ORIGINAL ARTICLE

EFFECT OF MESENCHYMAL STEM CELLS ON UTERUS AND PLACENTA

Mezenşimal Kök Hücrelerin Uterus ve Plasenta Üzerine Etkisi

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ABSTRACT

Objective: This study investigated the effects of mesenchymal stem cell (MSC) application on the expression of extracellular matrix proteins fibronectin, matrix metalloproteinase-2 (MMP-2), and matrix metalloproteinase-9 (MMP-9) at the implantation site in pregnant Wistar Albino rats.

Material and Methods: Thirty-two adult female Wistar Albino rats were divided into two groups (control, n=8; stem cell, n=8) on the 10th and 21st days of gestation. The stem cell group received MSCs derived from rat bone marrow, while the control group received Phosphate Buffered Saline (PBS). Implantation sites and placentas were collected, and fibronectin, MMP-2, and MMP-9 expressions were evaluated using immunohistochemistry.

Results: On day 10 of gestation, fibronectin reactivity in the decidual structure was similar in both control and stem cell groups, with no significant difference ($p>0.05$). On day 21, fibronectin expression was significantly elevated in the labyrinth area and spongiotrophoblasts of the stem cell group compared to the control group ($p=0.02$). MMP-2 immunoreactivity was reduced around trophoblastic giant cells in the stem cell group but remained consistent in decidua cells, gland epithelium, and vessel walls. MMP-9 levels were higher in trophoblastic giant cells in the stem cell group, though the difference was not statistically significant ($p>0.05$). On day 21, both MMP-2 and MMP-9 activities were less intense in both groups.

Conclusion: MSC administration significantly increased fibronectin expression in the labyrinth area and spongiotrophoblasts on day 21 of gestation, suggesting a role for MSCs in enhancing extracellular matrix remodeling at the implantation site. However, MSC treatment did not significantly alter MMP-2 or MMP-9 immunoreactivity, indicating that their effects may be specific to fibronectin-mediated processes during placental development.

Keywords: *Implantation; Mesenchymal Stem Cell; Fibronectin; MMP-2; MMP-9*

ÖZET

Amaç: Bu çalışmada, mezenkimal kök hücre (MKH) uygulamasının gebe Wistar Albino sıçanlarda implantasyon bölgesinde hücre dışı matriks proteinleri fibronektin, matriks metalloproteinaz-2 (MMP-2) ve matriks metalloproteinaz-9 (MMP-9) ekspresyonu üzerindeki etkileri araştırılmıştır.

Gereç ve Yöntemler: Otuz iki yetişkin dişi Wistar Albino sıçan gebeliğin 10. ve 21. günlerinde iki gruba ayrılmıştır (kontrol, n=8; kök hücre, n=8). Kök hücre grubuna sıçan kemik iliğinden elde edilen MKH'ler verilirken, kontrol grubuna Fosfat Tamponlu Salin (PBS) verilmiştir. İmplantasyon bölgeleri ve plasentalar toplandı ve fibronektin, MMP-2 ve MMP-9 ekspresyonları immünohistokimya kullanılarak değerlendirildi.

Bulgular: Gebeliğin 10. gününde, desidual yapıdaki fibronektin reaktivitesi hem kontrol hem de kök hücre gruplarında benzerdi ve anlamlı bir fark yoktu ($p>0,05$). 21. günde, fibronektin ekspresyonu kontrol grubuna kıyasla kök hücre grubunun labirent alanında ve spongiotrofoblastlarında önemli ölçüde artmıştır ($p=0,02$). MMP-2 immünoreaktivitesi kök hücre grubunda trofoblastik dev hücrelerin çevresinde azalmış, ancak desidua hücrelerinde, bez epitelinde ve damar duvarlarında sabit kalmıştır. MMP-9 seviyeleri kök hücre grubundaki trofoblastik dev hücrelerde daha yüksekti, ancak fark istatistiksel olarak anlamlı değildi ($p>0.05$). 21. günde, hem MMP-2 hem de MMP-9 aktiviteleri her iki grupta da daha az yoğundu.

Sonuç: MSC uygulaması, gebeliğin 21. gününde labirent bölgesinde ve spongiotrofoblastlarda fibronektin ekspresyonunu önemli ölçüde artırarak MSC'lerin implantasyon bölgesinde hücre dışı matriks yeniden şekillenmesini artırmada rol oynadığını düşündürmüştür. Bununla birlikte, MSC tedavisi MMP-2 veya MMP-9 immünoreaktivitesini önemli ölçüde değiştirmemiştir, bu da etkilerinin plasental gelişim sırasında fibronektin aracılı süreçlere özgü olabileceğini göstermektedir.

Anahtar Kelimeler: *İmplantasyon; Mezenşimal Kök Hücre; Fibronektin; MMP-2; MMP-9*

INTRODUCTION

Successful reproduction requires the blastocyst to implant into the endometrium, a process involving complex physiological, biochemical, and structural interactions between blastocyst trophoblasts and the uterine epithelium (1, 2). During the preimplantation period, the endometrial luminal epithelium and stroma undergo significant changes, including apposition, adhesion, and invasion (3). In rodents, the initial attachment response, observed by late day 4 in mice, coincides with increased stromal vascular permeability at the blastocyst attachment site (4). This triggers decidualization, a progesterone- and cAMP-driven transformation of endometrial stromal cells into decidual cells, which supports embryonic development (5, 6). In rats and mice, decidualization begins at the antimesometrial pole, where stromal cells form the primary decidual zone (PDZ) with an epithelial-like morphology. As implantation progresses, the PDZ regresses, giving way to the secondary decidual zone (SDZ), a layer of proliferating and differentiating stromal cells peaking around day 8 in mice (7, 8). This process facilitates epithelial-mesenchymal transition and immune adaptation critical for successful implantation (8).

The extracellular matrix (ECM) plays a pivotal role in implantation by mediating blastocyst-ECM interactions that maintain tissue architecture and regulate cell growth and differentiation (5). Matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, are key regulators of ECM remodeling, activating cell surface receptors, growth factors, cytokines, and adhesion molecules (9). These gelatinases degrade ECM components such as collagen IV, V, VII, XI, fibronectin, laminin, elastin, proteoglycans, and entactin, facilitating trophoblast invasion (10, 11). Studies highlight MMP-2's dominant role in early trophoblast invasion, while MMP-9 deficiency in mouse embryos impairs invasion, underscoring their importance in maternal-fetal communication (12-14). Fibronectin, a critical ECM protein, serves as a key adhesion molecule in connective tissue, promoting cell migration during embryogenesis and implantation (15-17).

This study aims to investigate the effects of mesenchymal stem cell (MSC) administration on the expression of fibronectin, MMP-2, and MMP-9 at the rat implantation site using immunohistochemistry, elucidating their roles in ECM remodeling and implantation success.

MATERIALS AND METHODS

In our study, 32 adult Wistar albino female rats weighing between 180-200 grams were used. The ethical approval of this study was granted by Adnan Menderes University (ADÜ) Animal Experiments Local Ethics Committee (ADÜ-HADYEK) (NO:64583101/2014/070-11.06.2014). The rat experiments were performed in ADÜ Animal Experiments Research Laboratory. The cell culture was performed in the ADÜ-BİLTEM laboratory, and the histology and immunohistochemical staining phases were performed in ADÜ Histology-Embryology Laboratory. All rats were maintained in plastic cages under 12:12 h day/night cycle at 23±2 temperature °C and 45±5% humidity with intake of water and food ad libitum. For the experiment, animals were divided into 2 groups of 16 rats, then the experimental procedure was performed as follows: 1. Control group (n=16): It was divided into 2 groups of 8 rats each on days 10 and 21. Female rats were kept in the same cage with male rats overnight, and those with spermatozoa found in vaginal smears the next morning were considered as day 0 of pregnancy and Phosphate Buffered Saline (PBS) was administered through the tail vein of the rats on the same day. 2. Stem Cell Group (n=16): The rats were divided into 2 groups of 8 rats each on days 10 and 21. Female rats were kept in the same cage with male rats for one night and those with spermatozoa in vaginal smears the next morning were considered as day 0 of pregnancy and 1x106 mesenchymal stem cells expressing Green Fluorescent Protein (GFP) were injected from the tail vein of the rats in 0.4 mL PBS on the same day. On days 10 and 21, all subjects were sacrificed under intraperitoneal anesthesia with 60 mg/kg ketamine and 5 mg/kg xylazine, after which the implantation site and placentas were removed and tissues were fixed in 10% neutral formalin.

Five female rats weighing 180-200 grams were used as donors to obtain mesenchymal stem cells. The rats were sacrificed by ketamine and xylazine administration and the femurs were removed after the hind and forelimbs were sterilized with 70% alcohol. The removed femurs

were cleaned from soft tissues and placed in 25 mL Hank's Buffered Slat Solution falcon tubes and kept for a period of time. In order to obtain stem cells, femurs were cut from both ends and placed vertically in tubes containing Minimum Essential Medium (MEM). After the bone marrow was transferred from the bones to the medium, it was centrifuged, 10 mL of medium was added to the pellet, pipetted and distributed into T25 flasks. The flasks were placed in the CO2 incubator and the medium was changed every three days and checked under light microscope (18).

In this study, retrovirus-mediated gene transfer was performed for permanent labeling of MSCs with Green Fluorescent Protein (GFP) before. For this, the retroviral mammalian expression vector MSCV (The Murine Stem Cell Virus) -IRES-GFP (6500 bp) carrying the cDNA of GFP was used. For this purpose, ecotropic Phoenix (gag+, pol+) packer cells were transfected with MSCV-IRES-GFP vector by calcium precipitation method. MSCs were infected with viral supernatant produced in transfected Phoenix cells for 2 days at 12-hour intervals. Then, cells were stained with Trypan blue (Sigma 93595, Germany) at a ratio of 1/1 for viability testing and cell counting was performed using a neubauer slide.

MSCs were identified by flow cytometry analysis using mesenchymal stem cell lineage surface expression markers (CD11b, CD29, CD45 and CD90) from passage number three. Cells attached to the surface of the culture dish were removed with 0.25% Trypsin-EDTA (Sigma T4049, Germany) centrifuged and the pellet was suspended with PBS. Cells were then incubated with fluorescein isothiocyanate (FITC)-conjugated monoclonal CD11b, CD29, CD45, and CD90 (BD, Biosciences, USA) antibodies specific for the identified cell surface markers for 45 minutes in the dark. After incubation and washing, the cell suspension was read on FACS Calibur Flow Cytometry (BD Biosciences) and analyzed with the BD CellQuestTM software program.

The tissue samples were embedded in OCT (Optimal Cutting Temperature) compound and kept overnight at +4ºC and then 5 µm sections were taken in a frozen (Cryostat A650) device. The sections were washed 3 times with PBS after 10 minutes in the dark with a drop of methanol and the sections were treated with the nuclear stain DAPI (Sigma-Aldrich D9542). Imaging was then performed under a fluorescence microscope (Leica DMI 4000).

For histologic follow-up, all tissues were fixed in 10% neutral formalin and then dehydrated in various alcohol series to prepare the tissues for microscopic examination. After transparency and paraffin blocking, serial sections of 5 µm thickness were taken with a microtome (Leica RM 2265, Germany). After deparaffinization, the tissue sections were stained with Hematoxylin-Eosin (H&E) and Alcian Blue stain (Sigma-Aldrich, 123657). The specimens obtained were subjected to general histopathologic evaluation under light microscope (Olympus-BX50). In immunohistochemical analysis, the immunoreactivity of Fibronectin, MMP-2 and MMP-9 proteins in the implantation site and placental tissues of the experimental groups were determined by Avidin-Biotin peroxidase method (19). Briefly, after deparaffinization of 5 µm thick sections, they were kept in citrate buffer 2 × 4 times (pH: 6.0) in a 300 W microwave oven to open epitopes and then kept in 3% hydrogen peroxide for endogenous peroxidase activity. To prevent unwanted staining, tissue sections were kept in Ultra V block and incubated with primary antibodies at +4ºC for 1 night. Biotinylated streptavidin-HRP and DAB secondary chromogens were then applied and sections were counterstained with hematoxylin Gill. They were dehydrated by passing through a series of ascending alcohol series and sections were covered with entellan. Sections were examined under an Olympus CX-31 light microscope. In all groups, the number of cells positive for Fibronectin (Abcam, ab32419), MMP-2 (Abcam, ab97779) and MMP-9 (Abcam, ab228402) immunoreactivity was evaluated by H-SCORE (Histologic scoring). Three sections were randomly selected for each group and four regions in each section were evaluated for analysis of immunohistochemical staining. Severity expression scoring: -(0): Negative, +(1): Weak, ++ (2): Moderate, +++ (3): Strong. Intensity expression scoring: 0,1: < %25, 0,4: < %26-50, 0,6: < %51-75, 0,9: < %76-100 (20). Immunoreactive score: the degree of intensity of positively stained cells multiplied by the scores obtained from the staining intensities H-SCORE for all groups was evaluated by three independent observers.

Statistical analysis was performed using the IBM SPSS

Statistics 15.0 software package (SPSS Inc., Chicago, IL, USA). Due to the relatively small sample size and the ordinal nature of the immunohistochemical scoring, a non-parametric approach was adopted. Comparisons between the control and stem cell groups were conducted using the Mann-Whitney U test. Descriptive statistics are presented as median values with interquartile ranges (25th–75th percentiles). A p-value of less than 0.05 was considered statistically significant (Table 1).

RESULTS

Immunophenotyping of MSCs was performed after the 3rd passaging. Immunophenotypic characterization of MSCs was determined by flow cytometry technique; CD45 and CD11b markers were negative and CD29 and CD90 markers were positive. Stromal markers: CD29 FITC (66.68%), CD90 (99.94%), CD45 PE (2.79%) and CD11b FITC (17.48%).

Experimental animals that were previously treated with MSCs transfected with GFP gene were sacrificed on days 10 and 21 and tissue sections were taken. Tissue sections were then labeled with DAPI and visualized with a fluorescence microscope (Leica DMI 4000). Day 10 and 21, the presence of MSCs in the implantation site of the rat uterus was demonstrated by the presence of GFP positive (Figure 1).

On the 10th day of gestation, the uterus mainly consists of 2 parts: antimesometrial region and mesometrial region (21). Towards the mesometrial region, the cryoallontoic placenta was observed and fetal membranes were prominent. In the antimesometrial region, stromal cells were dense but small in size compared to decidual cells (Figure 2A). Trophoblastic giant cells with large cytoplasm and abundant intensely stained granules with vacuoles in their cytoplasm were observed (Figure 2B). As the trophoblastic giant cells progressed towards the myometrium layer, they were observed to transform into stromal cells and the cytoplasm became smaller and differentiated as multinucleated cells with clear borders (Figure 2B). In the mesometrial region, stromal (decidua) cells differentiated into glycogen-rich cells to enable the formation of a glycogenic zone (Figure 2C). The stromal cells in the enlarged decidual area were shown to have multiple nuclei with a clear border with Alcian

Table 1. Group mean immunoreactivity scores and statistical evaluation (S: Severity, I: Intensity, NA: not available)

Day 10	Fibronectin				MMP-2				MMP-9			
	Control Group		Stem Cell Group		Control Group		Stem Cell Group		Control Group		Stem Cell Group	
	S	I	S	I	S	I	S	I	S	I	S	I
Vascular Wall	2	0,4	1	0,1	2	0,4	2	0,4	2	0,4	2	0,4
Gland Epithelium	2	0,4	2	0,1	2	0,1	2	0,1	2	0,1	2	0,1
Trophobalstic Giant Cells	1	0,1	2	0,4	3	0,6	2	0,4	2	0,4	2	0,4
Stromal Desidua cells	2	0,4	2	0,4	2	0,4	2	0,4	3	0,4	2	0,4
Day 21	**Fibronectin**				**MMP-2**				**MMP-9**			
	Control Group		Stem Cell Group		Control Group		Stem Cell Group		Control Group		Stem Cell Group	
	S	I	S	I	S	I	S	I	S	I	S	I
Vascular Wall	2	0,1	2	0,1	2	0,1	2	0,1	2	0,4	2	0,1
Gland Epithelium	2	0,4	2	0,1	2	0,1	2	0,1	2	0,4	2	0,4
Trophobalstic Giant Cells	2	0,1	2	0,1	2	0,1	2	0,1	1	0,4	2	0,1
Spongiotrophoblasts	1	0,4	1	0,1	1	0,1	2	0,1	2	0,4	2	0,4

Severity scoring		Intensity scoring	
Strong	3	Strong	0,9 <%76-100
Moderate	2	Moderate	0,6 <%51-75
Weak	1	Weak	0,4 <%26-50
Negative	0	Negative	0,1 <%25

Protein	Region	10-Day Control Group (n = 16)	10-Day Stem Cell Group (n = 16)	p-value (10-day)	Region	21-Day Control Group (n = 16)	21-Day Stem Cell Group (n = 16)	p-value (21-day)
Fibronectin	Vascular Wall	0,8 (0,8–0,8)	0,6 (0,3–0,9)	0,630	Vascular Wall	0,2 (0,2–0,8)	0,1 (0,1–0,2)	0,132
	Glandular Epithelium	0,8 (0,1–0,8)	0,5 (0,1–0,9)	0,937	Spongiotrophoblast Cells	1,2 (0,8–1,2)	0,8 (0,8–1,2)	0,394
	Trophoblastic Giant Cells	0,8 (0,1–0,8)	1,2 (0,8–1,3)	0,260	Giant Cells	0,2 (0,2–0,5)	0,2 (0,2–0,2)	NA
	Stromal Cells	0,8 (0,8–1,2)	0,8 (0,4–1,3)	0,699	Labyrinth (Stromal) Cells	NA	0,53 (0,3–0,8)	**0,020**
MMP-2	Vascular Wall	0,8 (0,8–0,9)	0,8 (0,7–1,3)	0,937	Vascular Wall	0,2 (0,2–0,3)	0,2 (0,2–0,3)	1,000
	Glandular Epithelium	0,2 (0,2–0,4)	0,2 (0,1–0,4)	0,818	Spongiotrophoblast Cells	NA	0,73 (0,4–1,0)	0,700
	Trophoblastic Giant Cells	1,2 (1,1–1,3)	1,2 (0,8–1,3)	0,699	Giant Cells	0,2 (0,2–0,3)	0,2 (0,2–0,9)	0,394
	Stromal Cells	1,2 (1,1–1,8)	0,8 (0,8–1,3)	0,180	Labyrinth (Stromal) Cells	NA	NA	NA
MMP-9	Vascular Wall	0,8 (0,8–1,2)	0,8 (0,6–1,2)	0,818	Vascular Wall	NA	0,73 (0,4–1,0)	NA
	Glandular Epithelium	0,3 (0,2–0,4)	0,2 (0,2–0,9)	1,000	Spongiotrophoblast Cells	0,8 (0,8–0,9)	1,0 (0,8–1,8)	0,310
	Trophoblastic Giant Cells	0,8 (1,2–1,2)	1,8 (1,1–1,8)	0,093	Giant Cells	0,4 (0,4–0,5)	0,5 (0,2–0,9)	0,937
	Stromal Cells	1,2 (1,2–0,8)	1,2 (0,8–1,8)	0,589	Labyrinth (Stromal) Cells	NA	0,73 (0,4–1,0)	0,221

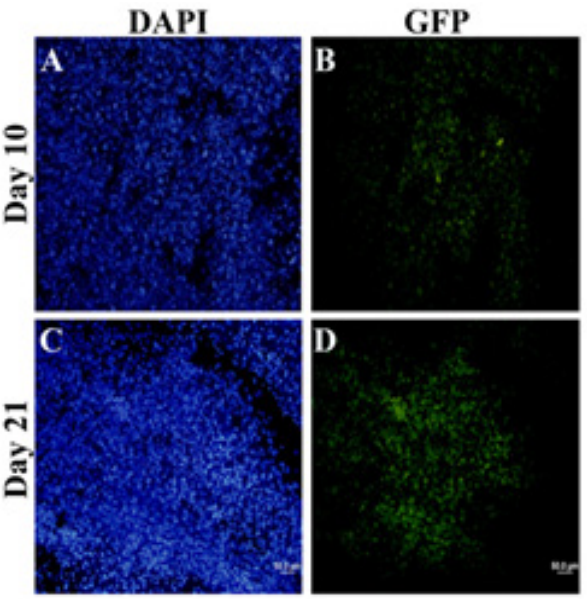


Figure 1. Demonstration of the presence of mesenchymal stem cells on days 10 and 21 by GFP positivity. A, B; Day 10 rat uterine implantation site. B: GFP positive cell line. C, D; Day 21 rat uterine implantation site. D: GFP positive cell line. Magnification: 20×, bar = 50 µm.

Blue stain (Figure 2C, D). 21-day-old rat placenta was observed to consist of 3 layers: maternal decidua area, spongiotrophoblast (basal zone) and labyrinth area (Figure 2E). Glycogen rich cells were observed in the labyrinth area, more in the spongiotrophoblast area, and their number was higher compared to 10-day-olds (Figure 2F,J). Giant trophoblastic cells were localized in the maternal decidua region, had large cytoplasm and intensely stained granule structures with vacuoles in the cytoplasm as on day 10 (Figure 2F,H). In addition, fetal and maternal blood vessels were observed in the spongiotrophobalst zone and fetal blood islets were observed in the labyrinth area (Figure 2G,J).

In the day 10 control group, fibronectin expression in the implantation site of rats showed weak immunoreactivity in giant trophoblastic cells and moderate immunoreactivity in decidual (stromal) cells (Figure 3A1,B1). Fibronectin expression was also seen in the luminal epithelium (Figure 3A1). Fibronectin immunoreactivity of the vessel wall and gland epithelium was moderate (Figure 3C1). MMP-2 immune reactivity of decidual cells was slightly more intense than in the stem cell group (Figure 3D1). Immunoreactivity of giant trophoblastic cells was moderate. (Figure 3E1). MMP-2 immunostaining showed no significant difference in immune reactivity in vessel wall and gland epithelium (Figure 3F1). In general, MMP-9 immunoreactivity examination showed intense ground staining in tissue sections. When the expression intensity for MMP-9 was analyzed, it was found to be intensely expressed in

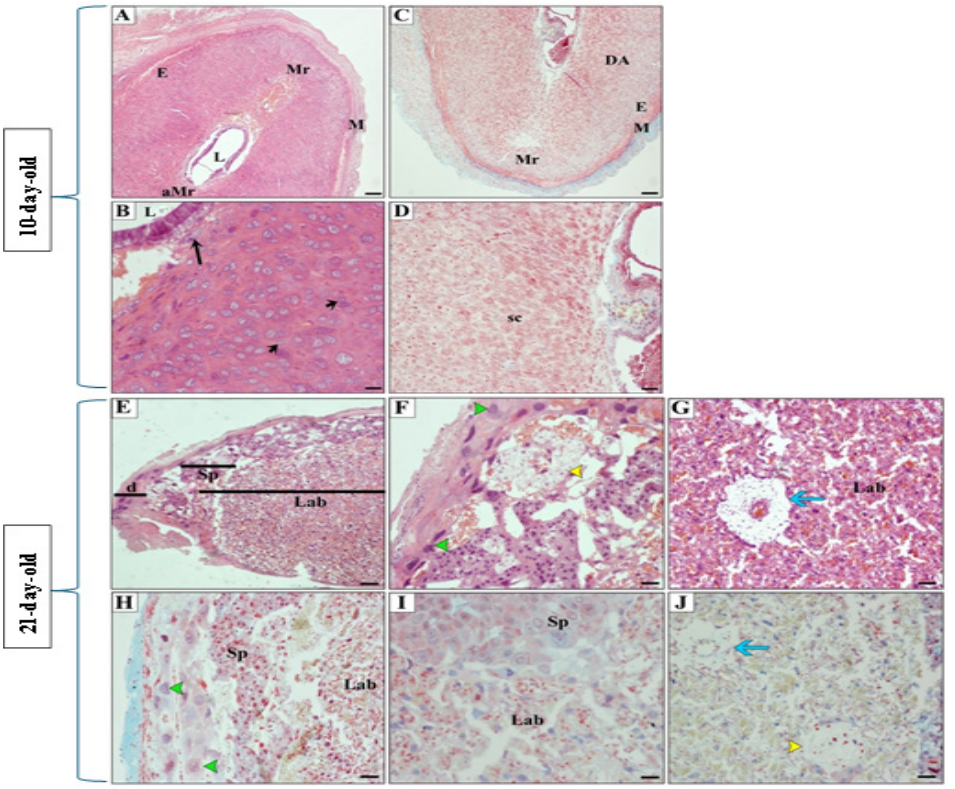


Figure 2. H&E and Alcian Blue staining images of 10-day implantation and 21-day placenta. A; General view - H&E Staining (aMr: Antimesometrial region, Mr: Mesometrial region, L: Lumen, E: Endometrium, M: Myometrium). Magnification: 10×. B; Trophoblastic Giant Cell (long arrow), L: Lumen, H&E staining, Magnification: 40×. C; Mr: Mesometrial region, DA: Decidual Area, E: Endometrium, M: Myometrium, Alcian Blue Staining, Magnification: 10×. D; sc: stroma cells, Alcian Blue Staining, Magnification: 40×. E; Lab: Labyrinth area, Sp: Spongiotrophoblast (basal zone), d: decidua area. Green arrowhead: giant trophoblastic cell. Yellow arrowhead: Glycogen cells. Blue arrow: Glycogen cells around the vessel. Figure E,F,G: H&E staining, Figure H,I,J: Alcian Blue staining. Magnification E: 10×, Magnification H: 20×, Magnification F,G,I,J: 40×

decidual cells (Figure 3G1) and moderately expressed in giant trophoblastic cells (Figure 3H1). In the vessel wall, moderate uptake was observed (Figure 3I1). The localisation densities are given in Table 1.

In the 10-day stem cell group, fibronectin immune reactivity was low in trophobalstic giant cells (Figure 3A2), whereas immune reactivity was moderate in vessel wall endothelium (Figure 3B2) and decidual cells (Figure 3C2). Immunohistochemical evaluation of MMP-2 showed moderate MMP-2 intensity in trophoblastic giant cells (Figure 3D2) and vascular endothelium (Figure 3E2) and low expression in decidual cells (Figure 3F2). MMP-9 immunolocalisation showed that MMP-9 intensity was low in trophoblastic giant cells (Figure 3G2) and decidual cells (Figure 3I2), whereas it was localised moderately in vascular endothelium (Figure 3H2). However, when the 10-day control and stem cell groups were compared, no significant difference was found in terms of immunlocalisation densities. The localisation densities are given in Table 1.

When the immunoreactivity of fibronectin was examined in the control group on the 21st day of gestation, it was observed that the immunoreactivity of trophoblastic giant cells in the maternal decidua (Figure 4A1) was of moderate intensity and staining was also observed in the subfloor connective tissue. On the other hand, it was observed that labyrinthine trophoblastic cells contained more than one nucleus and were larger in the transition zone from the attachment zone to the labyrinth layer (Figure 4B1). Fibronectin expression was less intense in the vessel wall (Figure 4C1). When MMP-2 immunoreactivity was analysed, immune reactivity of trophoblastic giant cells in the maternal decidua region of the rat placenta was moderate. The immunoreactivity of spongiotrophoblasts in the junctional zone was weak (Figure 4D1). Immunoreactivity of the vessel wall was moderate (Figure 4E1) and clusters of glycogen-rich cells in the labyrinth layer were detected (Figure 4B1,F1,G1,H1). When MMP-9 immunoreactivity was analysed, it was observed that the immune reactivity of trophoblastic giant cells in the maternal decidua region of the rat placenta was weak. The immunoreactivity of spongiotrophoblasts in the junctional zone was found to be moderate (Figure 4G1). MMP-9 was also localised in the vessel wall and decidua cells at medium density (Figure 4H1). At the same time, immunoreactivity of labyrinthine thorofoblastic cells showed moderate intensity (Figure 5I). The localisation densities are given in Table 1.

When the immunoreactivity of fibronectin was examined in the stem cell injected group on the 21st day of gestation, it was observed that ground staining was active in the maternal decidua in the outermost

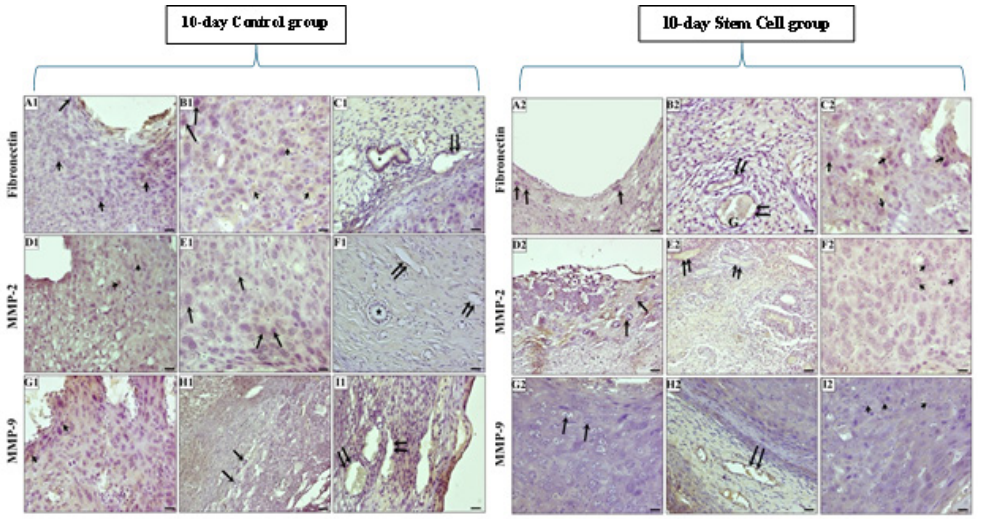


Figure 3. Fibronectin, MMP-2 and MMP-9 immunohistochemical expression images in the implantation site of control and stem cell group rats on day 10. Long arrow: Giant trophoblastic cells, Short arrow: decidual (stromal) cells, Double arrow: vascular wall (endothelium), Star: gland epithelium. Magnification A1,D1,F1,H1,I1 : 20×, Magnification B1,C1,E1,G1 : 40×. Magnification A2,B2,D2,E2,H2,I2 : 20×, Magnification C2,F2,G2 : 40×

Bozok Tıp Derg 2025;15(4):438-447
Bozok Med J 2025;15(4):438-447

layer, in the connecting zone just below it and the labyrinth layer (Figure 4A2). MMP-9 expression was low in trophoblastic cells (Figure 4B2), whereas the expression intensity was moderate in the vessel wall and decidua cells in the labret area (Figure 4C2). MMP-2 expression was found to be moderate in trophoblastic giant cells (Figure 4D2), moderate in labyrinth trophoblastic cells in the labyrinth layer (Figure 4E2) and in the vessel wall (Figure 4E2,F2). MMP-9 expression was moderate in trophoblastic giant cells (Figure 4G2, H2) and spongiotrophoblasts in the junctional zone (Figure 4H2) and weakly expressed in the vessel wall (Figure 4I2). The localisation densities are given in Table 1.

DISCUSSION

Embryo implantation is a critical process involving synchronized molecular and cellular interactions between the blastocyst and the endometrium, culminating in placenta formation and fetal development (3, 22). In rats, blastocyst attachment to the endometrium by late day 4 triggers stromal cell differentiation into decidual cells, forming the implantation chamber at the antimesometrial pole (23, 24). This decidualization process is essential for establishing a receptive uterine environment and supporting trophoblast invasion.

Fibronectin, a key extracellular matrix (ECM) glycoprotein, mediates cell adhesion, migration, and tissue remodeling during implantation (25). It is highly expressed in human placental tissue and extravillous trophoblasts (26, 27). Studies using fibronectin knockdown models demonstrate that its absence impairs trophoblast migration, vascularization, and embryo implantation, highlighting its critical role at the maternal-fetal interface (27, 28). Additionally, the transition from villous to extravillous trophoblasts is marked by increased expression of fibronectin receptors (α5β1) and decreased laminin receptors, influencing trophoblast morphology and behavior (29). In our study, fibronectin expression was observed in the uterus and placenta on days 10 and 21 of gestation. Notably, on day 21, fibronectin expression in the labyrinth area and spongiotrophoblasts was significantly higher in the mesenchymal stem cell (MSC)-treated group compared to the control group ($p=0.02$). This suggests that MSC administration may enhance fibronectin-mediated vascularization and ECM remodeling, potentially improving implantation success. These findings warrant further investigation into the signaling pathways, such as Src and integrin-mediated pathways, that may regulate fibronectin

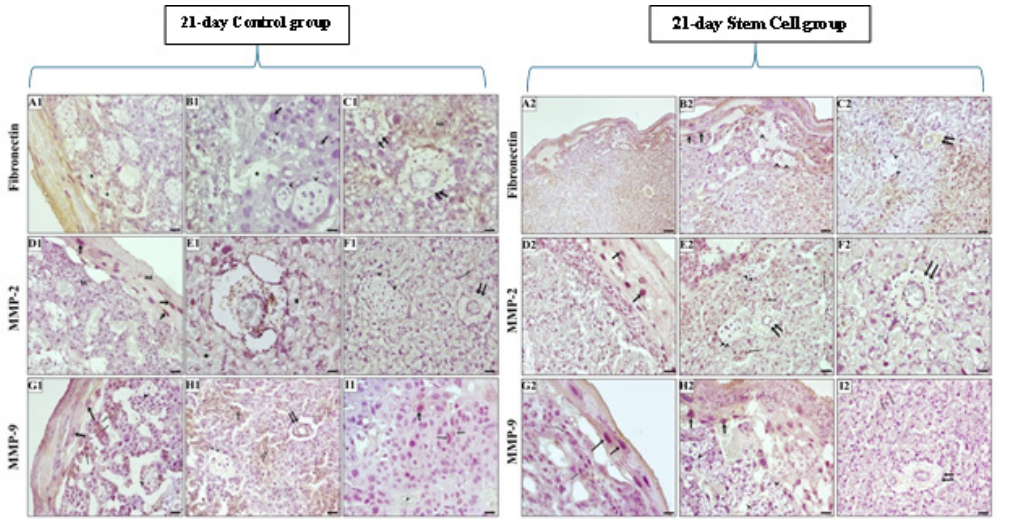


Figure 4. Fibronectin, MMP-2 and MMP-9 immunohistochemical expression images in the implantation site of control and stem cell group rats on day 21. Single long arrow: Trophoblastic giant cell, Double arrow: Vascular endothelium, Short arrow: Spongiotrophoblasts cell, Yellow star: glycogen-rich cell group. Magnification A1,-D1,F1,H11 : 20×, Magnification B1,C1,E1,G1,I1 : 40×. Magnification A2 : 10×, Magnification B2,C2,D2,E2,I2 : 20×, Magnification F2,G2,H2 : 40×.

expression in response to MSC treatment.

Matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, are essential for ECM remodeling, vascularization, and trophoblast invasion during pregnancy (30, 31). MMP-9 is critical for trophoblast invasion and decidual development, with its transcripts and proteins detected in trophoblast cells and the mesometrial decidua by mid-pregnancy (31, 32). MMP-2, expressed in stromal cells and primary trophoblast giant cells from day 8 in mice, also promotes trophoblast spreading and invasion (15, 32-35). In our study, MMP-9 was expressed in decidual cells, trophoblastic giant cells, vessel walls, and gland epithelium on day 10 in both control and MSC-treated groups. However, MMP-2 expression was lower in decidual cells in the MSC-treated group. On day 21, MMP-2 expression was minimal in trophoblastic giant cells in both groups, while MMP-9 expression was moderate in trophoblastic giant cells and spongiotrophoblasts in the MSC-treated group but weak in vessel walls. No significant differences in MMP-2 or MMP-9 expression intensity were observed between groups ($p>0.05$), suggesting that MSC treatment does not significantly alter MMP expression.

Previous studies have reported increased MMP-2 and MMP-9 expression in pregnant rat aorta, uterine artery, and myometrium from the fourth week, facilitating vascular dilation and ECM reorganization, including fibronectin degradation (36). Fibronectin has also been shown to enhance integrin-mediated adhesion and stimulate MMP-2 and MMP-9 production via Src tyrosine kinase and Ras/MAPK/PI3K signaling pathways (37). The significant increase in fibronectin expression in our MSC-treated group on day 21 may be linked to these pathways, necessitating further molecular studies. Additionally, vascular endothelial growth factor (VEGF) regulates MMP-2 and MMP-9 expression, with its inhibition reducing uterine MMP levels and impairing vascular dilation at the implantation site (38). Although vascularization increased in both groups by day 21 in our study, the lack of significant changes in MMP expression suggests that MSCs may preferentially influence fibronectin-mediated processes rather than MMP activity. Future studies should explore the molecular mechanisms, including VEGF and integrin signaling, underlying MSC effects on ECM dynamics during implantation.

CONCLUSION

Mesenchymal stem cell (MSC) administration significantly increased fibronectin expression in the labyrinth area and spongiotrophoblasts on day 21 of gestation in rats ($p=0.02$), suggesting a role in enhancing ECM remodeling and vascularization at the implantation site. However, MSC treatment did not significantly alter MMP-2 or MMP-9 expression, indicating that its effects may be specific to fibronectin-mediated processes rather than broad ECM degradation. These findings highlight the potential of MSCs to modulate key ECM components during implantation, which may improve pregnancy outcomes. Further research is needed to elucidate the molecular signaling pathways, such as Src, integrin, and VEGF, that mediate these effects and to explore their therapeutic implications for reproductive success.

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