venous blood samples from naturally pregnant women were collected at Al-Zahraa Teaching Hospital during routine antenatal visits, while samples from pregnant women undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) were obtained from the Fertility Center at Al-Sadr Hospital.

A total of 90 participants were included in the study: 36 naturally pregnant women (mean age 25.31 ± 5.70 years), 34 pregnant women who conceived via IVF/ICSI (mean age 30.65 ± 3.70 years), and 20 married, non-pregnant women with a history of previous childbirth as a control group (mean age 33.15 ± 7.35 years).

A structured questionnaire was administered to naturally pregnant participants to collect demographic and clinical data, including age, height, weight, medication use, gestational age, gestational diabetes status, pregnancy duration, parity, and potential risk factors for miscarriage. A similar questionnaire was used for the IVF/ICSI group, with additional information on duration of infertility and number of previous treatment attempts.

Written informed consent was obtained from all participants prior to data and sample collection.

Exclusion criteria

The experiment did not include women with diabetes or pregnant women who had a history of thrombotic problems. Women who had previously conceived via IVF and later became spontaneously pregnant were excluded, as were those who were naturally pregnant with a prior history of infertility before the current pregnancy.

Collection of blood sample

Venous blood samples were taken aseptically from the antecubital vein and placed into an EDTA tube and used immediately for the CBC analysis.

Complete Blood Count (CBC) analysis

The URIT-3000 Plus automated hematology analyzer (URIT Medical, China), utilizing optical and flow cytometric methods for leukocyte differentiation and electrical impedance for cell enumeration, was employed to assess the CBC.

Statistical Analysis

All statistical analyses were performed using SPSS, version 27 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± SD. For the comparison of three or more groups, one-way analysis of variance (ANOVA) followed by post-hoc analysis, when necessary, was applied.

3. Results and discussion

Table (1) illustrates the hematological parameters among women who are pregnant through ICSI/IVF, those with normal pregnancies, and non-pregnant control women.

A significant rise ($p=0.001^*$) in white blood cell (WBC$\times10^3/\mu L$) was noted in ICSI/IVF pregnancies ($10.37 \pm 2.90\times10^3/\mu L$) and normal pregnancies ($9.88 \pm 2.20\times10^3/\mu L$), which were higher than that of healthy non-pregnant women ($6.19 \pm 0.99\times10^3/\mu L$).

It has previously been established by the research in Port Harcourt, Nigeria, that pregnant women display elevated levels of WBC count, which gradually rises with advancing gestation [11]. The level of WBC count was determined throughout the initial seven weeks of gestation, and subsequently, it rose consistently up to 15 weeks of gestation, signifying consistent stability of

confirmation. Thereafter, it gradually rose to the highest level at 25 weeks of gestation, namely 9.9 × 10**9**/L, and then started declining gradually up to 40 weeks of gestation. The increase in the level of WBC count during the initial two trimesters was significantly high (all P values < 0.001), starting from 6.25 × 10**9**/L in pre-gestational weeks to 8.73 × 10**9**/L in the first trimester and to 9.33 × 10**9**/L in the second trimester. However, the third trimester WBC count is approximately equal to that of the second trimester (9.35 × 10**9**/L) [12].

Changes in the total and differential counts of leucocytes could be a sign of the body's defensive mechanism's physiological adjustment. The physiologic stress brought on by pregnancy is the cause of the increase in WBC that happens during pregnancy despite hemodilution [13]. It is thought to be a sign of an elevated inflammatory response [14].

The lymphocyte counts (× 10^3/μL) differed significantly (p = 0.009) across the three groups: ICSI/IVF pregnant women (2.50 ± 0.70 × 10^3/μL), naturally pregnant women (2.24 ± 0.58 × 10^3/μL), and control women (1.99 ± 0.39 × 10^3/μL).

Most studies contradicted our findings, but if we look back at the results a little, we find that the normal pregnancy value was 2.24 ± 0.58×10^3/μL, pregnancy with tubal fertilization was 2.50 ± 0.70×10^3/μL, and non-pregnancy was 1.99 ± 0.39×10^3/μL. In the analyses we performed using a CBC analyzer, the normal reference range was 1.0 - 4.11$0^3$/μL. This means that the pregnancy values were within the normal range and did not rise to a pathological level. All values are in 10^3/μL. It should also be noted that most studies measure the percentage, not the absolute number, of lymphocytes. The number of lymphocytes is expressed as an absolute number or as a percentage of white blood cells, with the normal reference range being between 20% and 40% of white blood cells in adults, or 1000 to 4000 cells/microliter [15]. Since WBC counts, especially neutrophils, increase during pregnancy, the percentage of lymphocytes may decrease, although the absolute number may remain normal or increase slightly.

In terms of granulocytes (×10^3/μL), a significant difference was found (p = 0.002*), with healthy non-pregnant women showing lower values (4.21 ± 0.89×10^3/μL) compared to naturally pregnant women (7.35 ± 2.09×10^3/μL) and those undergoing ICSI/IVF (7.90 ± 2.52×10^3/μL).

As concluded by an older study that took its samples from the cervical mucus during pregnancy. The number of granulocytes increased and also rose. after 38 weeks of pregnancy and during labor [16].This is the same result we obtained from the blood samples. Also at low Altitude, neutrophils increased in the third trimester [17]. Granulocytes consist of eosinophils, neutrophils, and basophils [18]. The gradual increase in neutrophil counts begins in the first three months of pregnancy, while eosinophil and basophil counts remain unaffected [5]. Numerous studies have connected moderate neutrophilia to pregnancy, and this increase in peripheral granulocyte numbers is the primary cause of this rise in leukocyte counts [19]. In pregnancy, neutrophils are crucial, particularly for placental growth and implantation. Vascular endothelial growth factor, which promotes angiogenesis and placental expansion, is one of the important cytokines and growth factors they produce. By regulating the production of cytokines and chemokines, neutrophils also govern the maternal immunological response. They produce the anti-inflammatory cytokine interleukin-10 (IL-10), which aids in preventing embryonic rejection and suppressing excessive maternal immune reactions. Additionally, the risk of infection rises during pregnancy due to immunological and hormonal changes, and neutrophils defend the mother by phagocytosing and eradicating invasive germs [20].

Red blood cell (RBC ×10^6/μL) also demonstrated a significant decrease (p = 0.001*) in ICSI/IVF pregnancies (4.08 ± 0.42×10^6/μL) and normal pregnancies (4.03 ± 0.39×10^6/μL) when compared to the control group (4.67 ± 0.55×10^6/μL).

The numbers of RBCs of pregnant women show a considerable variance, where the mean of RBC count in non-pregnant women are more than the average RBC count in pregnant women in northwestern Morocco [21].

For example, in another study, the researchers conducted the examination on pregnant women who were in their second trimester, and the data collected clearly indicated that the number of red blood cells was remarkably lower in pregnant women compared to healthy adult females. The level of white blood cells and neutrophils in the pregnant women was remarkably high [22]. This phenomenon is known as hemodilution because plasma expands in volume by 40–50%, whereas red cells expand by 15-20% [23].

It is well known that a rise in estrogen during pregnancy results in a reduction in erythropoietin production [24]. causes some red blood cells to be stimulated by this

hormone [25]. This causes some decrease in RBC in pregnant women.

The significant increase (p=0.001*) in hemoglobin (HGB) among pregnant women undergoing ICSI/IVF (11.54 ± 1.0g/dl) was observed when compared to those with natural pregnancies (10.81 ± 1.3g/dl), while it decreased in comparison to non-pregnant women (12.16 ± 0.5 g/dl). Additionally, there is a lower hemoglobin level in normal pregnant women compared to control women. And there is a significant increase in Hematocrit (HCT) (0.001*) during ICSI/IVF pregnancies (34.32 ± 2.89%) compared to normal pregnancies (31.50 ± 3.13%), but this value is lower than that of healthy non-pregnant women (37.02 ± 3.00%), and it is also lower in natural pregnancies compared to a healthy non-pregnant women.

Some research works have found that the HGB and HCT values among pregnant ladies are less as compared to other adults. As per an experiment, there is a significant statistical difference ($p<0.05$) between the mean HGB and HCT levels of the second-trimester and non-pregnant group as opposed to the controls. Furthermore, there is a marked distinction ($p<0.05$) between the mean HGB and HCT levels of third-trimester ladies in comparison to the controls [26]. Another research work discovered that the levels of HGB and HCT fell in the first trimester; reached their peak level in the second trimester and then again started rising in the third trimester [27].

HCT is the volume proportion of erythrocytes in the blood [28].Only red blood cells contain the protein hemoglobin. Hemoglobin's main serves to carry oxygen from the lungs to each and every cell in the body [29]. Therefore, when the value of red blood cells decreases, so will the HCT and HGB.

Blood volume rises throughout pregnancy to support the placenta's and the fetus's growth and development. CBC parameters are diluted as a result of this increase in blood volume. Because of this dilutional impact, the RBC count, HGB concentration, and HCT levels all drop during pregnancy [30]. To date, there are no clear scientific studies on HGB and HCT levels in pregnancies following IVF/ICSI compared to natural pregnancies.

The ICSI-IVF protocol begins with ovarian stimulation [31]. Ovaries become hyperstimulated when hCG is administered to induce ovulation. Increased capillary permeability brought on by an increase in different interleukins causes fluid to move from the intravascular to the extravascular compartment. Additionally, the quick fluid transfer results in hemoconcentration and hypovolemia. The increase in the hematocrit level reflects the hemoconcentration that takes place [32] and we believe the same applies to hemoglobin.

Mean Corpuscular Volume (MCV) measurements exhibited significant variation (P=0.001*) among the three groups, with IVF pregnancies demonstrating higher values (84.77 ± 6.8fL) than natural pregnancies (78.03 ± 6.3fL) and non-pregnant women (80.09 ± 8.8fL), while indicating a non-significant reduction in normal pregnancies compared to healthy non-pregnant women. For Mean Corpuscular Hemoglobin Concentration (MCHC), there is also significant variation (P=0.001*); however, IVF pregnancies exhibit diminished values (33.54 ± 0.9 g/dl) compared to normal pregnancies (34.28 ± 2.04g/dl) and show an increase from control women (32.63 ± 1.5g/dl). Additionally, MCHC levels are higher in normal pregnancies compared to those in healthy non-pregnant women. Mean Corpuscular Hemoglobin (MCH) showed a significant variation (0.009), in ICSI/IVF pregnancy (28.42 ± 2.2pg) was highest from normal pregnancy (26.82 ± 2.82pg) and healthy non pregnant (26.35 ± 2.91pg).

The Reference Interval to the MCHC 30.0–35.0 g/dl [33]. And the result us was between this range so Although there is a statistically significant difference between normal and IVF pregnant and non-pregnant women However, it is within the normal range. In one of the study the MCHC value was in pregnant women 32.52 ± 0.76 g/dl and this was Close to the results we have reached [34].

In pregnant women, hemoglobin levels and MCV are decreased in the first trimester when compared with non-pregnant women; thus, there is clinical significance in the parameters calculated based on these two, namely MCH and hematocrit, when compared with non-pregnant women. Furthermore, both white blood cells and granulocytes are clinically more increased [35].

In a study carried out at Omdurman Al Saudi Maternity Hospital between May 3 and June 6, 2011, the hematological parameters of pregnant Sudanese women were assessed and established. Pregnant women had considerably lower MCV, MCH, and MCHC than non-pregnant women (Pvalue <0.05) [36]. MCV does not alter much during pregnancy, but it does slightly rise in comparison to the control group. This increase peaks between weeks 30 and 35 and does not indicate a

vitamin B12 or folate shortage. Increased RBC production to satisfy pregnancy's demands. During pregnancy, MCH and MCHC did not change [37].

In a study conducted by Ainadis Alemu et al., they reached conclusions that contradicted our findings on natural pregnancy but were consistent with pregnancy induced by ICSI/IVF. This study showed that pregnant women had higher MCV and MCH level than control .The increase in MCV indices is a reflection of physiological stress resulting in increased variation in size and shape of RBCs and platelets [34].

Pregnant women showed considerably lower RBC counts, HGB, HCT, and PLT and significantly greater MCV and MCH than non-pregnant women [38]. The MCV, MCH and MCHC were low in 60.1%, 59.5% and 59.5% respectively, in pregnant female while high in few[39].

The findings in figure (1) indicated a substantial elevation ($p=0.001$) in platelet count among ICSI/IVF pregnant individuals ($358.50 \pm 88.33 \times 10^3/\mu L$) in contrast to the normal pregnant group ($267.86 \pm 64.46 \times 10^3/\mu L$) and the non-pregnant ($235.70 \pm 44.93 \times 10^3/\mu L$). According to a study on platelet indicators higher in non-pregnant compered normal pregnant and women conducted in a tertiary care hospital in Northern India [40]. Additionally, the platelet counts higher were not statistically significant in another study conducted in Yemen [41]. The findings of a study carried out between 2011 and 2014 at Oklahoma University Medical Center. When compared to women who are not pregnant, it revealed a drop in platelet count during a typical pregnancy [42].

Several physiologic processes that take place during pregnancy, such as platelet sequestration and utilization in the placental vasculature and platelet dilution caused by the expansion of maternal blood plasma volume, account for the continuous fall in the average platelet count [43]. It has been proposed that increased platelet turnover during pregnancy lowers platelet counts [44]. This means that the rate of platelet turnover and functional consumption increases during pregnancy, which may be compensated for by increased production to maintain numbers close to normal.

Research on platelet count (PLT) during pregnancy after IVF is very rare, making our study extremely important. Our research found that PLT levels are higher during pregnancy after ICSI/IVF than in natural pregnancies. There are several reasons for this elevated PLT, including:

1.hemorrhage:Hemorrhage-related thrombocytosis (secondary thrombocytosis) [45]. First-trimester bleeding is more common with IVF/ICSI conception than with spontaneous conception [46]. And also that may cause an increase in PLT in ICSI/IVF pregnant women in the study.

2. iron deficiency : One possible prothrombotic condition is severe iron deficiency anemia with subsequent thrombocytosis [47]. which is dose-dependently reversed by iron replacement therapy [48].

3.Inflammatory: Chronic inflammatory diseases and infections are secondary causes of thrombocytosis [49]. Since inflammation is likely to increase during IVF due to the embryo retrieval, transfer, and embryo transfer protocols, this may be a significant reason for elevated platelet counts during IVF compared to natural pregnancy.

Figure (2) showed a notable increase ($p=0.028$) in PLR between ICSI/IVF group (151.06 ± 46.35) compared with natural pregnancies (127.37 ± 48.68), and non-pregnant women (121.91 ± 25.27). Also in figure (3) Neutrophil to lymphocyte ratio was significantly increase (0.001) in the pregnancy using IVF (3.32 ± 1.27) and natural pregnancy (3.51 ± 1.38) compared to the control group (2.19 ± 0.55) .

Drawing from the Japan Environment and Children's Study, which included 76,853 singleton pregnancies (between 28 and 41 weeks), the distribution of the NLR and PLR indices was monitored. By tracking measurement curves at specific intervals (8–11, 12–17, and 18–21 weeks), The findings showed that as the pregnancy went on, the levels of these two indices gradually and significantly increased [50].

Pregnancy is a state of inflammation. The NLR [51] and PLR, are peripheral serum markers commonly utilized as cost-effective indicators of inflammation [52]. Due to the increase in neutrophils and the temporary decrease in lymphocytes, the NLR rises. In a similar vein, the PLR ratio rises because platelets can be mobilized in response to inflammation even though they were originally intended for blood coagulation [53]. Between weeks five and twenty of pregnancy, blood cortisol levels rise [54]. Leukocyte recruitment from the circulation to tissues and increased cortisol secretion brought on by the hypothalamic-pituitary-adrenal axis might result in a drop in the absolute lymphocyte count and an enhanced PLR [55].

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Table 1: A comparative study of hematological parameters in women conceived through ICSI/IVF compared to women conceived naturally and a control group of non-pregnant women.

Study parameters \ Study groups	(Mean ± SD) ICSI/IVF pregnancy N=34	Normal Pregnancy N=36	Healthy Non pregnant N=20	p-value
WBC (×10³\µL)	10.37 ± 2.90 (a)	9.88 ± 2.20 (a)	6.19 ± 0.99 (b)	0.001*
Lymphocytes (×10³/µL)	2.50 ± 0.70 (a)	2.24 ± 0.58 (a)	1.99 ± 0.39 (b)	0.009
Granulocytes (×10³/µL)	7.90 ± 2.52 (a)	7.35 ± 2.09 (a)	4.21 ± 0.89 (b)	0.002*
RBC (×10⁶ /µl)	4.08 ± 0.42 (a)	4.03 ± 0.39 (a)	4.67 ± 0.55 (b)	0.001*
HGB (g/dl)	11.54 ± 1.0 (a)	10.81 ± 1.3 (b)	12.16 ± 0.5 (c)	0.001*
HCT (%)	34.3 ± 2.89 (a)	31.5 ± 3.13 (b)	37.02 ± 3.00 (c)	0.001*
MCV (fL)	84.77 ± 6.8 (a)	78.03 ± 6.35(b)	80.09 ± 8.8 (b)	0.001*
MCH (pg)	28.42 ± 2.2 (a)	26.82 ± 2.8 (b)	26.35 ± 2.9 (b)	0.009
MCHC (g/dl)	33.54 ± 0.9 (a)	34.28 ± 2.04(b)	32.63 ± 1.5 (c)	0.001*

The values are given as mean ±SD, SD: standard deviation.* substantial deference (P<0.05).No notable differences are indicated by similar letters. Significant differences are indicated by different letters.

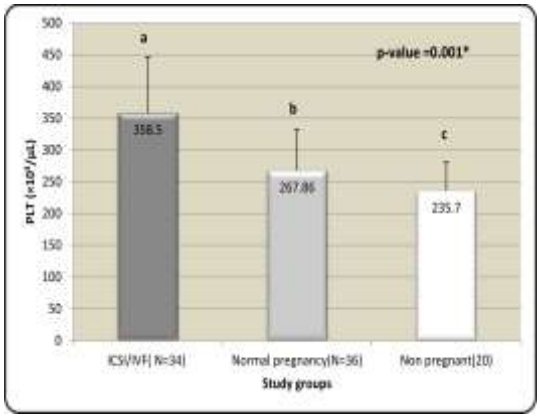

Fig 1: Platelet counts of ICSI/IVF pregnant, normal pregnant, and non-pregnant control women are compared. The values are given as mean ±SD, SD: standard deviation.*significant deference (P<0.05). No notable differences are indicated by similar letters. Significant differences are indicated by different letters.

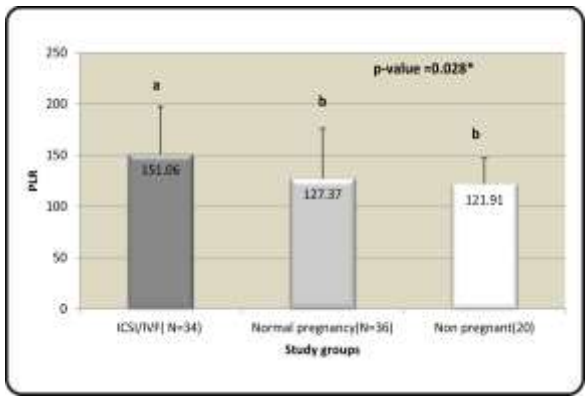

Fig 2: Comparative Study on the Platelet to Lymphocyte Ratio in Pregnant Women Undergoing ICSI/IVF, Normal Pregnant Women, and Non-Pregnant Women. The results are represented as Mean ± SD. SD = Standard Deviation.*Significant deference (P<0.05).Same letter means there is no significant deference.

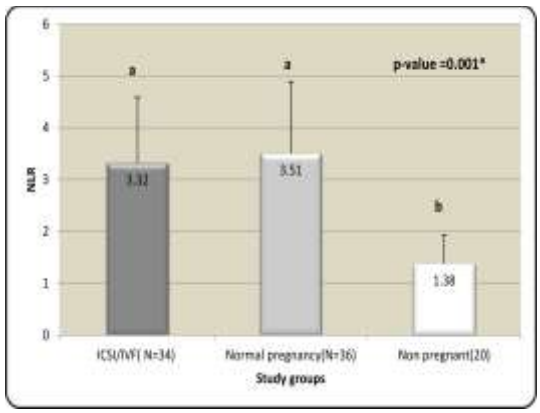

Fig 3: Neutrophil/lymphocyte ratio comparison among ICSI/IVF pregnant, normally pregnant, and non-pregnant controls. Data represented in mean ±SD, SD: Standard Deviation.*statistical difference (P<0.05).Same letter shows no statistical difference. Difference in letter denotes statistical difference.

Conclusion

This study demonstrates significant differences in selected hematological and inflammatory parameters between natural pregnancies and those achieved via IVF/ICSI. Women in the IVF/ICSI group exhibited significantly higher PLT, HGB, HCT, MCV, and MCH levels, whereas MCHC was significantly lower in natural pregnancies. Among the inflammatory markers, PLR

emerged as a significant discriminator between the two groups, while WBC, lymphocyte count, granulocyte count, RBC, and NLR showed no significant differences. These findings may reflect the physiological and inflammatory effects associated with hormonal stimulation protocols and alterations in coagulation pathways during assisted reproduction. Collectively, the results suggest that PLR and red blood cell indices could serve as accessible and cost-effective markers for monitoring pregnancy status in IVF/ICSI settings.

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Author's Contributions

The princeple investigator, **Zainab Mohanad Ali,** carried out the studies, collected data, and wrote the report. As academic supervisors, Professors **Dr.Suaad Mohammad Joda AL-Hadrawy** gave scientific advice, oversaw the study's design and interpretation, and critically revised the work for intellectual content. The manuscript was reviewed and approved by both authors before to submission.

Ethics

The University of Kufa's medical ethics committee gave its clearance for this study (2025). Parents gave both written and verbal consent, and participants and researchers agreed to publication.

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