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دراسة نسجية مرضية ومناعية هستوكيميائية للمشيمة البشرية في الثلثين الأول والثاني من الحمل المصاحب للإجهاض التلقائي في محافظة واسط

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المستخلص:

ترتبط التبدلات المناعية أثناء الحمل بفقدان الحمل المتكرر لأسباب مجهولة. تهدف هذه الدراسة إلى دراسة الدور المناعي والخلوي للخلايا في المشيمة لتحديد ما إذا كانت هناك علاقة لنشاطها الخلوي بارتفاع مخاطر الإجهاض في الثلثين الأول والثاني من الحمل. تم جمع ٣٢ عينة من انسجة المشيمة بعد تعرض النساء للإجهاض التلقائي في محافظة واسط وتم تحضيرها نسجيا باستخدام صبغتي الهيماتوكسيلين والايوسين لدراسة التغيرات العامة الى جانب تطبيق تقنية الكيمياء النسيجية المناعية باستخدام المضاد للكشف عن معدلات تعبير الخلايا البلعمية. أظهرت النتائج تغيرات نسجيه واضحة في الزغابات والخلايا المشيمية المتأثرة متزامنة مع زيادة ملحوظة في التعبير المناعي للبلاعم، خاصة في الثلث الثاني من الإجهاض. وهذا يقودنا إلى استنتاج أن الاستجابة المناعية والالتهابية تلعب دورًا مهمًا في الإجهاض. علاوة على ذلك، تساهم هذه الدراسة في توضيح العلاقة بين التغيرات الكيميائية المناعية وآلية فقدان الحمل، مما قد يمكننا في المستقبل من تطوير طرق تشخيصية وعلاجية أفضل للحفاظ على الحمل.

الكلمات المفتاحية: التغيرات النسيجية المرضية، الكيمياء النسيجية المناعية، المشيمة، الاجهاض التلقائي، واسط

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Histopathological and Immunohistochemical Study of Human Placenta in First and Second Trimester of Pregnancy Associated with Spontaneous Abortion in Wasit Governorate

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Abstract

Immunological variables during pregnancy are associated with recurrent pregnancy loss of unexplained origin. This study aims to investigate the immunological and cellular roles of placental cells to determine whether their cellular activity is linked to an increased risk of miscarriage during the first and second trimesters. 32 placental tissue samples were collected from women who had experienced spontaneous abortion in Wasit Governorate and prepared histologically using hematoxylin and eosin staining to study general changes. In addition, an immunohistochemical technique using anti-CD68 was used to determine macrophage expression levels. The results revealed distinct histological changes in the affected villi and placental cells, coinciding with a marked increase in macrophage immunoreactivity especially in the second trimester of miscarriage. This leads us to conclude that the immune and inflammatory response plays a significant role in miscarriage. Moreover, the work contributes to clarifying the connection between immunohistochemical changes and the mechanism of pregnancy loss, which may enable us in the future to develop better diagnostic and therapeutic methods for preserving pregnancy.

Keywords: Histopathological change, Immunohistochemical technique, Placenta, Spontaneous abortion, Wasit

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1.Introduction

The World Health Organization defines abortion as the termination of pregnancy, either spontaneous or induced. Spontaneous abortion is estimated to occur in 15%–20% of clinically diagnosed pregnancies, making it the most frequent pregnancy problem (Camayo et al., 2011). Furthermore, early biochemical pregnancy loss is estimated to account for an additional 30% of failed pregnancies. Approximately 25–50% of women who are fertile will experience at least one miscarriage (Kolben et al., 2019). The placenta is a vital transient that allows for the exchange of gas and nutrients between the mother and the fetus. Part of the mother is the decidua basalis, while part of the fetus is the chorionic frondosum. The placenta is bounded by the chorionic plate on the fetal side and the decidua basalis on the other; the decidua plate is the most closely integrated part of this structure within the placenta (Sadler, 2012).

Furthermore, this organ functions throughout fetal development and has a dual blood circulation system. Moreover, placental trophoblasts contribute in placental vascular growth and differentiation in addition to hemostasis by expressing and producing coagulation components (Lanir et al., 2003).The placenta, a functional unit, the chorionic villi, supplies the fetus with nutrition and oxygen while also acting as an excretory unit (Fox, 1999). There is a fundamental villous structure that is unaffected by gestational age, the stage of progress and development of the villous tree, and the histological arrival of chorionic villi. The exterior surface of these villi is covered by a trophoblastic mantle made up of two layers: layers of syncytiotrophoblast on the outside and cytotrophoblasts on the inside (Vijay, 1994).

The most frequent pregnancy complication is abortion. 15% of pregnancies that are recognized end in spontaneous abortion. Early and undetected pregnancies may result in significantly more abortions (Christopher, 2004). After losing a pregnancy or a newborn, histopathological analysis of the placenta may offer an explanation for the

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pregnancy difficulties, loss of pregnancy, or death of a newborn, as well as information pertinent to the care of the present newborn and/or future pregnancies (Roberts and Oliva, 2006). The present study aims to elucidate the specific function of placental phagocytic cells (macrophages) and to determine whether there is a significant relationship between the localized activity of these immune cells and the increased risk of miscarriage in the first and second trimesters of gestation**.**

2. Materials and Methods

2.1.Tissue specimens and histological technique

Placental tissue samples were obtained immediately after miscarriage from Al-Zahraa Teaching Hospital, Al-Batoul Maternity Hospital, and Wasit Investment Hospital of Wasit Governorate. The study included 32 human placental samples comprising 4 samples for each gestational stage associated with miscarriage and 4 control placental samples collected from women aged 19-43 years spanning the period from March to August 2025. These patients underwent indicated therapeutic abortions during the 1st and 2nd trimester (between gestational weeks 6 and 24) via suction curettage.

The control group placental samples were collected from healthy women of the same age group who completed their pregnancies and delivered vaginally. Patients' informed consent was obtained before any samples were taken, and the medical history of all participants in this study was recorded. The work and tests were completed in the laboratories of the College of Science and the College of Medicine at the University of Wasit. Samples were prepared for paraffin sections, cut into 5µm slices, and stained with standard haematoxylin and eosin stain after being fixed in 10% neutral-buffered formalin (Bancroft and Layton, 2019).

2.2. Immunohistochemical technique

In this immunohistochemical study, to retrieve antigens, paraffin slices placed on adhesive slides were submerged in citrate solution and heated in a microwave oven in three cycles of 5 minutes each. After cooling for 10 minutes at ambient temperature, these sections were rinsed with phosphate-

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buffered saline. Subsequently, they were subjected to a 10-minute treatment with 3% hydrogen peroxide (H_2O_2). Following a rinse with distilled water, sections were washed with PBS (pH 7.6) and treated with mouse anti-human CD68 monoclonal antibody (clone PG-M1; Dako, Denmark) at a dilution of 1:50 to identify macrophages. Sections were incubated in the corresponding secondary antibody solution for 10 minutes following three washes with PBS (Kiernan, 2015). Following the final PBS wash, chromogenic solution was applied to the sections for 8 minutes. After rinsing the sections with distilled water, hematoxylin was applied for 2 minutes as a counterstain and assessed under a light microscope (Ermis, 2021; Tasin et al., 2021). The primary antibody was not used in the preparation of the negative control sections. For photography, a Canon digital camera was utilized. There were four categories for the staining intensity, which were semi-quantitatively graded into four: negative (-), weak (+), moderate (++) and strong (+++).

2.3. Digital image analysis

The QuPath programme for quantitative pathology analysis (version 0.4.4) was used to evaluate the placental anti-CD68 staining patterns (Hein et al., 2021). Slides stained with anti-CD68 were imported as bright-field images into the QuPath program. The average number of anti-CD68-positive tissue, area, and mean staining intensity for each slide were analyzed after a new image was manually produced by choosing three areas in the inflammatory area at random, according to previously published guidelines (Porter et al., 2023).

3. Results and Discussion

3.1. Histological results

3.1.1. Hematoxylin and eosin stain

Histological evaluation of the control group of placental specimens (collected at 38-40 weeks of gestation). After conducting a histological examination, we found extensive placental villi covered with cytotrophoblast cells, and separated by spaces between the villi that contained maternal blood. The presence of intact fetal blood vessels inside the chorionic villus

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containing red blood cells indicates the presence of a good blood supply, as shown in Figure1 (A,B).

The first group of miscarriages was a placenta in the first trimester (at 6 weeks of gestation), where numerous vacuolations appeared within the stroma and cells, giving an edematous appearance in addition to swelling of the chorionic villi. On the other hand, clear blood congestion and tissue filled with blood vessels were observed, indicating a lack of blood flow, as shown in Figure 2A. This result was consistent with findings of Al-Khazraji et al. (2023), who reported clear cellular necrosis and vacuolar degeneration in the cells and tissues of the placenta.

The results of histological investigation of the 8-week-old placenta revealed clear degenerative changes, as vacuolar degeneration was found within the placental stroma and the appearance of multiple vacuoles within trophoblast cells. Also, the appearance of fibrosis in the middle of the tissue section, as presented in Figure 2B below. The result of this pathological condition was consistent with that found (Khong et al., 2016).

Histological examination of the remaining cases at the 12th week of gestation revealed chorionic villi in different sizes with thickening of some walls. Their edges appeared irregular and curvy, containing small to no blood vessels. Degenerative and edematous changes appeared within these villi, Figure 2C. This closely matches the findings reported by Stallmach et al. (2001).

On the other hand, evaluation of the second-trimester miscarriage group (at the 16th week of gestation) and after microscopic examination, it was found that there was clear blood congestion within the intervillous spaces and an accumulation of red blood cells. Also, degenerative changes were observed in some trophoblast cells lining the villi, with mild edema in the villous stroma and fibroid deposition, as seen in Figure 3A. The results were consistent with the findings documented by Habek (2011). Furthermore, this result was in line with Sandoval (2016), who also noted villous edema and fibroid deposition. The histopathological examination of a placenta at 20

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weeks indicated the presence of clear degeneration of the chorionic villus cells, in addition to the loss of the cells' nuclei, as a clear sign of necrosis and death of the tissue as a result of interruption of blood supply. Likewise, notice dense red cell clusters indicating hemorrhage and severe congestion between the villi, as in Figure 3B. These results are in agreement with Petersen et al. (2006).

Meanwhile, histological examination results at the 22nd week of gestation revealed marked vascular proliferation as an abnormal finding resembling a hemangioma. Additionally, hypertrophy of the smooth muscle within the placenta was observed; this reaction stems from a disturbance in blood flow between the mother and the fetus, as illustrated in Figure 3C. These results are in agreement with Kraus (2013).

Placental samples from cases of SARS-CoV-2 (Rebutini et al., 2021), dengue virus (DENV) (Fernandes Ribeiro et al., 2017), maternal hypertension and diabetes mellitus (Aldahmash et al., 2021), and other congenital infections (Stanek et al., 2011; Heider, 2017; Roberts and Torous, 2022), also showed these alterations. The final group in this study included specimens at the 24th week of gestation, in which a marked density of inflammatory cells, predominantly polymorphonuclear cells, was observed within the intervillous spaces; this indicates acute placental inflammatory infiltration, likely attributable to a specific infection.

This finding was accompanied by congestion and hemorrhage within the intervillous spaces, in addition to fibrosis of the trophoblastic layer, as demonstrated in Figure 3D. Early pregnancy hypoxia can change placentation and trophoblastic differentiation. Depending on the degree of hypoxia, late gestational hypoxia can alter mitochondrial activities, endoplasmic reticulum stress, hormone production, nutrition, and angiogenic factor secretion (Colson et al., 2021). As seen in case 2, trophoblastic necrosis appears to be associated with an increase in perivillous fibrin (Chen and Roberts, 2018). Obstruction of maternal blood flow may be seen,

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depending on the degree of fibrinoid deposition, which could impact the fetus.

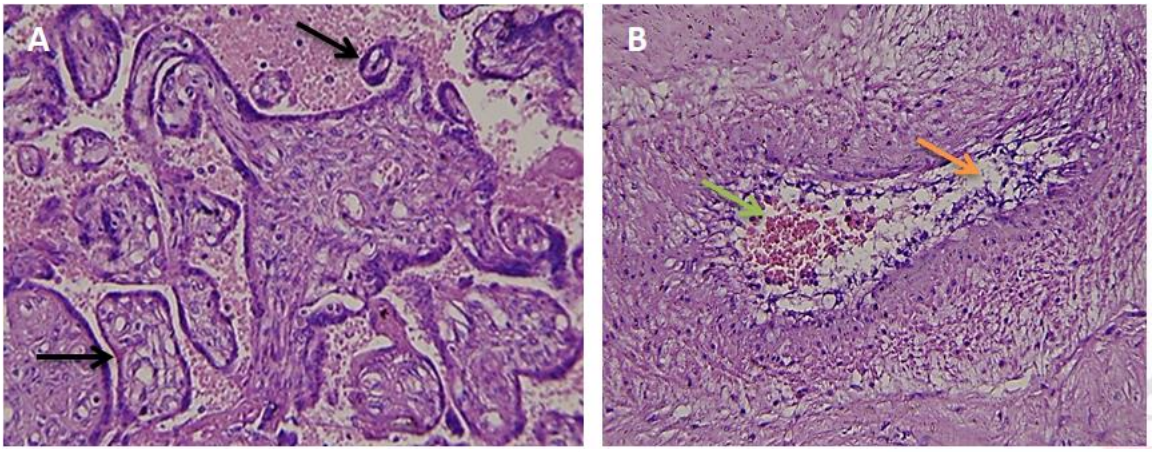
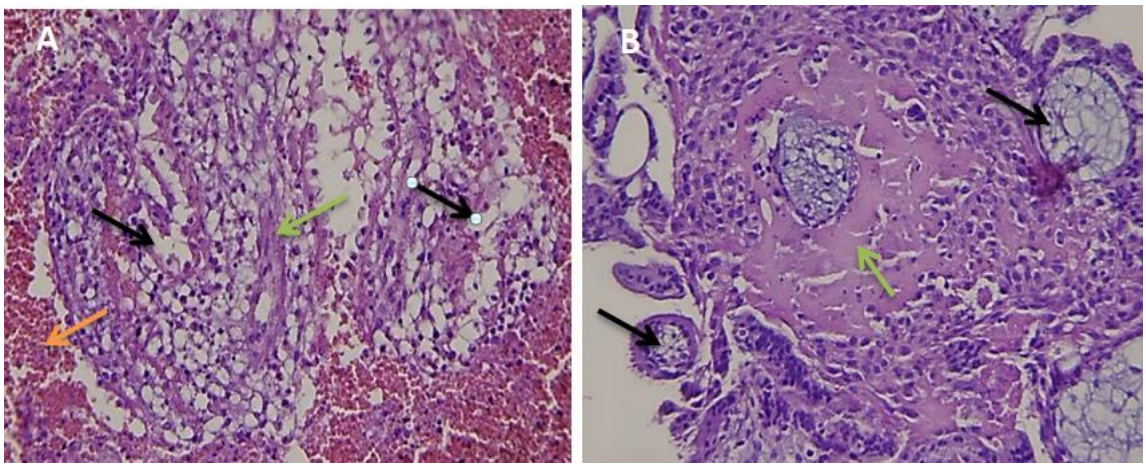


Figure 1. Photomicrographs of the control group placental tissue showed **A:**well-developed chorionic villi covered by syncytiotrophoblasts (black arrow). **B**: an abundance of fetal capillaries (green arrow) and stromal cells (orange arrow). H&E stain, ×400.



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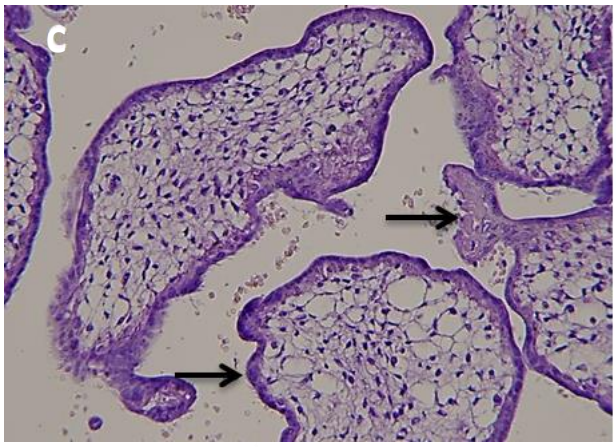
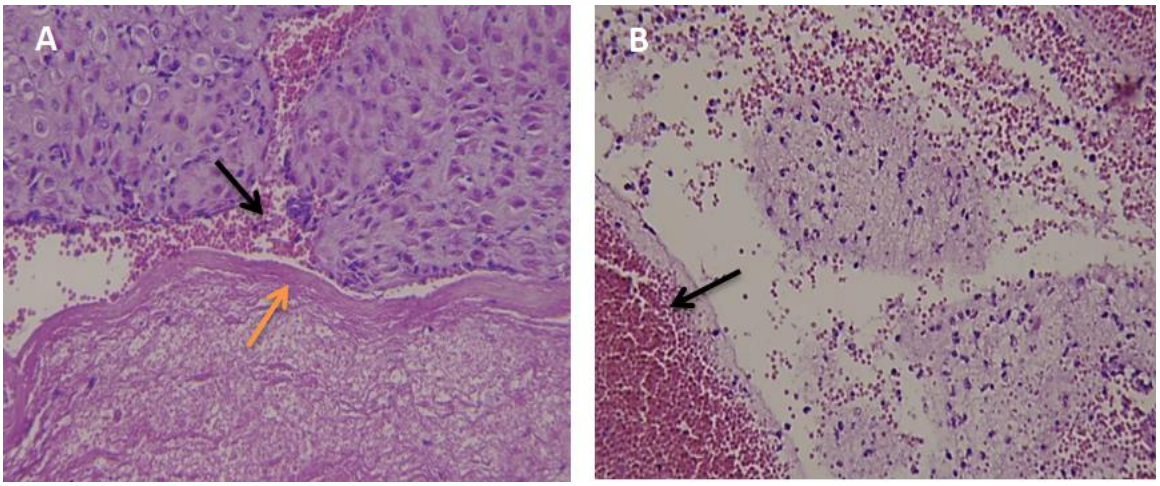


Figure 2. Photomicrograph of the first-trimester placental tissue showed: **A:** at the 6th week of gestation numerous vacuolation within the stroma (black arrow), focal stromal hemorrhage (orange arrow), fibrinoid deposition (green arrow). **B:** at 8 week showed clear degenerative changes as vacuolar degeneration (black arrow), fibrinoid deposition (green arrow). **C:** at the 12th week presence of chorionic villi in different sizes, thickening of some of the walls where their edges appear irregular and curvy (black arrow), small to no blood vessels. H&E stain, ×400.



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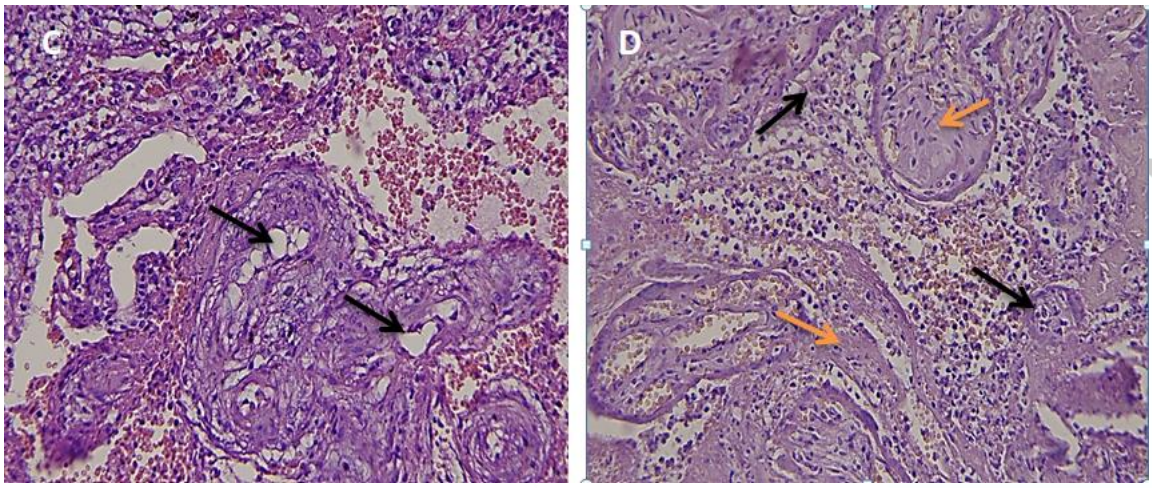

Figure 3. Photomicrograph of the second trimester placental tissue showed: **A**:at the 16th week of gestation clear blood congestion within the intervillous spaces (black arrow), fibrinoid deposition (orange arrow). **B**: at degeneration of the chorionic villus cells, dense the 20 week showed vascular proliferation hemorrhage (black arrow). **C:** at the 22 week showed dense of accumulation (black arrow). **D:** at the 24 week showed inflammatory cells (black arrow), fibrosis of the trophoblast layer (orange arrow). H&E stain, ×400.

3.2. Immunohistochemical results

The immunological localization of macrophages in decidual cells has been made easier by the application of IHC technology. In regards to mean immunostaining intensity, area of immune reactivity relative to cross sectional area, and number of positive cells was performed using QuPath digital pathology software as in Table 1 below. Concerning immunohistochemical staining of antibody CD68 cells, in our study found negative (-) results for macrophages infiltration of control groups, as shown in Figure 4A. As for, sections from placenta at 6 weeks of age showed weak (+) immunohistochemical expression and staining intensity compared to the control group, as can be seen in Figure 4B. While we observed moderate (++) immunological reactivity in placenta samples aged 8, 12, and 16 weeks,

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as in Figures 4 (C,D,E) respectively. On the other hand, immunostaining of samples aged 20, 22, and 24 weeks showed strong (+++) immune reaction and a large number of positive cells compared to the previous study groups, as in Figures 4 (F,G,H). The immunological profile of women who experience repeated pregnancy loss differs significantly from that of controls, according to our study's findings. Pregnancy loss syndrome appears to be caused by an immune system "malfunction" that leaves the fetus unprotected. Macrophages (CD 68+ cells) are known to have a part in the development of the feto maternal interface.

According to earlier research, macrophages in the human uterus take part in the phagocytosis of apoptotic cells (Abrahams et al., 2004), work with trophoblasts to perform vascular remodeling in the uterus (Harris, 2010), promote monocyte migration at the decidua basalis, and meaningfully increase the production and secretion of pro-inflammatory chemokine and cytokines (Fest et al., 2007).The findings of this study demonstrate the activation of immune expression, characterized by an increase in CD68+ cell expression, mostly by trophoblast and inflammatory cells located in the intervillous space and within fetal capillaries, in contrast to the negatively reactive control groups. These findings closely align with prior observations reported by Nunes et al. (2019) and Rabelo et al. (2020).

Table 1. Statistical data using QuPath software

Placental group	CD68 Staining Expression	Area%	Mean intensity	Number of positive cells
Control	-	1.452	29.722	141
6 week	+	2.958	96.522	763
8 week	++	13.035	99.628	1713
12 week	++	7.19	102.218	1829
16 week	++	17.758	98.188	3025
20 week	+++	7.182	130.698	8554

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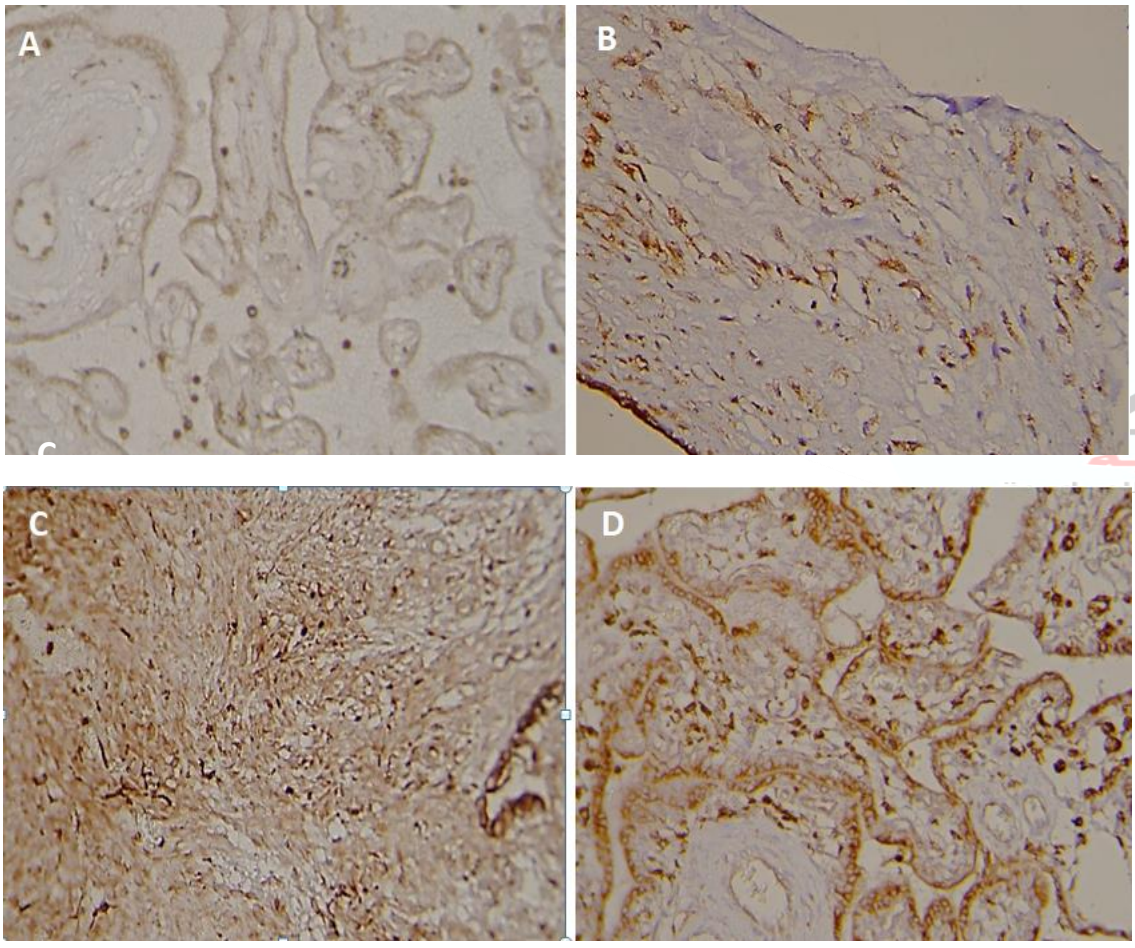
22 week	+++	18.337	106.589	8863
24 week	+++	14.638	106.259	9400



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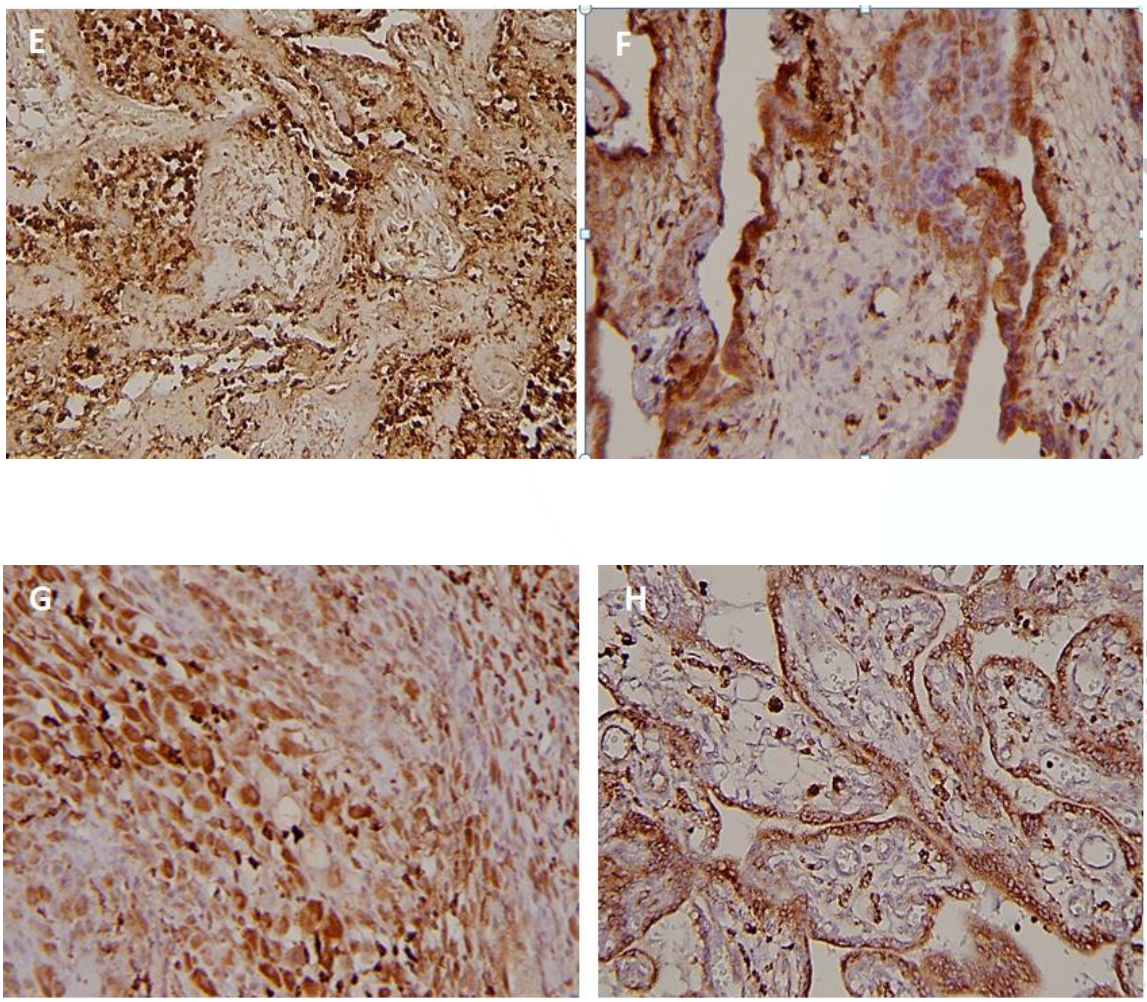

Figure 4. Representative photomicrographs of macrophage detection via anti-CD68 immunohistochemistry in placental tissues showed: **A**: no in the control group. **B:** at 6 week age of gestation showed weak expression expression of the immunostaining. **C:** at 8 week age showed moderate expression of the immunostaining. **D**: at 12 week age showed moderate expression of the tissues immunostaining. **E:** at 16 week age showed moderate expression of the immunostaining. **F:** at 20 week age showed strong expression of the immunostaining. **G:** at 22 week age showed strong expression of the immunostaining. **H:** at 24 week age showed strong expression of the immunostaining, ×400.

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4. Conclusions

In conclusion, this study successfully identified the quantitative dynamics and spatial distribution in the placenta throughout the pregnancy's first and second trimesters using CD68 antibodies. An increase in the infiltration of CD68-positive macrophages has been observed in the placentas of miscarriage cases; this may be associated with inflammatory changes contributing to pregnancy loss. These findings suggest that early immune changes could be crucial in the mechanism of miscarriage. Therefore, understanding this early immune response could open new avenues for early diagnosis and intervention in high-risk pregnancies.

Ethical Approvals

Consent was obtained from all patients prior to sample collection, following an explanation of the study's objectives. Furthermore, ethical approval was secured from the Wasit Directorate of Health.

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References

1. Camayo, F. J. A., Martins, L. A. B., & Cavalli, R. C. (2011). *Perda gestacional retida: Tratamento baseado em evidência*. *Femina, 39*(1), 49–56.

2. Kolben, T. M., Rogatsch, E., Hester, A., Kuhn, C., Schmoeckel, E., Czogalla, B., et al. (2019). Involvement of ILR4α and TLR4 in miscarriages. *Journal of Reproductive Immunology, 131*, 36–43. doi: https://doi.org/10.1016/j.jri.2018.12.001

3. Sadler, T. W. (2012). Fetal membranes and placenta. In *Langman's medical embryology* (12th ed., pp. 96–101). Wolters Kluwer.

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4. Lanir, N., Aharon, A., & Brenner, B. (2003). Haemostatic mechanisms in human placenta. *Best Practice & Research Clinical Haematology, 16*, 183–195. doi: https://doi.org/10.1016/S1521-6926(02)00098-1

5. Fox, H. (1999). Development of placenta and membranes. In J. Dewhursts, M. Deswiet, & G. Chamberlain (Eds.), *Basic sciences in obstetrics and gynecology*. Churchill Livingstone.

6. Vijay, V. (1994). *Handbook of placental pathology* (2nd ed.).

7 Christopher, P. C. (2004). The female genital tract. In R. S. Cotran, V. Kumar, & T. Collins (Eds.), *Pathologic basis of disease* (7th ed., pp. 1079–1080). WB Saunders.

8. Roberts, D. J., & Oliva, E. (2006). Clinical significance of placental examination in perinatal medicine. *Journal of Maternal-Fetal and Neonatal Medicine, 19*, 255–264. doi: https://doi.org/10.1080/14767050600676349

9. Bancroft, J. D., & Layton, C. (2019). The hematoxylins and eosin. In K. S. Suvarna et al. (Eds.), *Bancroft's theory and practice of histological techniques* (8th ed., pp. 126–138). Elsevier.

10. Kiernan, J. A. (2015). Immunohistochemistry. In *Histological and histochemical methods: Theory and practice* (4th ed., pp. 454–490). Scion Publishing.

11. Ermis, I. S. (2021). Losartan protects ovarian tissue against ischemia-reperfusion: An immunohistochemical study. *Analytical and Quantitative Cytopathology and Histopathology, 43*(5), 345–352.

12. Tasin, C., Ermiş, I. S., & Deveci, E. (2021). Endothelin-1 and APAF-1 expression in the umbilical cord of placenta previa cases. *Analytical and Quantitative Cytopathology and Histopathology, 43*(5), 439–445.

13. Hein, A. L., Mukherjee, M., Talmon, G. A., Natarajan, S. K., Nordgren, T. M., Lyden, E., Hanson, C. K., Cox, J. L., Santiago-Pintado, A., Molani, M. A., et al. (2021). QuPath digital immunohistochemical analysis of placental tissue. *Journal of Pathology Informatics, 12*, 40. doi: https://doi.org/10.4103/jpi.jpi_72_21

14. Porter, R. J., Din, S., Bankhead, P., Oniscu, A., & Arends, M. J. (2023). QuPath algorithm accurately identifies MLH1-deficient inflammatory bowel disease-associated colorectal cancers in a tissue microarray. *Diagnostics, 13*, 1890. doi: https://doi.org/10.3390/diagnostics13111890

15. Al-Khazraji, H. A., et al. (2023). A comparative study of the diagnosis of *Toxoplasma gondii* in human placenta by traditional method, restriction fragment length polymorphism, and the immunohistochemistry method. *The Egyptian Journal of Hospital Medicine, 90*, 1707–1712. doi: https://doi.org/10.21608/EJHM.2023.284278

16. Khong, T. Y., Mooney, E. E., Ariel, I., Balmus, N. C. M., Boyd, T. K., Brundler, M. A., et al. (2016). Sampling and definitions of placental lesions: Amsterdam placental workshop group consensus statement. *Archives of Pathology & Laboratory Medicine, 140*, 698. doi: https://doi.org/10.5858/ARPA.2015-0225-CC

17. Stallmach, T., Hebish, G., Meter, K., Dudenhausen, J., & Vogel, M. (2001). Rescue by birth: Defective placental maturation and late fetal mortality. *Obstetrics & Gynecology, 97*, 505–509. doi: https://doi.org/10.1016/S0029-7844(00)01208-4 .

18. Habek, D. (2011). Multiple intraplacental hematomas—Kline's hemorrhage: Case report. *Acta Clinica Croatica, 50*, 423–425.

19. Sandoval, O. (2016). *The placenta in a case of pregnant woman infected by Chikungunya virus*. *Journal of Virology and Retrovirology, 2*(1), 00009, 1–4.

20. Petersen, O. M., Heller, D. S., & Joshi, V. V. (2006). *Handbook of placental pathology*. Taylor & Francis.

21. Kraus, F. T. (2013). Fetal thrombotic vasculopathy: Perinatal stroke, growth restriction, and other sequelae. *Surgical Pathology Clinics, 6*, 87–100. doi: https://doi.org/10.1016/J.PATH.2012.10.001

22. Rebutini, P. Z., Zanchettin, A. C., Stonoga, E. T. S., Prá, D. M. M., de Oliveira, A. L. P., Dezidério, F. S., et al. (2021). Association between COVID-19 pregnant women symptoms severity and placental morphologic

features. *Frontiers in Immunology, 12*, 685919. doi: https://doi.org/10.3389/FIMMU.2021.685919

23. Fernandes Ribeiro, C., Lopes, V. G. S., Brasil, P., Pires, A. R. C., Rohloff, R., & Nogueira, R. M. R. (2017). Dengue infection in pregnancy and its impact on the placenta. *International Journal of Infectious Diseases, 55*, 109–112. doi: https://doi.org/10.1016/j.ijid.2017.01.002

24. Aldahmash, W. M., Alwasel, S. H., & Aljerian, K. (2021). Gestational diabetes mellitus induces placental vasculopathies. *Environmental Science and Pollution Research, 29*, 19860–19868. doi: . https://doi.org/10.1007/s11356-021-17267-y

25. Stanek, J., Sheridan, R. M., Le, L. D., & Crombleholme, T. M. (2011). Placental fetal thrombotic vasculopathy in severe congenital anomalies prompting EXIT procedure. *Placenta, 32*, 373–379. doi: https://doi.org/10.1016/J.PLACENTA.2011.02.002

26. Heider, A. (2017). Fetal vascular malperfusion. *Archives of Pathology & Laboratory Medicine, 141*, 1484–1489. doi: https://doi.org/10.5858/ARPA.2017-0212-RA

27. Roberts, D. J., & Torous, V. (2022). Placental pathology. In *Reproductive and developmental toxicology* (3rd ed., pp. 1399–1420). doi: https://doi.org/10.1016/B978-0-323-89773-0.00069-2

28. Colson, A., Sonveaux, P., Debiève, F., & Sferruzzi-Perri, A. N. (2021). Adaptations of the human placenta to hypoxia: Opportunities for interventions in fetal growth restriction. *Human Reproduction Update, 27*(3), 531–569. doi: https://doi.org/10.1093/humupd/dmaa053

29. Chen, A., & Roberts, D. J. (2018). Placental pathologic lesions with a significant recurrence risk—What not to miss! *APMIS, 126*(7), 589–601. doi: https://doi.org/10.1111/apm.12796

30. Abrahams, V. M., Kim, Y. M., Straszewski, S. L., Romero, R., & Mor, G. (2004). Macrophages and apoptotic cell clearance during pregnancy. *American Journal of Reproductive Immunology, 51*(4), 275–280. doi: https://doi.org/10.1111/j.1600-0897.2004.00156.x

31. Harris, L. K. (2010). Review: Trophoblast-vascular cell interactions in early pregnancy: How to remodel a vessel. *Placenta, 31*(Suppl.), S93. doi: https://doi.org/10.1016/j.placenta.2009.12.012

32. Fest, S., Aldo, P. B., Abrahams, V. M., et al. (2007). Trophoblast-macrophage interactions: A regulatory network for the protection of pregnancy. *American Journal of Reproductive Immunology, 57*, 55. doi: https://doi.org/10.1111/j.1600-0897.2006.00446.x

33. Nunes, P., Nogueira, R., Coelho, J., Rodrigues, F., Salomão, N., José, C., et al. (2019). A stillborn multiple organs' investigation from a maternal DENV-4 infection: Histopathological and inflammatory mediators characterization. *Viruses, 11*(4), 319. doi: https://doi.org/10.3390/v11040319

34. Rabelo, K., de Souza, L. J., Salomão, N. G., Machado, L. N., Pereira, P. G., Portari, E. A., et al. (2020). Zika induces human placental damage and inflammation. *Frontiers in Immunology, 11*, 2146. doi: 10.3389/fimmu.2020.02146