

Classification of recurrent implantation failure patients using peripheral blood immunological and metabolic markers

Sina Baharaghdam^{1,2}, Özkan Yildirim³, Mohammadbagher Pirouzpanah⁴, Danial Abbas Kabiri³, Shima Karimi^{1,2}, Mohammad Esfani Farahani⁵, Amirhossein Aghapour^{1,2}, Ali Hassanzadeh⁶, Amin Kamrani^{1,2}, Reza Mousavi Ardehaie⁴, Amir Mehdizadeh⁷, Parvin Hakimi⁸, Javad Ahmadian Heris⁹, Leili Aghebati-Maleki^{1,2}, Mehdi Yousefi^{4,2*}

¹Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

²Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

³Department of Histology and Embryology, Institute of Health Sciences, Atatürk University, Erzurum, Türkiye

⁴Molecular Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

⁵Department of Toxicology and Cancer Biology, University of Kentucky, Lexington, USA

⁶Department of Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

⁷Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

⁸Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

⁹Department of Allergy and Clinical Immunology, Pediatric Hospital, Tabriz University of Medical Sciences, Tabriz, Iran

Table S1. Primer pair sequences.

Gene	Primer	Sequence (5' → 3')
TWEAK	Forward	GGAATTCTCAGCCACTGCGG
	Reverse	ACCTGGAAGAGTCCGAAGTAGGT
Fn14	Forward	CGCAGGACGTGCACTATGG
	Reverse	CAGTCCATGCACTTGTCCAG
IL-15	Forward	TGTGGAACCACTGACTACTG
	Reverse	GGACATTATATGCCAGTTATTGC
IL-18	Forward	CAAGGAATTGTCTCCCAGTGC
	Reverse	AATAGTTTGTGCGAGAGGAAGC
CD56	Forward	AACCGCATTGGGCAGGAGTC
	Reverse	CTTACCAACTGCTCTCCACTCA
GAPDH	Forward	TCGGAGTCAACGGATTTGGT
	Reverse	TGAAGGGGTCATTGATGGCA

Abbreviations: TWEAK, Tumor necrosis factor-like weak inducer of apoptosis; Fn14, Fibroblast growth factor-inducible molecule 14; IL, Interleukin; CD, Cluster of differentiation; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase. The selected genes were chosen based on their established roles in endometrial immune regulation and implantation biology. IL-15 is a key cytokine involved in uterine NK-cell recruitment and activation, while IL-18 contributes to the modulation of Th1/Th2 balance and local inflammatory responses. TWEAK and its receptor Fn-14 participate in tissue remodeling, angiogenesis, and immune regulation within the endometrium, processes that are critical during the window of implantation. GAPDH was used as a housekeeping gene for normalization due to its stable expression in endometrial tissue.

Table S2. Baseline demographic and clinical characteristics of the participants in the studied groups at baseline. Abbreviations: FC, Fertile controls; RIF, Recurrent implantation failure; BMI, Body mass index; FSH, Follicle stimulating hormone; LH, Luteinizing hormone; AMH, Anti-Mullerian hormone; No, Number.

* Note: All values are presented as mean \pm SD and were analyzed using an independent sample t-test. A *p*-value < 0.05 was considered statistically significant.

Characteristic	FC (28 women)	RIF patients (163 women)	<i>p</i> -value
Age (years)	31.40 \pm 3.24	30.50 \pm 2.96	0.080
BMI (Kg/m ²)	26.31 \pm 1.68	27.40 \pm 2.27	0.072
Duration of Infertility (years)	0	6.3 \pm 2.6	-
Type of Infertility			
Primary	0	87.80% (143)	-
No. of embryos transferred	0	7.50 \pm 3.25	-
Failed prior IVF cycles	0	6 \pm 2.50	-
FSH (mIU/mL)	5.89 \pm 1.44	6.44 \pm 2.08	0.163
LH (mIU/mL)	3.84 \pm 1.45	4.48 \pm 1.64	0.081
Endometrial thickness (mm)	10.90 \pm 1.85	9.60 \pm 2.01	0.061
AMH (ng/ml)	2.8 \pm 1.12	2.6 \pm 1.84	0.564
No. of smoking patient	1 (3.57%)	2 (1.22%)	0.554
No. of smoking partners	10 (35.7%)	75 (46%)	0.048
Abnormal karyotype	0	0	-
Male infertility	0	0	-