

Assessment of hematological and immunological changes during first vs. third trimester of pregnancy

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ABSTRACT

The purpose of this study was to evaluate immunological and hematological changes in a sample of pregnant women that occurred between the first and third trimesters of pregnancy. To support fetal development and prepare the mother for delivery, pregnancy is a dynamic physiological state characterized by progressive changes in blood parameters, immune function, and renal physiology. Differentiating between pathogenic circumstances that may affect the health of the mother and fetus, and normal physiological changes requires an understanding of these changes. This study used a comparative cross-sectional analysis. Blood and urine samples were taken from 57 pregnant women in different places, including family and primary healthcare centers. between February and April, 28 of whom were in the first trimester and 29 of whom were in the third. In addition to a notable increase in positive urine cultures, especially *Escherichia coli*, there was a notable increase in proteinuria, glycosuria, and pyuria throughout the third trimester. These results point to a higher risk of renal physiological stress and UTIs during the latter stages of pregnancy. Red blood cell shape clearly changed throughout pregnancy, according to blood film analysis. Normal results and normocytic normochromic patterns predominated during the first trimester, Mild anemia and hypochromic microcytic anemia significantly increased by the third trimester. In line with physiological anemia of pregnancy, hemoglobin levels dramatically dropped throughout the third trimester. Additionally, platelet levels significantly decreased, most likely because of gestational thrombocytopenia. On the other hand, white blood cell counts dramatically rose, primarily due to neutrophilia, which is indicative of leukocytosis. Lymphocyte levels also dropped, suggesting immunological regulation that is necessary for preserving fetal tolerance and is typified by a change from cell-mediated immunity to humoral immunity.

Keywords: Pregnancy, First Trimester, Immunological Changes, Physiological Anemia, Gestational Thrombocytopenia.

Introduction

Significant anatomical, hormonal, and metabolic changes occur during pregnancy, which is a unique physiological state that is carefully planned to support the growth and development of a semi-allogeneic embryo. To ensure fetal life and growth without compromising the mother's ability to protect against foreign infections, this calls for a thorough and dynamic remodeling of both the hematopoietic and

immune systems (Li and Huang, 2009). Comprehending these intricate changes is not just a matter of academic interest; rather, it is an essential basis for deciphering the course of a typical pregnancy and identifying the early indicators of major pathological complications like recurrent miscarriage, pre-eclampsia, intrauterine growth restriction (IUGR), and preterm birth (Svensson-Arvelund et al., 2014). The oversimplified earlier

beliefs that saw pregnancy as a time of "immunodeficiency" or only a change to a Th2 adaptive response have been superseded by more advanced scientific hypotheses. The hematopoietic and immunological systems undergo closely controlled, interrelated alterations during gestation, with their profiles drastically changing between the start and conclusion of pregnancy, according to current evidence, which presents a more accurate and interactive picture (Branch, 1992). The innate immunity and coagulation systems serve as the organism's first line of defense against external threats, such as foreign antigens and mechanical damage, demonstrating the importance of this interaction (Svensson-Arvelund et al., 2014). The intricate relationships between these two systems have been thoroughly investigated; coagulation proteases have definite immunomodulatory effects, whereas immune cells are essential in starting the coagulation cascade (Kohli and Blois, 2025). When faced with external obstacles, this physiological crosstalk which is necessary for a healthy pregnancy can turn into a pathologically vicious loop that reinforces coagulation and inflammation (Svensson-Arvelund et al., 2014). Therefore, it has been suggested that hemostatic instability and immunological maladaptation are the main causes of many unfavorable pregnancy outcomes (Faas and de Vos, 2017). Fetal trophoblast cells penetrate the endometrial tissue in the first trimester of pregnancy (months 1-3), right after embryo implantation, and develop into the decidua, which secures the placenta and guarantees fetal nourishment (Branch, 1992). To preserve the integrity of the uterus, this trophoblastic invasion necessitates strict restrictions. As a result, the endometrium contains local immune cells prior to implantation, such as uterine natural killer (uNK) cells, macrophages, and regulatory T cells (Tregs), and their numbers rise and change right away following implantation encouraging trophoblast polarization and angiogenesis, these cells help placental establishment in addition to controlling the delicate balance between tolerating the trophoblast and restricting its invasion (Branch, 1992; Kohli and Blois, 2025). More importantly, there is a noticeable change in the hemostatic system that leads to a physiological "hypercoagulability" condition; platelet counts rise, coagulation factor levels like fibrinogen rise, and the action of natural anticoagulants falls (Kohli and Blois 2025; Faas and de Vos, 2017; Valdés, 2011). The intimate relationship between the immune and coagulation systems is reflected in this change, which shields the mother from severe bleeding after

placental separation. Coagulation proteases regulate immunological responses, while activated immune cells help start the coagulation cascade (Kohli and Blois, 2025). Studies on immunology show that innate immune activation persists and advances. The third trimester has shown an increase in nonclassical monocytes (CD14+, CD16+), which are thought to be more proinflammatory because of their capacity to produce higher levels of inflammatory cytokines including tumor necrosis factor (TNF) and IL-1.

The process of getting the uterus and cervix ready for labor is thought to include this increased innate immune activation, which partially parallels alterations that occur in systemic inflammatory disorders like sepsis (Valdés, 2011). Dendritic cells, on the other hand, have more tolerogenic qualities; they produce less interferon- α and express more markers linked to tolerance, such as CD200 (Branch, 1992). A more intricate paradigm involving a delicate balance between T helper cells (Th1, Th2 and Th17) and regulatory T cells (Tregs), which are highly concentrated at the maternal-fetal interface to promote immune tolerance, has replaced the straightforward Th2 shift model in the context of adaptive immunity. The scientific foundation for understanding normal pregnancy physiology is an integrated understanding of these parallel and overlapping alterations between the immune and hematopoietic systems throughout various stages of pregnancy. This knowledge is crucial for creating accurate biomarkers that can identify pregnancies that are at risk, like pre-eclampsia, whose pathophysiology is connected to malfunction in these intricate immuno-hematological interactions (Valdés, 2011). Additionally, figuring out these pathways opens up new avenues for finding possible therapeutic targets to enhance the management of vascular pregnancy problems and protect the health of the mother and fetus in the short and long term (Fadhil, 2025).

Materials and Methods

In order to assess hematological and immunological changes between the first and third trimesters of pregnancy, this study was planned as a comparative cross-sectional study. The study started in February and run through April. Data and samples were gathered from a variety of sources, including friends, family, and primary healthcare facilities. Samples of women's blood and urine were taken, transferred to our institution, and tested in the department's labs under the supervision of the supervising professor. For hematological analysis, two to three milliliters of venous blood were drawn under aseptic conditions

and placed in an EDTA tube. Urine samples from midstream clean catch were gathered in sterile containers. Samples were processed right away for routine urinalysis, microscopic analysis, and culture.

Results and Discussion

Age groups by trimester of pregnancy: The age distribution of pregnant women in the first and third trimesters is compared in Table (1), which reveals that most pregnant women in both groups are between the ages of 20 and 30. This result is in line with World Health Organization studies (WHO, 2023), which show that the majority of pregnancies worldwide take place in women between the ages of 20 and 34 because of optimal fertility and decreased obstetric risk compared to extremes of age. In the third trimester, older age groups (30–40 and over 40) saw a relative increase. This change may be the result of sample variation, but it is consistent with global demographic trends that indicate an increase in mother age. According to studies like those compiled by the American College of Obstetricians and Gynecologists (Walker et al., 2016), social, educational, and economic factors are contributing to the increasing prevalence of delayed childbirth. The third trimester group's apparent increase in this category may indicate that older women are more willing to continue their pregnancies under close medical supervision. While the percentage of women in the 20–30 age group dropped from 60.7% in the

first trimester to 48.3% in the third, older age groups (30–40 and >40 years) had a relative increase in the third trimester. This change may be the result of sample variation, but it is consistent with global demographic trends that indicate an increase in mother age. On the other hand, pregnancies among women under 20 were rare in this cohort and nonexistent in the third trimester group. This finding is in line with international data showing that teenage pregnancies, although still common in some areas, are frequently associated with increased chances of unfavorable outcomes, such as preterm delivery and fetal problems. Younger mother age is often linked to socioeconomic and healthcare access issues, which may affect study representation, according to United Nations Population Fund reports (UNFPA, 2022). Women between the ages of 20 and 30 make up the biggest percentage of prenatal participants, according to studies done in Baghdad and Basra; lesser percentages are seen at the extremes of age. Furthermore, recent observations in Iraqi populations that show a gradual shift toward delayed childbearing, especially in urban areas, due to socioeconomic and educational factors (Fadhil, 2025; Fadhil and Saheb, 2024), and numerous other Iraqi studies that come into contact with these results (Haider et al., 2013; Hashim et al., 2025; Atta and Jomah, 2025), are consistent with the slight increase in women aged ≥ 30 years in the third trimester group.

Table (1): Distribution of age groups among pregnancy trimesters in study samples

	Trimester			
	First		Third	
	No.	%	No.	%
Age				
< 20 years	2	7.1	0	0
20 - 30 years	17	60.7	14	48.3
30 - 40 years	7	25	10	34.5
> 40 years	2	7.1	5	17.2
Total	28	100	29	100
Chi-square test of independence (p – value) = 0.25				

Pregnancy stages and urine parameters: Using Chi-square analysis, the distribution of urine parameters among women in the first and third trimesters is shown in Table (2) as numbers and percentages. There is a statistically significant variation in urine protein between trimesters ($p = 0.008$). Only 3.5% of the women had 1+ levels during the first trimester, whereas nearly half (46.5%) had negative protein and 50% had trace levels. On the other hand, there is a noticeable shift toward greater protein levels

throughout the third trimester: negative cases drop to 13.8%, trace instances climb to 62%, and 1+ cases significantly increase to 24.2%. This suggests that proteinuria increases in frequency and severity in the latter stages of pregnancy, which may be due to physiological changes in the kidneys but also raises the possibility of hypertensive conditions such as pre-eclampsia (WHO, 2016; Wallis et al., 2008). Additionally, the results seem to indicate a highly significant difference in urine glucose ($p < 0.001$). In

the first trimester, every woman tested negative for glucose (100%), but in the third trimester, only 44.8% were still negative and 55.2% had trace glycosuria. This result may suggest a higher risk of gestational glucose intolerance in later stages and is consistent with the decreased renal threshold for glucose and elevated glomerular filtration rate during pregnancy (Cunningham et al., 2022 (There is a significant change in urine WBCs ($p = 0.013$). 21.5% had 1+, 42.8% had trace, and 35.7% had no WBCs throughout the first trimester; there were no instances of 2+. In the third trimester, fewer women had no WBCs (24.1%), trace was 34.5%, 1+ dropped to 10.3%, and a significant percentage (31.1%) had 2+. This change toward more severe pyuria points to a higher incidence of infection or inflammation of the urinary system in the latter stages of pregnancy. There is no discernible difference in urine RBCs ($p = 0.366$). The distribution is rather similar between trimesters: 53.6% of women in the first trimester had "nil" RBCs, compared to 41.4% in the third, while higher degrees (1+ and 2+) only slightly differ. This suggests that there is no significant correlation between hematuria and gestational age in this study. The results of the urine culture indicate a highly significant difference ($p < 0.001$). Only 3.6% of samples tested positive for *Escherichia coli* during the first trimester, and nearly

all samples (96.4%) showed no bacterial growth. On the other hand, no-growth instances drop to 48.3% during the third trimester, while *E. coli* growth sharply rises to 51.7%. This indicates a significant increase in UTIs during the latter stages of pregnancy, consistent with the physiological predisposition to urinary stasis and bacterial colonization (Cunningham et al., 2022; UNFPA, 2022). This is consistent with data from Iraq showing that the most common bacteria causing UTIs in pregnant women is *E. coli*, and that infection rates in Iraq are higher than in other nations (Hanoon, 2024). This validates the link between elevated pyuria and verified bacteriuria in the findings of our investigation. These results are in line with several Iraqi research that show how pregnancy progression affects renal physiology and the risk of infection. Iraqi epidemiology data provide substantial support for this conclusion. Urinary tract infections (UTIs) are very common among pregnant women, with an increased frequency as pregnancy progresses due to urinary stasis and hormonal effects, according to a meta-analysis done in Iraq. Bacterial growth is facilitated by progesterone-induced relaxation of the urinary system and the mechanical pressure of the expanding uterus (Hanoon, 2024; Tawfeeq et al., 2025).

Table (2): Distribution of urine parameters among pregnancy trimester in study samples

		Trimester				Total	Chi-Square
		First		Third			
		No.	%	No.	%		
Urine Protein	Negative	13	46.5	4	13.8	17	0.008
	Trace	14	50	18	62	32	
	1 ⁺	1	3.5	7	24.2	8	
	Total	28	100	29	100	57	
Urine Glucose	Negative	28	100	13	44.8	41	< 0.001
	Trace	0	0.0	16	55.2	16	
	Total	28	100	29	100	57	
	Nil	10	35.7	7	24.1	17	
Urine WBC (HPF)	Trace	12	42.8	10	34.5	22	0.013
	1 ⁺	6	21.5	3	10.3	9	
	2 ⁺	0	0.0	9	31.1	9	
	Total	28	100	29	100	57	
Urine RBC (HPF)	Nil	15	53.6	12	41.4	27	0.366
	Trace	7	25	6	20.7	13	
	1 ⁺	2	7.1	7	24.1	9	
	2 ⁺	4	14.3	4	13.8	8	
	Total	28	100	29	100	57	
Urine Culture	No growth	27	96.4	14	48.3	41	< 0.001
	<i>E. coli</i> growth	1	3.6	15	51.7	16	
	Total	28	100	29	100	57	

Examining blood films: The distribution of blood film results, including percentages, among women in the first and third trimesters is shown in Table (3), with a highly significant difference between groups (Pearson Chi-square, $p < 0.001$). Only the first trimester (6/28; 21.4%) had normal morphology, while the third trimester (0.0%) had no cases. This suggests that entirely normal hematological profiles are more prevalent in the early stages of pregnancy but tend to decline as the gestational period lengthens. The most common result in both trimesters is mild anemia, which affects 39.3% (11/28) of women in the first trimester and rises to 55.2% (16/29) in the third. This increase is a result of both physiological hemodilution and rising nutritional needs, especially in the later stages of pregnancy (Khalil et al., 2009). The normocytic normochromic pattern is missing in the third trimester (0.0%) and only occurs in the first trimester (39.3%; 11/28). Hypochromic microcytic anemia, on the other hand, is not present in the first trimester (0.0%) but becomes noticeable in the third trimester (44.8%; 13/29). Red blood cell morphology clearly and statistically significantly changed throughout pregnancy, according to the research. Normal blood films and normocytic normochromic anemia, which represent physiological adjustments, predominate in the first trimester. But during the third trimester, there is a noticeable shift toward hypochromic microcytic alterations and mild anemia, which are indicative of iron deficiency. These variations represent actual physiological and pathological changes throughout pregnancy, as confirmed by the highly significant p-value ($p < 0.001$). The idea of physiological anemia of pregnancy is consistent with the prevalence of normocytic normochromic cells, which implies that anemia at this

time is mostly caused by hemodilution rather than actual deficient situations (Cunningham et al., 2022; UNFPA, 2022). On the other hand, there is a noticeable change toward pathological red cell morphology during the third trimester. Due to increased fetal iron needs and maternal iron storage depletion in late pregnancy, hypochromic microcytic anemia is a powerful indicator of iron shortage (WHO, 2020; Milman, 2012). The shift from physiological to nutritional anemia is further supported by the third trimester's total lack of normocytic normochromic patterns. These results align with a number of studies conducted in Iraq and the surrounding area. For instance, a study carried out in Baghdad by (Al Husaini and Al Hilfy, 2025) found that women's microcytic hypochromic anemia significantly increased in the third trimester compared to the first, and they attributed this to insufficient iron supplementation (Tawfeeq et al., 2025). Similarly, iron deficiency anemia was the most common hematological anomaly in late pregnancy, according to research from Basra by Hasan et al. (2025), with blood film examination showing a high frequency of microcytic hypochromic cells. These regional findings support the present findings and emphasize the persistent problem of maternal anemia in Iraqi public health. Additionally, the results are consistent with published literature on a global scale. According to the World Health Organization, anemia becomes more common as pregnancy progresses, especially in low- and middle-income nations where iron deficiency is still the primary cause. Additionally, research by Milman (2012) has highlighted that women are at risk for microcytic hypochromic anemia because their iron needs increase dramatically during the third trimester, frequently surpassing food intake.

Table (3): Distribution of blood films among pregnancy trimesters in study samples

		Trimester			
		First		Third	
		No.	%	No.	%
Blood Film	Normal	6	21.4	0	0.0
	Mild anemia	11	39.3	16	55.2
	Normocytic normochromic	11	39.3	0	0.0
	Hypochromic microcytic	0	0.0	13	44.8
	Total	28	100	29	100
Pearson Chi-Square = < 0.001					

A one-way ANOVA comparison of two groups across multiple hematological parameters is shown in Table (4). Between the first and third trimesters, hemoglobin levels dropped dramatically from 12.65 g/dL to 10.96 g/dL ($p < 0.001$). This decrease reflects

the well-known physiological anemia of pregnancy, which is mostly caused by hemodilution. Hemoglobin concentration decreases progressively during pregnancy due to a disproportionate increase in plasma volume relative to red cell mass. This result is

in line with well-established research that demonstrates hemoglobin usually falls during pregnancy, peaking in the third trimester. (Rahman and Mohammed, 2018; Saleem et al., 2022; Tawfeeq et al., 2025; Khalil, 2017). Significant decreases may point to iron shortage or other pathogenic causes, even when moderate anemia is physiological. The platelet count decreased significantly from $260 \times 10^9/L$ to $204.96 \times 10^9/L$ ($p < 0.001$). Gestational thrombocytopenia, which results from hemodilution, increased platelet consumption, and increased splenic sequestration, is frequently blamed for this decrease. In simple pregnancies, platelet counts typically stay within the normal range despite the decline and are not linked to bleeding risk. The WHO states that anemia during pregnancy is defined as a hemoglobin level of less than 11.0 g/dl, with two severity levels: mild anemia (Hb level 9 to 10.9 g/dL) and severe anemia (Hb level 7 to 8.9 g/dL) (WHO, 2020). The WBC count rose from $7.66 \times 10^9/L$ to $10.14 \times 10^9/L$ ($p < 0.001$). This increase is indicative of pregnancy-related physiological leukocytosis, which is primarily caused by hormonal shifts and increased bone marrow activity. It is also seen as an adaptive

mechanism to strengthen the immune system of the mother. Neutrophils are the primary cause of the rise. The percentage of neutrophils rose dramatically from 56.16% to 67.52% ($p < 0.001$). This increase is indicative of neutrophil dominance in leukocytosis associated with pregnancy. Hormonal factors, particularly cortisol and estrogen, increase the generation of neutrophils and decrease apoptosis. An improved innate immune response is a result of this. The percentage of lymphocytes dropped dramatically from 32.32% to 29.16% ($p = 0.015$). During pregnancy, the immune system shifts from cell-mediated immunity (Th1) to humoral immunity (Th2), which is linked to the decrease in lymphocytes. Because the fetus is immunologically semi-allogeneic, this adaptability aids in preventing fetal rejection. These alterations are a reflection of physiological modifications to promote fetal growth, preserve maternal health, and regulate immunological tolerance. All of these findings are consistent with numerous studies conducted in Iraq (Fadhil, 2025; Haider et al., 2013; Hameed and Ali, 2023; Saleem et al., 2022; Tawfeeq et al., 2025; Hasan et al., 2025).

Table (4): Distribution of complete blood count and blood film among pregnancy trimester in study samples

		Trimester		Sig.
		First	Third	
Hemoglobin (g/dL)	Mean	12.648	10.956	< 0.001
	SD	0.604924	0.620537	
WBC ($\times 10^9/L$)	Mean	7.66	10.144	< 0.001
	SD	0.919239	1.537877	
Platelets ($\times 10^9/L$)	Mean	260	204.96	< 0.001
	SD	28.04758	25.68313	
Neutrophils (%)	Mean	56.16	67.52	< 0.001
	SD	4.129972	4.54716	
Lymphocytes (%)	Mean	32.32	29.16	0.015
	SD	4.308132	4.552289	

Conclusions

Significant physiological and immunological changes occur during pregnancy, intensifying from the first to the third trimester. The study found that third-trimester women exhibited lower platelet counts and hemoglobin concentrations, along with higher neutrophil and total white blood cell counts, indicating normal immune modulation. Urine analysis revealed increased proteinuria, glycosuria, pyuria, and Escherichia coli urinary tract infections in late pregnancy, suggesting renal stress. A blood film examination showed a shift from normal red blood cell morphology in the first trimester to hypochromic microcytic anemia in the third trimester, indicative of

iron depletion. These findings underscore the importance of routine hematological and urine monitoring to identify pathological conditions and improve maternal and fetal health outcomes through early intervention.

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