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Research Article

Histopathological Changes in The Placentas of Pregnant Women with Premature Rupture of Membranes (PROM) and Preterm PROM

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Abstract

Introduction: Premature rupture of membranes (prom) and preterm prom (pprom) are critical obstetric complications associated with placental dysfunction. Histological changes in PPROM placentas have not been studied much in studies compared to PROM. This study aimed to investigates histopathological alterations in placental tissues of premature rupture of membranes of PROM and preterm PROM patients .

Methods: This experimental non-randomized case-control study was conducted with 30 PROM patients , PPROM pregnant women and 30 healthy pregnant women at Dicle University Faculty of Medicine between May 2024 and May 2025. Placental tissues obtained following cesarean delivery were subjected to routine tissue monitoring. Placental samples were stained with hematoxylin-eosin and examined histopathologically using a light microscope.

Results: When histopathological findings were compared between the PROM, PPROM and control groups, villous integrity in control groups, congestion in blood vessels, fibrinoid accumulation, inflammation and intervillous hemorrhage were higher in the PPROM group than in the control group ($p<0.001$).H&E revealed preserved villous integrity in controls, partial atrophy/fibrin deposition in PROM, and severe degeneration/edema in preterm PROM.

Conclusion: Preterm PROM placentas display pronounced histopathological damage. The intricate relationship between PROM and placental pathology underscores the profound impact of metabolic changes on placental structure and function throughout pregnancy. Understanding these pathological changes is crucial for elucidating the progression of PPROM and its implications for maternal and fetal health, highlighting the importance of comprehensive placental analysis in managing pregnancies complicated by PROM.

Keywords: Placenta, Premature Rupture of Membranes, Preterm Premature Rupture of Membranes, Histopathology, Hematoxylin-Eosin Staining

Introduction

Premature rupture of membranes (PROM) and Preterm rupture of membranes before the 37th week of gestation, termed preterm premature rupture of membrane (PPROM), is a common obstetric complication which occurs in approximately 3–4.5% of all pregnancies [1]. PPROM is associated with 30% of neonatal morbidities and mortalities in preterm delivery [2], and remains a challenge for the obstetrician [3]. Over the past decade, studies have emerged associating maternal upper genital tract infection with PPROM and spontaneous preterm delivery [4,5].PPROM carries higher risks of neonatal complications due to inflammation and tissue degradation[6,7].

The placenta is an essential organ generated during gestation, one of its primary roles being the transfer of oxygen and macro- and micro-nutrients from the mother to the fetus and the removal of waste products from the fetus for fetal development and well-being [8]. Placentation was described as the 'approximation or combination of an embryo's tissues with those of its natural or surrogate parent for physiological interchange [9]. The placenta has crucial immunoregulatory characteristics, which are clearly presented by the tolerance of the fetus by the maternal immune system in a process that includes excessive inflammation inhibition following blastocyst implantation, modulated by uterine natural killer cells and proliferation of regulatory T cells [10]. Alterations that occur in the placenta during pregnancy should be compatible with the functions that the placenta

undertakes during pregnancy. Defective implantation and placentation are associated with adverse pregnancy outcomes [11]. Moreover, placentas have antibacterial, anti-inflammatory, and anti-scarring activities. All these features have encouraged an interest in utilizing placentas for applications in cell therapy and regenerative medical treatment modalities [10].

Studies have shown that histopathological changes in the fetal membranes, including alterations in collagen structure, inflammation, and vascular abnormalities, contribute to the weakening of the membranes and their subsequent rupture. For instance, a study by Menon et al. demonstrated increased levels of inflammatory markers, such as interleukin-6, in the amniotic fluid of women with PPROM compared to women with intact membranes [12].

Another study by Kim et al. investigated the role of oxidative stress and apoptosis in the pathophysiology of PPROM, highlighting the presence of oxidative damage and cell death in the fetal membranes of affected pregnancies. Furthermore, research by Gomez-Lopez discussed the importance of histological evaluation of the membranes in identifying biomarkers for predicting and managing PPROM. [13,14]

Understanding the histopathological changes associated with PPROM is essential for improving clinical management and developing targeted interventions to prevent preterm birth and its complications. Further research in this field will enhance our knowledge of the underlying mechanisms of PPROM and pave the way for more effective strategies to reduce the incidence and adverse outcomes of this condition.

Therefore, in this study, we aimed to examine the histopathological changes in the placentas of patients with PROM and PPROM compare them with the placentas of the healthy control group.

MATERİALS AND METHODS

This experimental non-randomized case-control study was conducted with 90 patients at the Department of Histology and Embryology, Dicle University Faculty of Medicine (Diyarbakır, Turkey) between May 2024 and May 2025. The study group included singleton pregnant women who had not previously been diagnosed with PPROM and PROM. Thirty healthy singleton pregnant women who had no history of PROM and PPROM or any complications in their current or previous pregnancies were included in the control group. Inclusion criteria: age 18–40 years, no systemic/respiratory diseases.

This study is part of Deniz Balsak's doctoral thesis from the Department of Histology and Embryology at Dicle University. Ethical approval for this study was obtained from the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee. The ethics number is 2024/175. The placentas used as materials in the study were obtained from patients delivered at Dicle University Faculty of Medicine, Gynecology and Obstetrics Clinic. Tissue tracking and histological evaluation of the study was carried out at Dicle University Faculty of Medicine Histology and Embryology Research Laboratory.

Routine histological tissue preparation

Placentas obtained following cesarean delivery were transferred to the laboratory under appropriate conditions. Placental tissues taken in small pieces were subjected to routine tissue preparation. Tissues were placed in a 10% Formalin fixative (catalogue no. Z2902-3.75L, Sigma, Germany) solution and kept for 24 hours to fix. Then, the tissues were kept under running water overnight and dehydrated by passing through increasing alcohol series (50%, 70%, 80%, 90%, 96% and absolute ethyl alcohol). Samples were kept in xylene for 3x30 minutes and the alcohol was removed. The tissues were incubated in paraffin wax at 58°C for 3 × 45 minutes and then embedded in paraffin blocks. Four µm thick sections from the blocks were taken on a positively charged slide with the help of a microtome (catalogue number: Leica RM2265, Wetzlar, Germany) and histochemical staining was performed.

Hematoxylin-Eosin staining

Placental tissue sections taken from paraffin blocks were placed in a boiler set at 37°C. The sections were kept in an oven at 58-62°C for 6 hours to melt excess paraffin on the slide. After the sections were deparaffinized in xylene for three 15-minute intervals, they were passed through a decreasing alcohol series (100%, 96%, 90%, 70%, and 50% ethyl alcohol) for 10 minutes each, then transferred to distilled water and kept for 5 minutes. Hematoxylin-eosin (H&E) stain was applied to the sections. After the staining phase, the sections were quickly immersed in an increasing alcohol series (passing through 80%, 90%, and 96% ethyl alcohol series) and kept in absolute alcohol for 2 minutes. Finally, the sections were kept in xylene for 3x15 minutes and the tissue was covered with a coverslip by dropping mountain medium on it.

RESULTS

Hematoxylin-eosin staining findings

Control Group

In the placental tissue of the control group, the integrity of the villous structures is preserved. Chorionic villi have regular contours, and no significant edema or degenerative changes in the stroma are observed. The syncytiotrophoblast layer uniformly covers the surface of the villi. No inflammatory cell infiltration or vascular disorders were observed. Villous fibrinoid accumulation and syncytial node are minimal (Figure 1).

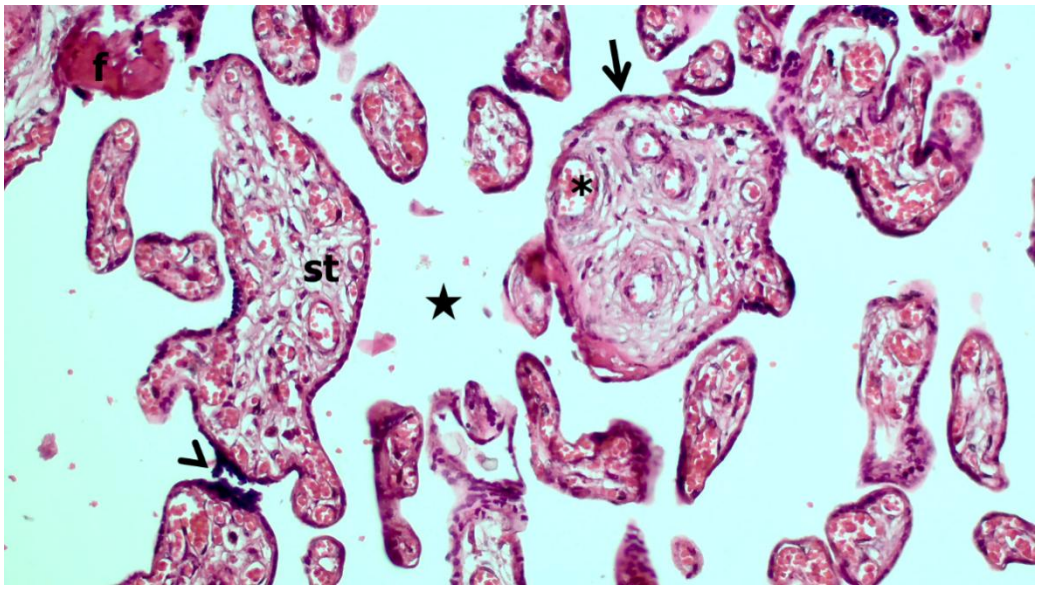

Figure 1: Hematoxylin-eosin (H&E)-stained placental tissue sample from the control group.

Chorionic villi (arrow), Syncytial knot (arrowhead), stroma(st), villous capillaries (asteriks), intervillous area (star), fibrinoid accumulation (f), (Magnification: 20x, scale bar: 50 µm).

PROM Group

In the placental section belonging to the PROM group, the villous structures appear to be partially distorted and atrophyed in places. Increased accumulation of fibrinoid material in the intervilli-area and inflammatory cell infiltration in the stroma are noted. Irregularity in the syncytiotrophoblast layer and cell loss in some areas have been observed. There is a significant congestion in the vascular structures and a hemorrhage-like appearance in some areas (Figure 2.). Histopathologies in this group were increased compared to the control group.

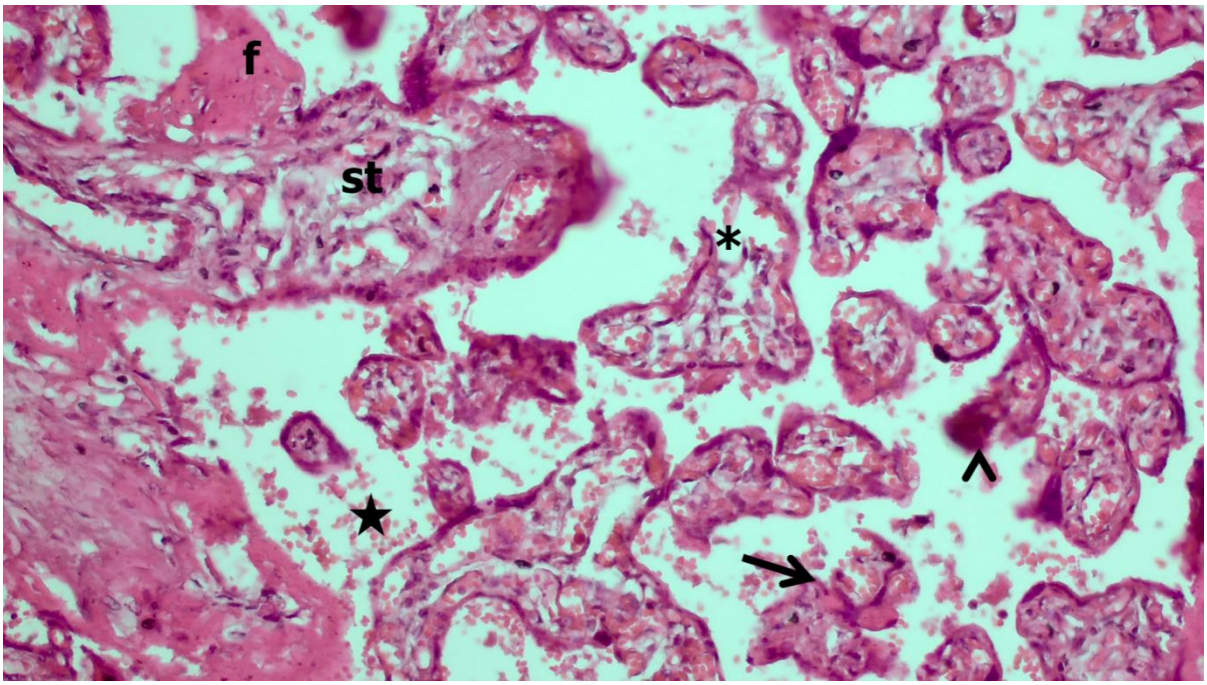

Figure 2: Hematoxylin-eosin (H&E) stained placental tissue sample belonging to the PROM group.

Chorionic villi (arrow), Syncytial knot (arrowhead), stroma(st), villous capillaries (asteriks), intervillous area (star), fibrinoid accumulation (f), (Magnification: 20x, scale bar: 50 µm).

Preterm PROM Group

In the placental specimen belonging to the preterm PROM group, irregularities in the villous structures, degeneration and significant fibrin accumulations are noteworthy. Thinning and separation are observed in the syncytiotrophoblast layer. There is inflammatory cell infiltration and edema concentrated in the stromal regions. In addition, in some areas, narrowing and congestion of the lumens of the villous vessels are observed (Figure 3). According to the control and PROM groups, the most intense histopathology was observed in this group.

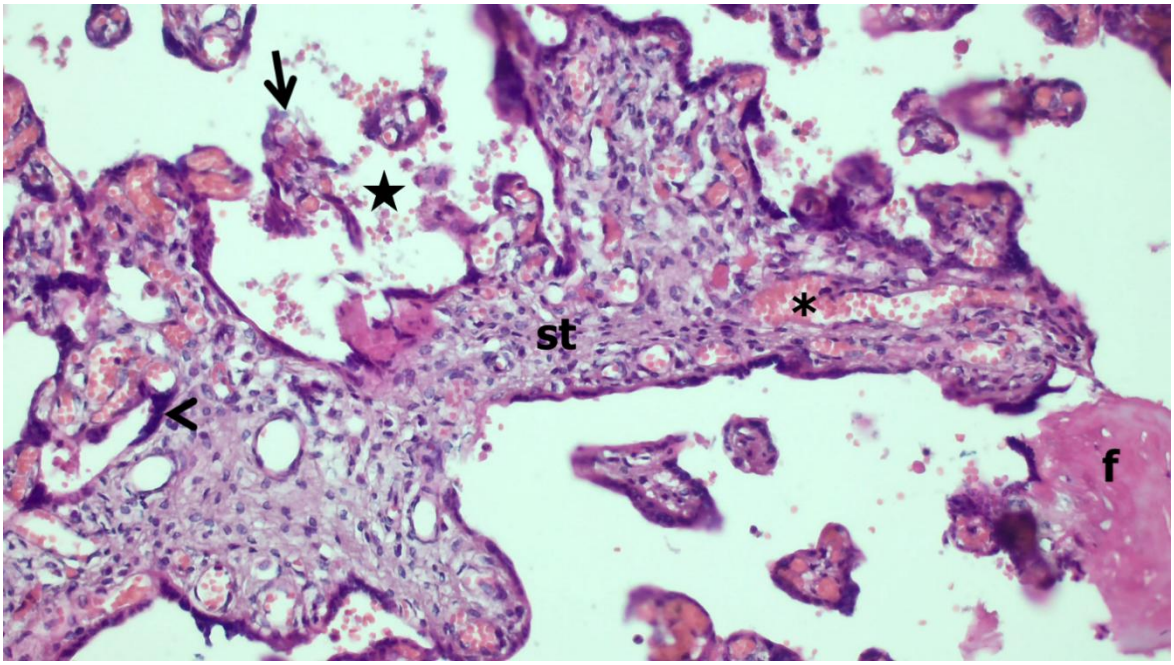

Figure 3: Hematoxylin-eosin (H&E) stained placental tissue sample belonging to the preterm PROM group.

Chorionic villi (arrow), Syncytial knot (arrowhead), stroma(st), villous capillaries (asteriks), intervillous area (star), fibrinoid accumulation (f), (Magnification: 20x, scale bar: 50 µm).

DISCUSSION

Histopathological changes such as villous degeneration, syncytiotrophoblast thinning, edema, congestion and inflammatory infiltration are frequently observed in the placenta in cases of preterm premature rupture of membranes (PPROM) and PROM.

In this study, hematoxylin-eosin (H&E)-stained placental sections showed more significant villous degeneration, syncytiotrophoblast thinning, edema and congestion in the preterm PROM group compared to the control group and the PROM group. In the control group, villous structures were observed in a regular and integrated manner, while partial atrophy, fibrinoid accumulation and inflammation were observed in the PROM group. These findings indicate that PROM, and especially preterm PROM, may adversely affect maternal-fetal exchange by disrupting placental tissue integrity.

The villous atrophy and degeneration we observed may reflect the destructive effect of inflammatory processes and oxidative stress on placental tissue, consistent with studies in the literature [15]. PROM leads to the entry of bacteria into the uterus with premature rupture of the amniotic membrane and, accordingly, the triggering of an inflammatory response. Especially in cases of preterm PROM, this inflammatory response is thought to be more severe and affects the placenta at an earlier stage [16]. Enzymes such as inflammatory mediators (e.g., interleukins, TNF-α) and matrix metalloproteinases (MMPs) can contribute to placental tissue destruction, resulting in disruption of the villous structure and syncytiotrophoblast thinning [17].

Syncytiotrophoblast thinning can lead to a weakening of the barrier between maternal blood and fetal blood, thereby reducing the passage of oxygen and nutrients into the fetus. This can lead to fetal hypoxia and developmental problems [18]. Syncytiotrophoblast thinning was more pronounced in the preterm PROM group, supporting that this group is at higher risk in terms of fetal prognosis.

Fibrinoid accumulation is a finding associated with placental aging and inadequate maternal blood flow [19]. The fibrinoid accumulation observed in the PROM group in our study suggests that placental function is partially impaired and maternal-fetal exchange is affected. In the preterm PROM group, the fact that fibrinoid accumulation is less pronounced can be interpreted as tissue destruction is more prominent and fibrinoid accumulation is not yet sufficiently developed.

The edema and congestion observed in the placental tissues may be associated with increased vascular permeability and impaired blood flow resulting from inflammation. Especially in the preterm PROM group, the

congestion is more intense, indicating that inflammatory processes affect vascular structures more and placental perfusion is more severely impaired.

Among the limitations of this study are the relatively small sample size and the fact that only histopathological evaluation was performed. In future studies, performing immunohistochemical and molecular analyses with larger sample groups will help us to understand the placental effects of PROM and preterm PROM in more detail.

CONCLUSİON:

In conclusion, this study shows that PROM, and especially preterm PROM, lead to significant changes in placental tissue, and these changes may increase the risk of fetal morbidity and mortality. A deeper understanding of the pathophysiological mechanisms underlying placental damage may lead to the development of new strategies for preventing and treating these complications.

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REFERENCES:

1. Lee T, Silver H. Etiology and epidemiology of preterm premature rupture of the membranes. Clin Perinatol. 2001;28:721-34. https://doi.org/10.1016/S0095-5108(03)00073-3 PMid:11817185

2. Gopalani S, Krohn M, Meyn L, et al. Contemporary management of preterm premature rupture of membranes: determinants of latency and neonatal outcome. Am J Perinatol. 2004;21:183-90. https://doi.org/10.1055/s-2004-828609 PMid:15168316

3. Garite TJ. Management of premature rupture of membranes. Clin Perinatol. 2001;28:837-47. https://doi.org/10.1016/S0095-5108(03)00081-2 PMid:11817193

4. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000;342:1500-7. https://doi.org/10.1056/NEJM200005183422007 PMid:10816189

5. Asrat T. Intra-amniotic infection in patients with preterm prelabor rupture of membranes. Pathophysiology, detection, and management. Clin Perinatol. 2001;28:735-51. PMid:11817186 https://doi.org/10.1016/S0095-5108(03)00074-5

6. Della Rosa, P. A., Miglioli, C., Caglioni, M., Tiberio, F., Mosser, K. H. H., Vignotto, E., Canini, M., Baldoli, C., Falini, A., Candiani, M., & Cavoretto, P. A hierarchical procedure to select intrauterine and extrauterine factors for methodological validation of preterm birth risk estimation. BMC pregnancy and childbirth, 2021;21(1):306. https://doi.org/10.1186/s12884-021-03654-3 PMid:33863296 PMCid:PMC8052693

7. Delorme, P., Lorthe, E., Sibiude, J., & Kayem, G. Preterm and term prelabour rupture of membranes: A review of timing and methods of labour induction. Best practice & research. Clinical obstetrics & gynaecology, 2021;77:27-41. https://doi.org/10.1016/j.bpobgyn.2021.08.009 PMid:34538740

8. Rani A, Wadhwani N, Chavan-Gautam P, Joshi S. Altered development and function of the placental regions in preeclampsia and its association with long-chain polyunsaturated fatty acids. Wiley Interdiscip Rev Dev Biol. 2016;5(5):582-97. https://doi.org/10.1002/wdev.238 PMid:27239793

9. Mess A, Carter AM. Evolution of the placenta during the early radiation of placental mammals. Comp Biochem Physiol A Mol Integr Physiol. 2007;148(4):769-79. https://doi.org/10.1016/j.cbpa.2007.01.029 PMid:17347003

10. Lobo SE, Leonel LC, Miranda CM, Coelho TM, Ferreira GA, Mess A, et al. The Placenta as an Organ and a Source of Stem Cells and Extracellular Matrix: A Review. Cells Tissues Organs. 2016;201(4):239-52. https://doi.org/10.1159/000443636 PMid:27050810

11. Turco MY, Moffett A. Development of the human placenta. Development. 2019;146(22):dev163428. https://doi.org/10.1242/dev.163428 PMid:31776138

12. Menon, R., & Fortunato, S. J. Infection and the role of inflammation in preterm premature rupture of the membranes. Best practice & research. Clinical obstetrics & gynaecology, 2007;21(3):467-478. https://doi.org/10.1016/j.bpobgyn.2007.01.008 PMid:17448730

13. Kim, C. J., Romero, R., Chaemsaithong, P., Chaiyasit, N., Yoon, B. H., & Kim, Y. M. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. American journal of obstetrics and gynecology, 2015;213(4 Suppl), S29-S52. https://doi.org/10.1016/j.ajog.2015.08.040 PMid:26428501 PMCid:PMC4774647

14. Gomez-Lopez, N., Romero, R., Galaz, J., Xu, Y., Panaitescu, B., Slutsky, R., Motomura, K., Gill, N., Para, R., Pacora, P., Jung, E., & Hsu, C. D. Cellular immune responses in amniotic fluid of women with preterm labor and intra-amniotic infection or intra-amniotic inflammation. American journal of reproductive immunology (New York, N.Y. : 1989;82(5):e13171. PMCid:PMC6788958 https://doi.org/10.1111/aji.13171 PMid:31323170

15. Redline, R. W. Placental pathology: a systematic approach. *Modern Pathology, 2008;21(3):214-221.

16. Romero, R., Espinoza, J., Mazor, M., Manaster, I., Kusanovic, J. P., & Diamond, M. P. The preterm parturition syndrome. *BJOG: An International Journal of Obstetrics & Gynaecology, 2006;113(s3):17-42. https://doi.org/10.1111/j.1471-0528.2006.01120.x PMid:17206962 PMCid:PMC7062298

17. Fortunato, S. J., & Menon, R. Preterm premature rupture of membranes: a cascade of events leading to fetal inflammation. *American Journal of Obstetrics and Gynecology, 2001;185(6 Suppl):S53-S63.

18. Mayhew, T. M., Ohadike, C. I., Baker, P. N., & Crocker, I. P. Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction. *Placenta, 2000;21(2-3):143-153.

19. Benirschke, K., & Kaufmann, P. Pathology of the Human Placenta. Springer. 2000. https://doi.org/10.1007/978-1-4757-4199-5