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Immunohistochemical Profiling of CD68 and VEGF in First-Trimester Miscarriage Placentas

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ABSTRACT

Background: Miscarriage occurs when a pregnancy ends spontaneously before the fetus is viable outside the uterus. Early miscarriage, sometimes referred to as first-trimester miscarriage, which happens before 12 weeks. Vascular endothelial growth factor (VEGF), an angiogenic protein necessary for blood vessel formation, and cluster of differentiation 68 (CD68), a marker for macrophages, are vital to the immunological response. This research aimed to evaluate VEGF and CD68 expression patterns in first-trimester miscarriage in placental tissues and decidua in a bid to ascertain their potential function in immune response and angiogenesis in miscarriage.

Methods: The study included 60 women who experienced spontaneous abortion during the first trimester, 30 with missed abortion, and 30 with incomplete abortion. Using immunohistochemical staining, the expression of CD68 and VEGF in decidual and placental tissues was investigated. Expression levels were scored semi-quantitatively by averaging data from five fields per slide and subjected to statistical analysis.

Results: Building on these methods, immunohistochemical staining confirmed significantly elevated CD68 expression in both placental and decidual tissues from missed and incomplete abortions, indicating enhanced macrophage infiltration. Widespread staining (>50%) was seen in 50% of incomplete abortion decidua and 30% of missed abortion tissues. In contrast, VEGF expression was found to be predominantly negative in all samples, with no case showing positive staining, indicating impaired angiogenesis.

Conclusion: The findings highlight a dual pathological mechanism in early miscarriage characterized by enhanced inflammatory macrophage infiltration and deficient angiogenic support. This imbalance may contribute to placental dysfunction and pregnancy failure. These results underscore the potential diagnostic and therapeutic relevance of immune–angiogenic pathways in early miscarriage

Keywords: CD68; Imunohistochemical staining; Miscarriage; VEGF

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INTRODUCTION

Miscarriage is defined as a spontaneous and unintentional loss of a pregnancy before the fetus is viable. It replaced the term "abortion" as the medical definition of miscarriage (1, 2). Miscarriage before the twentieth week of pregnancy is caused by chromosomal abnormalities (3) or uterine or cervical disorders (4). Infections and other medical conditions account for approximately 15% of early and 66% of late miscarriages. Immunological and environmental factors, maternal age, chronic health conditions, and lifestyle factors like smoking, alcohol consumption, obesity, and stress can increase the risk of miscarriage, particularly in mothers aged 35 and older (5, 6). Miscarriage typically occurs in the first three months of pregnancy, with common symptoms including vaginal spotting and passing blood clots (7). Decidua is differentiated into three regions that include the decidua parietalis, decidua capsularis, and decidua basalis, in accordance with the assertion of Yin et al. 2024 (8). The placenta, a crucial fetal organ, rapidly develops during pregnancy, secreting signaling molecules that govern maternal-fetal interactions, influencing fetal development, growth, and immunity (9, 10). Additionally, the placenta plays a central role in providing nutrients, disposing of metabolic wastes, and ensuring a healthy pregnancy by facilitating complex communication between the fetus and mother, involving immune-related tasks (11). The interaction between the fetus and the maternal immune system is crucial for protecting against immune rejection. Factors like immune tolerance, placental macrophages, and cytokine release have been reported to be involved in miscarriages (12-14). The delicate balance between immune tolerance and immune responses to infections in a pregnancy is crucial for a healthy pregnancy, and any condition disturbing this balance can lead to miscarriage.

Cluster of differentiation 68 (CD68), a receptor and soluble protein, is a promising candidate for therapeutic purposes (15). CD68 is a marker of macrophage presence and activation in tissues and helps diagnose immune reactions that accompany pregnancy or disease-associated miscarriages (Ref). Vascular endothelial growth factor (VEGF), a cytoprotective and anti-apoptotic factor, is essential in the formation of blood vessels in the placenta, ensuring adequate blood flow to the developing fetus. It plays a role in modifying the immune environment, protecting the fetus from mother's immune responses. Abnormal VEGF levels can lead to pregnancy complications including miscarriage (16-20). Immunopathological mechanisms in miscarriage include dysregulated cytokine signaling, altered macrophage activation, and defective angiogenesis. Pro-inflammatory cytokines such as interleukin- IL)-18 and IL-23 can shift the immune environment toward a Th1-dominant response, promoting tissue damage and trophoblast apoptosis (21, 22). Concurrently, aberrant expression of angiogenic factors such as VEGF compromises placental vascularization, and altered macrophage activity, reflected by CD68 expression, contributes to inflammatory infiltration and impaired tissue remodeling (23).

The present study aimed to investigate the immunohistochemical expression patterns of CD68 and VEGF in placental tissues and decidua from first-trimester miscarriages to elucidate their potential roles in the pathophysiology of miscarriage.

MATERIALS AND METHODS

Study Population

A total of sixty women attending the Fatima Al Zahra Hospital and Al-Liqa in Baghdad, Iraq, were recruited. The participants included thirty women with missed abortion and thirty with incomplete abortion. These women experienced abdominal pain and vaginal bleeding in the first trimester of their pregnancies, which led to their hospitalization.

Inclusion and Exclusion Criteria

The study included women aged 18-40 years who presented with Rh-positive blood type and no known cause of vaginal bleeding, while the exclusion criteria included samples of women who smoked or consumed alcohol, were receiving ongoing treatment for chronic diseases, had anemia (hemoglobin <8 g/dL), and had a negative Rh blood group. As women were brought for uterine evacuation at the women's emergency department of the hospital the products of conception were taken from the patients.

Ethical Approval

Ethical approval for the present study was obtained from the Ethics Committee of the Baghdad University's College of Women's Science (Approval number: 7902/22) on December 21, 2023. The study was approved by the scientific committee of Bagdad University, college of science (no. 7902-22 on 21/12/2023). Their medical histories were recorded, and clinical examinations were conducted before enrollment. Participants were then assigned to different groups.

Immunohistochemical Analysis of CD68 and VEGF

Curettage was used to create placenta specimens, which were embedded in paraffin blocks, preserved in 10% formalin, sliced into 5 µm pieces, deparaffinized in xylene, rehydrated, and incubated with 3% hydrogen peroxide at 37°C for 10 minutes to inhibit peroxidase activity. Slides were washed twice in phosphate buffer saline, rinsed with distilled water for 5 minutes, and incubated with primary antibodies against CD68 and VEGF. Slides were then washed three times in PBS, then rinsed twice in PBS and distilled water. The study involved applying mouse anti-CD68 and mouse anti-VEGF conjugates (Dako, Denmark), incubating at 37°C, and performing four PBS rinses. The mixture was then applied to tissue, rinsed twice, and counterstained with Harris Hematoxylin. The slides were then dehydrated, mounted, and examined under a light microscope (24).

Assessment of the Immunohistochemical Staining

Two experienced pathologists evaluated slides separately using a semi-quantitative grading program. Based on their cytoplasmic staining, placenta, and decidual stromal cells were categorized as negative (0), slightly positive (+1), moderately positive (+2), or strongly positive (+3). A noticeable brown stain receives a positive score. When more than 50% of the cells showed strong staining, the result was recorded as strong positive. Additionally, when less than 20% of the cells showed significant staining, the result was reported as slightly positive. When 20% to 50% of the target cells showed strong staining, the staining result was moderately positive (+2) (25).

Statistical Analysis

At the end of the study, all data was compiled and tabulated. Appropriate tests were used for statistical analysis, such as Fisher's test for the immunohistochemical data and the Pearson chi-square (free statistics software).

RESULTS

Demographic Data

The mean age of the participants was 27.13±5.4 years in the missed abortion group, and 27.83±6.5 years in the incomplete abortion group with no significant differences. Additionally, there was no significant differences in pregnancy status and parity between the two groups, Table 1.

Expression of CD68 and VEGF in Placental and Decidual Tissues

The immunohistochemical analysis revealed that the highest expression of CD68 in incomplete abortion cases was observed in the decidua, with strong expression (+++, >50%) detected in 15 out of 30 samples (50%).

Table 1: The Number and percentage of Pregnancy status, children, and maternal among women participating in the study

Variables		Missed abortion		Incomplete abortion		P-value
		Frequency No. (30)	%	Frequency No. (30)	%	
Pregnancy status	1-2	9	30%	5	16.6%	0.24447 NS
	3-4	12	40%	10	33.3%	0.6317 NS
	≥5	9	30%	15	50.0%	0.2190 NS
Children	0-1	10	33.3	9	30.0%	0.8146 NS
	2-3	15	50%	10	33.3%	0.2354 NS
	>3	5	16.6	11	36.3%	0.1038 NS
Maternal age (years)	Mean±SD	27.13±5.4		27.83±6.5		0.1NS

Table 2. The intensity of CD68 expression in decidual and placental tissues

Grade (intensity of CD68)	Decidua (incomplete abortion; (n = 18)	Placenta tissue (incomplete abortion (n = 12)	Decidua (missed abortion; (n = 12)	Placenta tissue (missed abortion; (n = 18)	Statistical test
Negative (0) <10	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Weak (+; 10-20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Moderate (++; 20-50%)	3 (10%)	12 (40%)	3 (10%)	9 (30%)	X^2=0.75 (NS)
Strong (+++; >50%)	15 (50%)	0 (0%)	9 (30%)	9 (30%)	Fisher test= 0.78 (NS)
Total	18 (60)	12 (40)	12 (40)	18 (60)	0.12 (NS)

CD68: Cluster of differentiation; X^2: Chi-square; NS: non-significant; *p-value<0.05: significant; **p-value < 0.01: highly significant

In contrast, 12 samples (40%) exhibited moderate expression (++ staining, 20–50%) in the placental tissue, as shown in Table 2. Meanwhile, the missed abortion group exhibited strong expression (+++, >50%) in both decidual and placental tissues, with 9 out of 30 samples (30%) in each region. Additionally, moderate expression (++ staining, 20–50%) was observed in the placental area of 9 out of 30 samples (30%). In cases of missed and incomplete abortion, moderate expression (++ staining, 20–50%) was less frequent in the decidua, occurring in only 10% of the samples.

In cases of incomplete abortion, 60% of the decidual samples showed negative (0) VEGF expression, while 40% of placental tissues in the same group exhibited negative VEGF expression. For missed abortion cases, 40% and 60% of the decidual and placental tissues displayed negative VEGF staining, respectively. None of the samples exhibited weak (0-20%), moderate (20-50%), or strong (>50%) positive VEGF staining. In total, 60% of decidual samples from incomplete abortion cases and 60% of placental tissues from missed abortion cases showed negative VEGF expression. Similarly, 40% of both decidual and placental samples from missed abortion cases exhibited negative staining.

Immunohistochemical Evaluation of Placental and Decidual Tissues

Fig. 1 presents the immunohistochemical expression of CD68 across different study groups. Fig. 1A shows moderate CD68 expression in the placental tissue from an incomplete

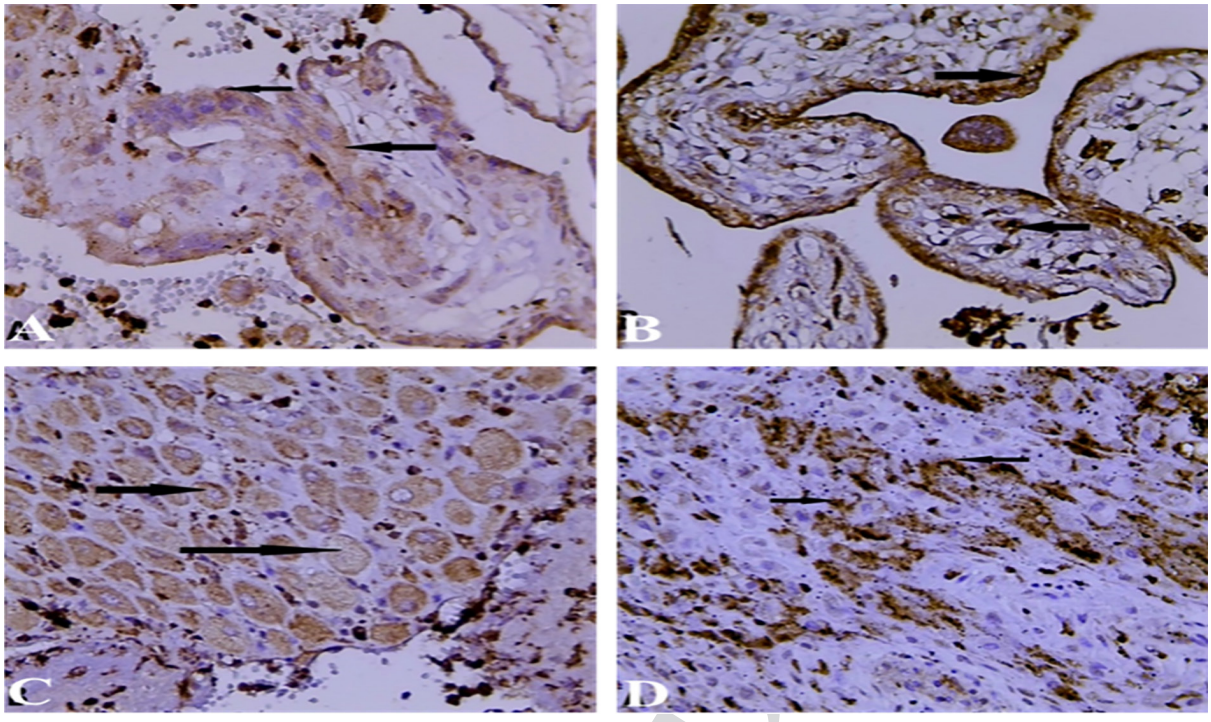

Fig. 1. Immunohistochemical expression of CD68 in placental and decidual tissues. Representative micrographs showing moderate CD68 expression in: (A) placental tissue from an incomplete abortion, (B) placental tissue from a missed abortion,(C) decidual tissue from an incomplete abortion, and (D) decidual tissue from a missed abortion (×40). CD68: Cluster of Differentiation 68.

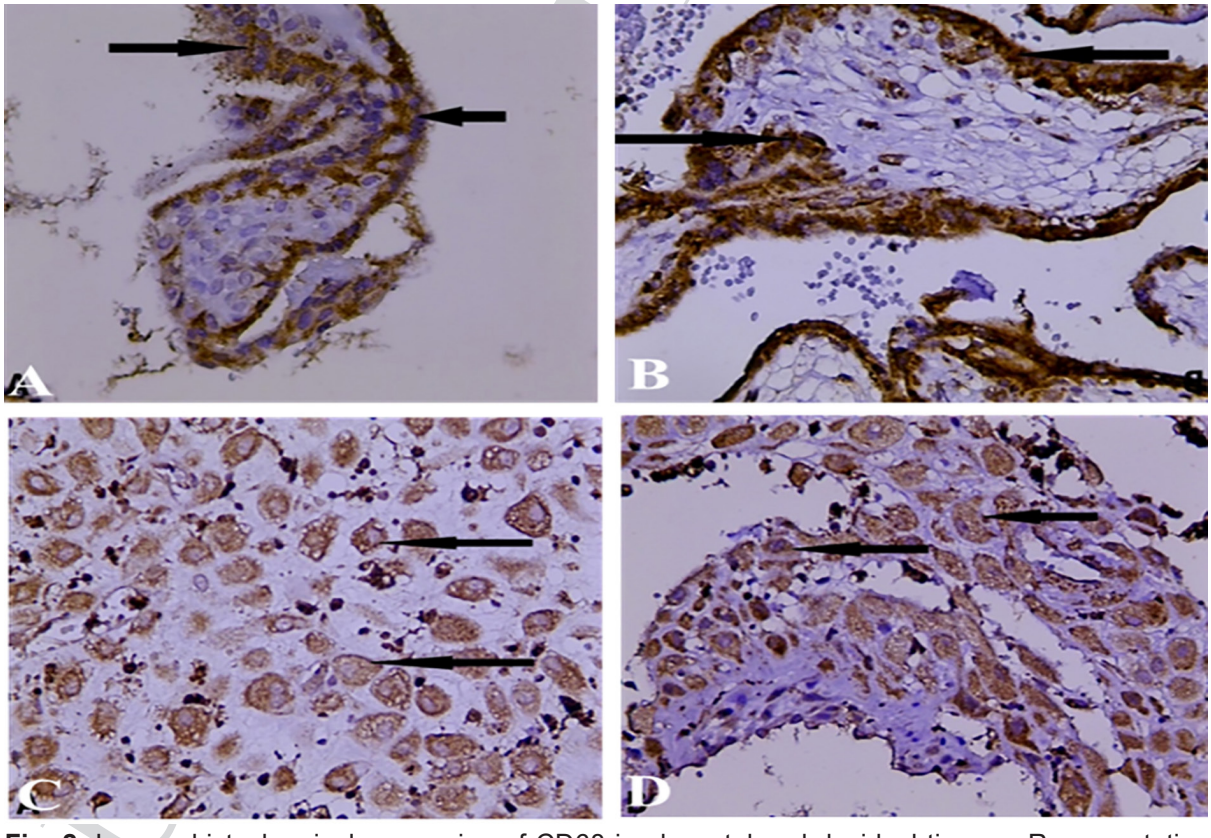

Fig. 2. Immunohistochemical expression of CD68 in placental and decidual tissues. Representative micrographs showing strong CD68 immunoreactivity in: (A) placental tissue from an incomplete abortion, (B) placental tissue from a missed abortion, (C) decidual tissue from an incomplete abortion, and (D) decidual tissue from a missed abortion (×40). CD68: Cluster of Differentiation 68.

abortion case. In Fig. 1B, moderate CD68 expression was observed in placental tissue from a missed abortion case. Fig. 1C illustrates moderate CD68 expression in the decidua of an incomplete abortion case. Finally, the decidua in a missed abortion case has moderate expression of CD68, as shown in Fig. 1D. The tissue of incomplete abortions expressed high levels of CD68 (Fig. 2A). Similarly, Fig. 2B displays strong expression of CD68 in the placental tissue of missed abortion cases. In Fig. 2C, strong CD68 expressions were observed in the decidua of incomplete abortion cases, while Fig. 2D shows strong expressions in the decidua of missed abortion cases. Fig. 3 shows the immunohistochemical expression of VEGF in the study groups. Fig. (3A) displays negative VEGF expression in the placental tissue of incomplete abortion cases. Similarly, Fig. (3B) shows negative expression in the placental tissue of missed abortion cases. A negative VEGF expression was observed in the decidua of incomplete and missed abortion cases, as shown in Figs. 3C and D.

DISCUSSION

A previous study by Zhang and colleagues (2022) did not demonstrate a linear relationship between the risk of spontaneous abortion and maternal age (appeared J-shaped relationship). Additionally, this study showed that the optimal threshold for maternal age is around 29 years, and maternal age over 30 years is accompanied with increased risk of spontaneous abortion (26). An increase in Spontaneous abortion is strongly correlated with age and parity and both the number of children and the frequency of abortion increase in grand multiparas (27).

The present study provides insight into the immunological and angiogenic alterations associated with first-trimester miscarriage by examining CD68 and VEGF expressions in decidual and placental tissues from incomplete and missed abortions. The findings highlight a dysregulated immune–vascular environment that may contribute to miscarriage.

The marked CD68 expression observed in

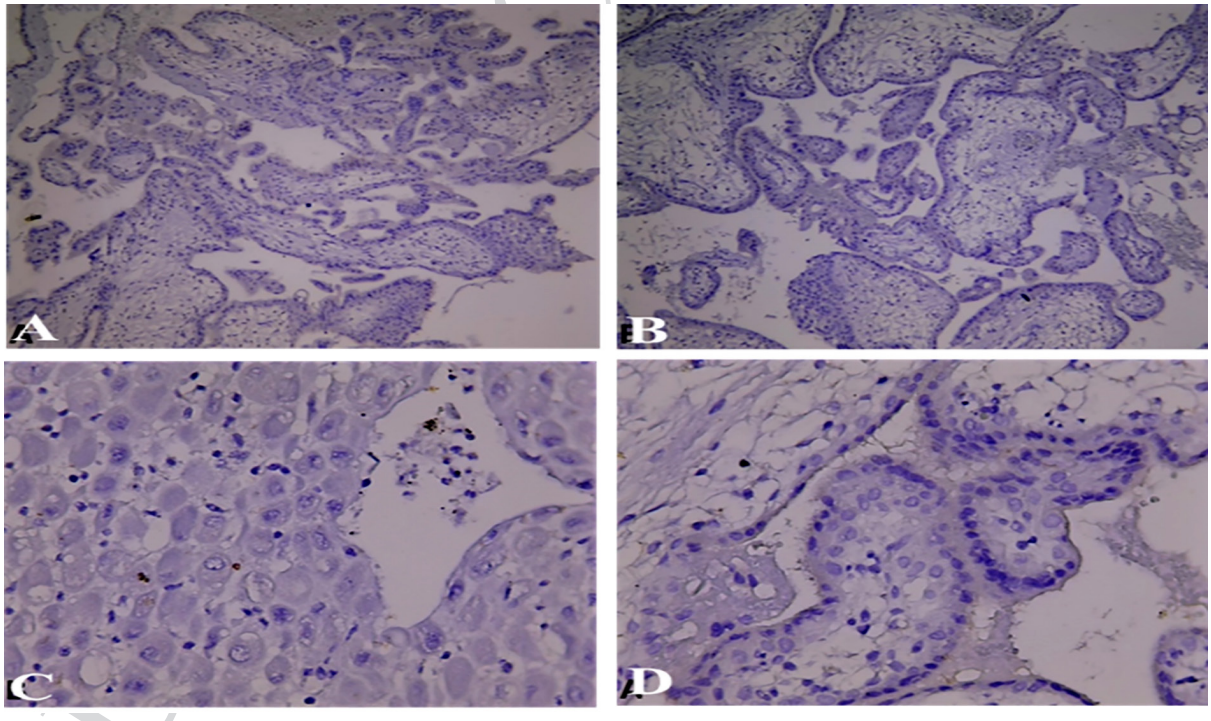

Fig. 3. Immunohistochemical expression of VEGF in placental and decidual tissues. Representative micrographs showing negative VEGF immunoreactivity in: (A) placental tissue from an incomplete abortion, (B) placental tissue from a missed abortion, (C) decidual tissue from an incomplete abortion, and (D) decidual tissue from a missed abortion (×40). VEGF: Vascular Endothelial Growth Factor.

both decidual, and placental tissues indicates enhanced macrophage activity, supporting the concept that immune imbalance plays a central role in miscarriage. Although macrophages are essential for implantation, tissue remodeling, and maintenance of maternal–fetal immune tolerance, excessive accumulation or polarization toward a pro-inflammatory (M1) phenotype can be detrimental (28, 29). The high proportion of samples showing strong CD68 expression in the present study suggests that uncontrolled macrophage activation may contribute to tissue damage, trophoblast apoptosis, and impaired placental function.

In contrast, VEGF expression was largely absent in both decidual and placental tissues, indicating compromised angiogenic signaling. VEGF plays a critical role in placental vascular development and fetal growth, and its downregulation has been associated with placental insufficiency and adverse pregnancy outcomes (30, 31). The concurrent high CD68 expression and low VEGF levels supports the concept that excessive immune activation may suppress angiogenic pathways, leading to inadequate placental vascularization. This imbalance between inflammation and angiogenesis may represent a key mechanism underlying miscarriage. The histological evidence of increased CD68-positive macrophages suggests an inflammatory environment in which immune cells may clear apoptotic debris or respond to tissue injury, but cause further placental damage (32). Simultaneously, reduced VEGF expression indicates impaired vascular support, reinforcing the concept that failed pregnancies may result from a shift from immune tolerance and angiogenic support toward inflammation and vascular insufficiency.

Previous studies support the present findings that immune dysregulation and impaired angiogenesis play key roles in early miscarriage. Increased infiltration of CD68-positive macrophages has been consistently reported in decidual and placental tissues from spontaneous and missed abortions, suggesting an exaggerated inflammatory response that disrupts trophoblast function and maternal–fetal tolerance (33-35). In parallel, reduced VEGF expression has been associated with defective placental vascular development and poor pregnancy outcome (36, 37). Additionally, inflammatory cytokine activity has been shown to suppress angiogenic signaling, linking immune activation to vascular insufficiency (38). These findings support our observation that increased macrophage activity combined with reduced VEGF expression contributes to the pathogenesis of first-trimester miscarriage.

Study Limitation

Finally, this study is limited by its small sample size, restriction to Rh-positive women, and lack of healthy first-trimester control placentas. These factors limit the generalizability of the findings and prevent definitive comparisons with normal placental development.

Semi-quantitative immunohistochemical scoring was used to evaluate CD68 and VEGF expressions because this method allows simultaneous assessment of both staining intensity and tissue localization within placental and decidual structures. This approach is widely used in histopathological studies when spatial distribution of immune cells and angiogenic markers is relevant. Although digital image analysis and molecular techniques such as RT-PCR or ELISA provide more objective quantification, they do not preserve tissue architecture or allow cell-specific localization. Nevertheless, we acknowledge that the lack of molecular quantification represents a limitation, and future investigations will incorporate digital image analysis and complementary molecular assays to strengthen quantitative validation of these findings. We acknowledge that the use of only chi-square and Fisher's exact tests without adjustment for multiple comparisons or confounding factors represents another

limitation in our study. Due to the limited sample size and the categorical nature of the immunohistochemical data, more advanced statistical modeling was not feasible. Future studies with larger cohorts will include multivariate analyses and appropriate correction methods (e.g., Bonferroni or false discovery rate adjustments) to improve statistical rigor

CONCLUSION

The present study reveals that first-trimester miscarriage placentas, from both incomplete and missed abortions, have a unique immunohistochemical profile. They feature high CD68 and low or absent VEGF expressions. The strong CD68 staining in decidual tissues indicates an active inflammatory process. In contrast, the near-total lack of VEGF expression in all tissue samples points to a serious impairment in angiogenesis, which is crucial for placental development and fetal support. The concurrent increased CD68 and decreased VEGF expressions suggests an environment where immune activation coincides with a lack of vascular support. These findings highlight the roles of immune dysregulation and impaired angiogenesis in miscarriage; however, the limited sample size and cross-sectional design restrict causal interpretation. Future studies incorporating larger cohorts and functional analyses are needed to clarify underlying mechanisms and therapeutic potential.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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