DOI: https://doi.org/10.31351/vol34iss4pp113-124

Implantation outcome is Associated with *Mucin-16, MiR- 1226* and *MiR-210* Expression during IVF of Iraqi Females

Israa M.Majeed[1] and Asmaa M. Salih Almohaidi [*,1]

[1] Department of Biology ,College of Science for Women, University of Baghdad, Baghdad, Iraq.

[*]**Corresponding author**

Received 10/6/2024, Accepted 6/10/2024, Published 20/12/2025

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Abstract

One of the main obstacles to early pregnancy and assisted reproduction is embryo implantation failure. Endometrial receptivity and the interactions between the embryo and the mother determine implantation. The control of implantation involves several molecules. One such molecule is microRNA (miRNA), which is known to have a role in embryo implantation as a transcriptional regulator of gene expression. The *mucin-16* gene produces a protein that belongs to the mucin family and is crucial for creating a mucous barrier. Mucins attached to the uterine membrane are essential for the implantation of embryos .The current study evaluates the Expression level of *miR-210* and *miR-1226* with *mucin-16* in infertile females under the In vitro fertilization (IVF) program and its influence on embryo implantation by measuring the fold change. This study included 128 Iraqi females, fertile and infertile, who were sorted into 26 successful implantations and 58 failed implantations under an in vitro fertilization program and 44 fertile females under the IVF program and considered as controls. The first conclusion in the current work is that the infertility cases under the IVF program with fertile failure implantation cases related to significant up-regulation of *mucin-16. In contrast,* successful fertile cases recorded a significant down-regulation. There is a significant positive correlation between *mucin16* and *miR-210-HG* in failure embryo implantation in infertile females and a negative correlation between *mucin16* with *miR-210*-HG and *miR-1226* in failure embryo implantation in fertile females. These results recorded that up-regulation of *miR-210* and *miR-1226* gene expression in infertile females and up-regulation of *miR-1226* gene expression in fertile females undergoing IVF programs may affect embryo implantation by regulating *mucin-16* gene expression.

Keywords: Gene Expression, Implantation, *Mucin-16* Gene, IVF, *Microrna-210*, *Microrna-1226.*

Introduction

The process of embryo implantation, the "window of implantation," is the short period during which the uterine endometrium is receptive to blastocyst implantation, a critical stage in the formation of pregnancy [(1)] and only lasts for a short while. Synchronous development and bidirectional interaction occur between the uterus and the embryo after implantation, eventually establishing a structural connection and material exchange[(2)].

Understanding the mechanism of implantation has a profound effect on improving reproductive efficiency. The efficiency of pregnancy in humans remains relatively low (~30%), and implantation failure accounts for 75% of pregnancy loss [(3)]. Therefore, many factors related to implantation failure need to be investigated. Some of them are genetic factors The mucins family serves as implantation inhibitors [(4)]. Within the mucins family, mucin-16, also known as CA125, is a trans-membrane glycoprotein that protects and lubricates the epithelial surfaces of the reproductive organs [(5)]. Mucin-16 protects epithelial cells and also has a function in different disorders, and *mucin-16* expression was shown to be positively associated with inflammation [(6)]. Previously, *mucin-1* expression association with infertility was proved [(7–9)]. Another local investigation found a link between the hormonal state of *mucin1* and *mucin4* in infertile Iraqi females [(7,10)]. The scientists discovered *mucin-16* appeared to be a more targeted inhibitor, with a total reduction of expression across the pinopods throughout the implantation phase[(11)]. Few researchers have investigated the involvement of *mucin-16* in the endometrium during embryo implantation[(12–14)]. Iraqi studies recorded differences in the endometrial size of females under IVF related to IVF output [(15–18)]. On the other hand, a study showed that the fold changes in *mucin-1* were positive in the infertile group and negative in the

fertile and pregnant groups [8]. MicroRNAs are a class of highly conserved noncoding small RNAs (miRNAs) comprising around 22 nucleotides. They target the regulation of gene expression by base-pair pairing, thereby participating in several biological processes such as cell division, cell growth, and differentiation [19–21]. A previous research indicates that *miR-1226* plays a crucial role in inflammation [22,23] mir-210 was first identified in trophoblast cells as an iron metabolism regulator responding to hypoxia stress and implicated in defective placentation [24,25]. This study aims to determine the effect of microRNA and mucin-16 in implantation during gene retreading in IVF.

Materials and Methods

Blood Sampling

The blood sample collection and the practical work of this study extended from November 2022 to July 2023. The patient group consists of females who have no children or have had a problem conceiving in the last few years. They were enrolled in public hospitals, infertility centers, and private IVF in Baghdad, Iraq. 128 Iraqi females who were fertile and infertile and underwent IVF were sorted into:

-Group 1- included 26 infertile females who underwent successful IVF.

-Group 2- included 58 infertile females who underwent unsuccessful IVF.

-Group 3- included 21 fertile females who underwent successful IVF as control.

- Group 4- included 23 fertile females who underwent unsuccessful IVF as a control

RNA Extraction

Using the Trans Zol Up Plus RNA Kit (Trans Gen, biotech. ER501-01), total RNA was extracted from the whole blood sample following the manufacturer's procedure.The 2000c nanodrop spectrophotometer (Thermo Fisher Scientific, USA) was used to evaluate the concentration and purity of extracted RNA. The RNA concentration of the samples ranged from 73-147ng/µl. An A260/A280 ratio of around 2.0 suggested that the RNA sample was pure. Using the Easy Script® One-Step gDNA Removal and cDNA Synthesis Super Mix Kit, total RNA was reverse-transcribed to complementary DNA (cDNA). According to the manufacturer's instructions, the operation was performed in a reaction volume of 20 µl. (4µl) of total RNA had to be reversely transcribed. The components of this kit are mRNA/miRNA, Anchored Oligo(dT)18 Primer (0.5µg / µl), Random Primer (0.1µg/µµl), GSP, 2xES Reaction Mix, EasyScript® RT/RI Enzyme Mix, gDNA Remover, and RNase-free Water.They incubated a random primer for 10 minutes at 25°C. For qPCR, an anchored oligo (dT) 18 primer and GSP were incubated for 15 minutes at 42°C. To inactivate enzymes, they were incubated for 5 seconds at 85°C. Alpha DNA Ltd. (Canada) created and lyophilized the primers. All of the primer sequences used in the assays for this study are shown in Table 1.

Table 1. Primers used in the current study.

Primer	Sequence (5′→3′ direction)	primer bp	Tm °C	References
Mucin16 (Gene Expression)				
Forward	5GCCTCTACCTTAACGGTTACAATGAA 3	26	58°C	[26]
Reverse	5 GGTACCCCATGGCTGTTGTG 3	20		
GAPDH –Glyceraldehyde 3-phosphate dehydrogenase				
Forward	5TGAGAAGTATGACAACAGCC3	20	58°C	[27]
Reverse	5TCCTTCCACGATACCAAAG3	19		
miR - miR210HG (Gene Expression)				
Forward	5GCTTGGTAGAGTGTCACGCC3	20	58°C	[28]
Reverse	5CATCTGACCGAGCCAGTTTG3	20		
miR-1226 (Gene Expression)				
Forward	5CCCCTGCGTGTTTTATGAAG3	20	56°C	[22]
Reverse	5CCTGTACTGGGGAAGTTCA3	19		
U6 (Gene Expression)				
Forward	5CTCGC TTCGGCAGCACA3	17	56°C	[29]
Reverse	5AACGCTTCACGAATTTGCGT3	20		

Quantitative Real-Time PCR (qRT–PCR) runs:

The quantitative real-time PCR (qRT–PCR) was carried out using the QIAGEN Rotor gene Q real-time PCR system (Germany). The expression levels and fold changes of the *Mucin16, GAPDH, miR-210 HG,miR-1226,* and *miRU6* genes were assessed using the TransStart®Top Green qPCR Super Mix kit and measuring the threshold cycle (Ct). Every reaction was performed twice. The *mucin-16, GAPDH, miR-210HG, miR-1226,* and *miRU6* gene expression experiments used the following quantitative real-time PCR components and volume: 2xTransStart®Top Green qPCR Super Mix (10 µl), Nuclease free water (6 µl), Forward Primer (10 µM) (1 µl), Reverse Primer (10 µM) (1 µl), and cDNA (2 µl). The cycling protocol was programmed for the following optimized cycles according to the thermal profile of *Mucin-16, GAPDH, miR-210HG, miR-1226* and *miRNAU6* gene expression; the gene's expression stages and Temperature were found to be 94°C for 30 seconds, which acts as a hold temperature to activate the polymerase; subsequently, a 35–40 cycle consisted of 94°C for 5 seconds (denaturation), 56-58°C for 15 seconds (annealing), and 72°C for 20 seconds as (extension phase). A dissociation curve was measured in a range of Temperatures between 55-95c.

Statistical analysis

The threshold cycle was used to quantify gene expression levels. The $2^{\wedge-\Delta\Delta CT}$ method was used to calculate the fold change of target gene expression. [30] SPSS version 25 was used for statistical data analysis. Duncan's multiple range test is a statistical test that compares the means of various groups. In contrast, the Pearson correlation coefficient (PCC) test assesses the linear correlation between two data sets.

Results and Discussion

The success, failure, and total embryo implantation of infertile females under IVF and the success, failure, and total embryo implantation of fertile females under IVF were the six groups whose mucin16 gene expression was assessed using the reference gene *GAPDH.*

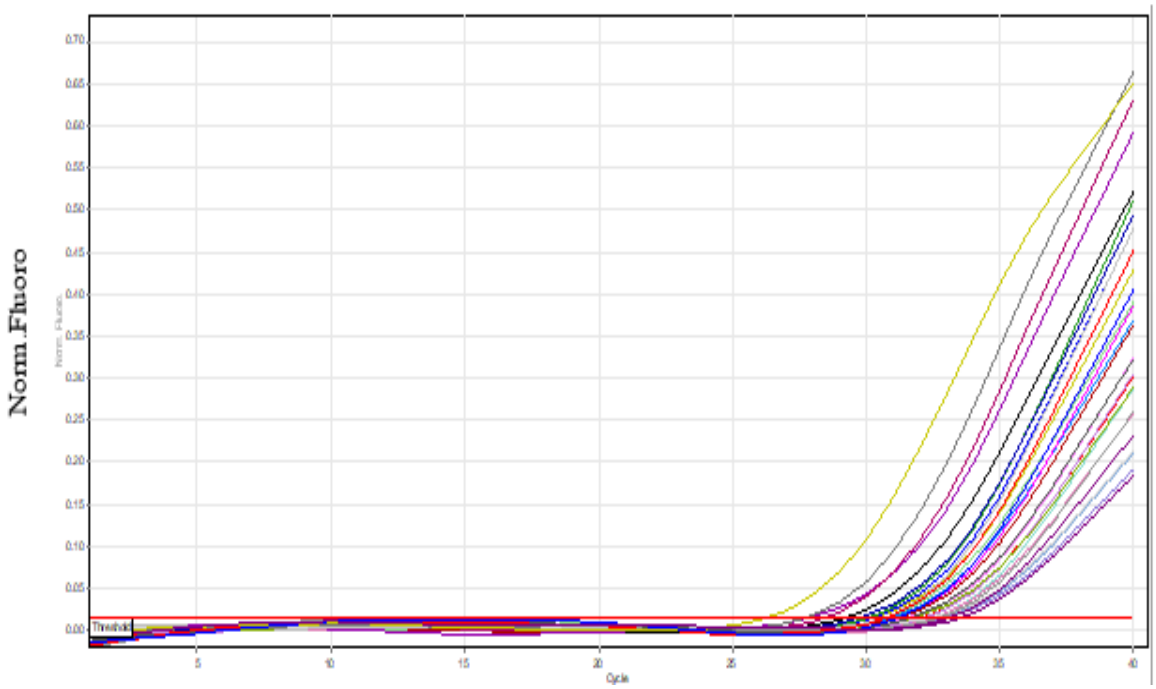

Figure 1. *Mucin16* gene amplification was plotted using qPCR samples that covered all research groups.The CT values varied between 24 and 34The photograph was taken directly from Qiagen Rotor gene qPCR machine Cycle=Number of cycle Norm.Fluoro=Normal Fluorescence

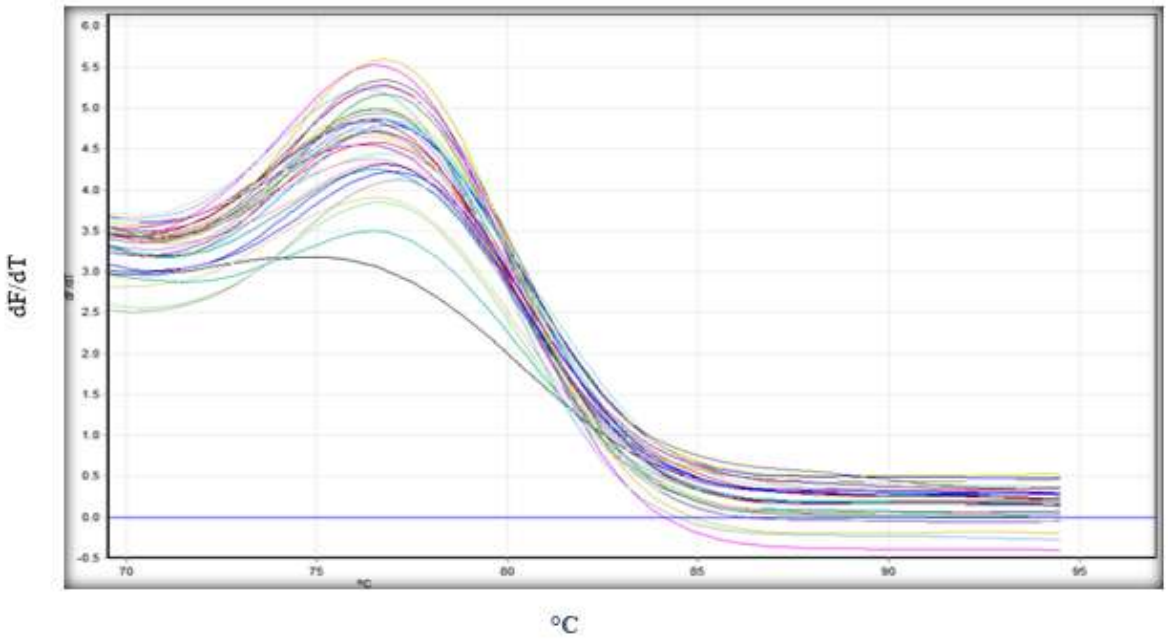

Figure 2. *Mucin16* gene dissociation curves using qPCR samples that covered all research groups. The images were captured using the Qiagen Rotor-Gene Q qPCR apparatus.°C =Temperature. dF/dT=Fluorescence melting peaks obtained by plotting the negative derivative of fluorescence over Temperature

The mean Ct of the *mucin-16* gene for success, failure, total embryo implantation in infertile females and success, failure, and total embryo implantation in fertile females is 27.26, 24.99, 26.13, 31.84, 31.75, and 31.8, respectively. The current study results indicate that the fold change in *mucin-16* gene expression was upregulated in success implantation, 23.43; failure implantation, 98.36; total implantation, 47.84 in infertile females and failure implantation, 1.19 in fertile females, while the fold change down-regulated in success implantation, 0.84 in fertile.

Table 2. The fold of *MUC16* gene expression depends on the 2-$^{\Delta\Delta Ct}$ method in infertile and fertile Females under the IVF program.

Study groups		Mean ±SD							
		Ct of *mucin-16*	**Ct of GAPDH**	**ΔCt**	**Ct of calibrator**	**ΔΔCt**	**$2^{\wedge-\Delta\Delta Ct}$**	**Experimental group/control group**	**Fold of gene expression**
Infertile	Success (26)	27.26^{c}	20.40	6.86	9.91	-3.05	8.28	8.28/0.35	23.43
	Failure (58)	24.99^{a}	20.20	4.79	9.91	-5.12	34.78	34.78/0.35	98.36
Total infertile (84)		26.13^{b}	20.30	5.83	9.91	-4.08	16.91	16.91/0.35	47.84
Fertile	Success (21)	31.84^{d}	20.17	11.67	9.91	1.76	0.30	0.30/0.35	0.84
	Failure (23)	31.75^{d}	20.59	11.16	9.91	1.25	0.42	0.42/0.35	1.19
Total Fertile (44)		31.8^{d}	20.39	11.41	9.91	1.5	0.35	0.35/0.35	1

Similar letters mean no significant differences, and different letters mean significant differences. The current study found that *MUC16* expression was more upregulated in success, failure, and total embryo implantation in infertile females under the IVF program than in fertile females. The *MUC16* gene fold change for infertile females (success, failure) is more than one, indicating that it is positively associated with infertility. However, when comparing success and failure in embryo implantation, the fold change in implant failure is more significant than implant success, indicating that *mucin16* gene expression is positively associated with implant failure. This research concludes that mucin-16 folding may be related to the failure of implantation in females under the IVF program, and the expression of mucin16 around the time of implantation is an implantation inhibitor. The function of *MUC-16* is as an implantation inhibitor. A few experimental studies showed that glycoprotein (*MUC16)* acted as an inhibitor [31]. Gipson and her team discovered that *MUC16* seemed a more particular indicator of inhibition [13].The current results go with previous Iraqi studies that studied genetic factors and emphasized that genetic variation in Integrin Alpha2, Integrin Bbeta3 and Leukemia Inhibitory Factor (LIF) genes are related to the success or failure of embryo implantation [15–17,32].The current study is consistent with the study finding that the expression of *MUC16* in females who conceived after In vitro fertilization/embryo transfer (IVF/ET) was lower than failure [11]. Furthermore, it was shown that a proposed scoring system might be utilized to predict the outcome of embryo transfer because the proposed score is favourably connected with implantation and clinical pregnancy [33]. The *MUC 16* expression is inversely linked with clinical pregnancy and is consistent with the hypothesis that pinopode is an implantation promoter and *MUC16* is an implantation inhibitor [11]. The authors found that removing MUC16 from the proposed promotes adhesion in human luminal uterine epithelial cells [13]. Karen and colleagues found that a decrease in *MUC16* expression produced by Human Chorionic Gonadotropin (HCG) injection might increase trophoblast-epithelial contact [34]. Another study discovered that *MUC16* expression was considerably greater in females with high progesterone on the day of the hCG trigger than in females with normal progesterone. Given that progesterone has been found to influence *MUC1* gene expression [35], the variations in *MUC16* expression reported might partly be explained by progesterone [11]. According to, *MUC16* is expressed on the surface of the luminal epithelium and glandular epithelium throughout the menstrual cycle, consistent with the idea that the endometrium is usually unreceptive to implantation except during the implantation window LH+6, LH+8 (days of surge LH) when expression of *MUC16* is reduced (and expression of other putative promoters is increased) [14].The scientists also discovered that *MUC16* is a membrane component of the non-receptive luminal uterine surface that hinders cell attachment and that removing it from the pinopode enhances trophoblast adhesion [11]. This corresponds with the total loss of expression over the pinopodes throughout the implantation period, whereas in the case of *MUC1*, the loss was partial. Furthermore, using an in vitro model, the scientists demonstrated that *MUC16's* anti-adhesion capability appeared greater than *MUC1's* [36].The infertile females in cases of success, failure, and total embryo implantation records of highly significant up-regulation of *mucin-16* folding from other groups; this may be attributed to a variety of factors not previously studied and may be related to infertility.

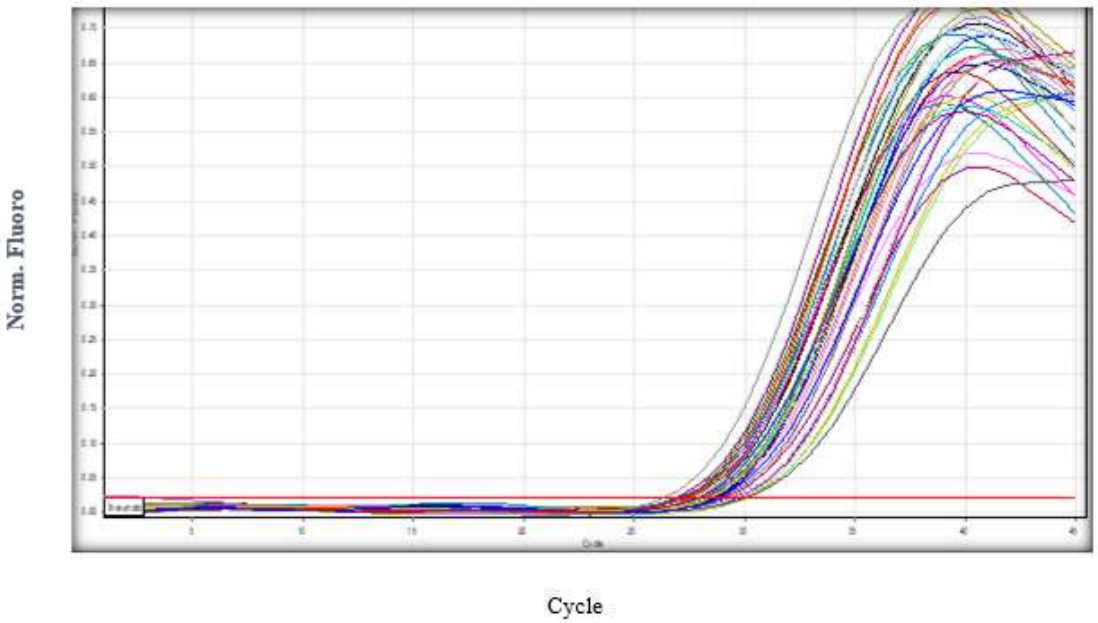

Figure 3. *miR-210HG* gene amplification was plotted using qPCR samples that covered all research groups. The CT values varied between 15 and 23. The photograph was taken directly from Qiagen Rotor gene qPCR machine.Cycle=Number of cycle Norm. Fluoro =Normal Fluorescence.

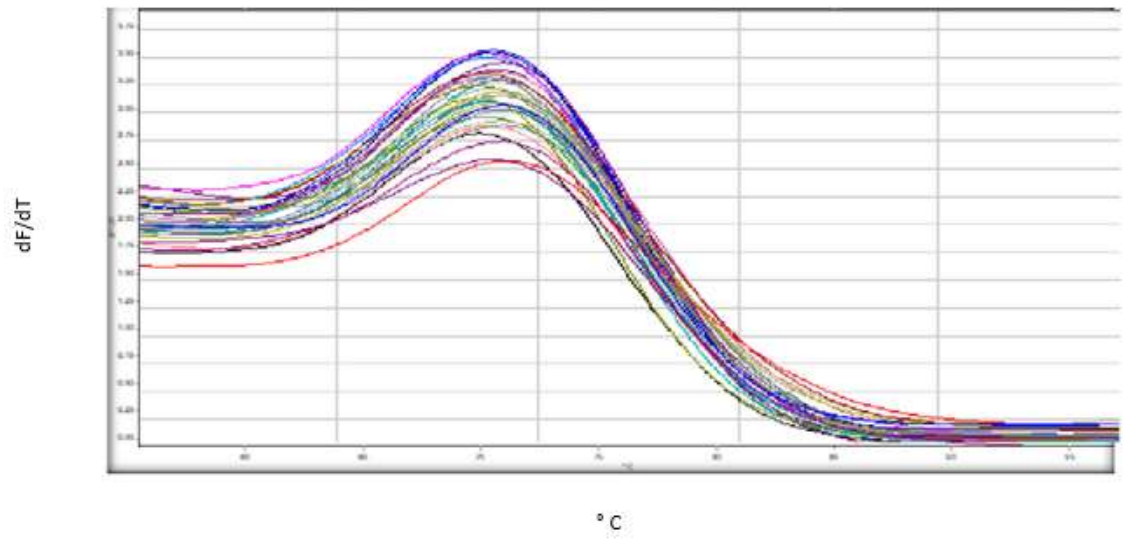

Figure4. *miR- 210HG* gene dissociation curves using qPCR samples that covered all research groups. The images were captured using the Qiagen Rotor Gene Q qPCR apparatus.° C =Temperature dF/dT=Fluorescence melting peaks obtained by plotting the negative derivative of fluorescence over Temperature.

Table 3. The fold of *miR210* gene expression depending on the 2-$^{\Delta\Delta Ct}$ method in infertile and fertile Females under IVF program.

Study groups		Mean ±SD							
		Ct of *miR210*	Ct of *U6*	ΔCt	Ct of calibrator	ΔΔCt	$2^{-\Delta\Delta Ct}$	Experimental group/control group	Fold of gene expression
Infertile	Success (26)	28.35[b]	20.83	7.51	9.01	-1.50	2.82	2.82/0.50	5.61
	Failure (58)	27.20[a]	21.23	5.97	9.01	-3.04	8.23	8.23/0.50	16.35
Total infertile (84)		27.77[a]	21.03	6.74	9.01	-2.27	4.82	4.82/0.50	9.58
Fertile	Success (21)	31.06[d]	19.83	11.23	9.01	2.22	0.21	0.21/0.50	0.43
	Failure (23)	29.60[c]	20.72	8.88	9.01	-0.13	1.10	1.10/0.50	2.18
Total Fertile (44)		30.30[c]	20.30	10.00	9.01	0.99	0.50	0.50/0.50	1

Similar letters mean no significant differences, and different letters mean significant differences.The fold change in *miR210HG* was upregulated in the success group at 5.61, failure at 16.35, total embryo implantation at 9.58 in infertile females and failure at 2.18 in fertile females under the IVF program. At the same time, it was down-regulated in the success group at 0.43 in fertile females.The current study concluded that up-regulation of *miR-210* gene expression is positively associated with implantation failure, even in fertile females under IVF programs, which may be a response to genetic, environmental, and physiological factors. The miRNAs in the placenta, such as *miR-210*, respond to the changes in these factors during pregnancy, and altered expression of *miR-210* leads to pregnancy disorders.The result of the fold change in the present study agrees with the studies reported that enhanced expression of *miR-210* in response to a hypoxic placenta controls the migration and invasion of trophoblast cells and modifies the pathway connected to inflammation [(24)].*miR-210* levels have risen approximately 13-fold in hypoxia-induced cell lines [(37)]. Current results go with the previous studies that found that the expression of *miR-210* was upregulated in the placenta, suppressing trophoblast cell invasion by down-regulating multiple target gene expressions [(38)], trophoblasts invasion ability abnormally leads to placenta implantation failure and causing a series of pregnancy disorders, such as recurrent pregnancy loss (RPL) [(39,40)]. The fold of *mir-210* gene expression is upregulated in pregnant females with preeclampsia, and it was found to be higher in severe than in mild preeclampsia [(41)]. The significant up-regulation of *miR-210* may be related to the increasing chance of failure implantation in females under the IVF program; miR-210 up-regulation may prevent implantation by regulating the expression of *mucin-16*-gene.

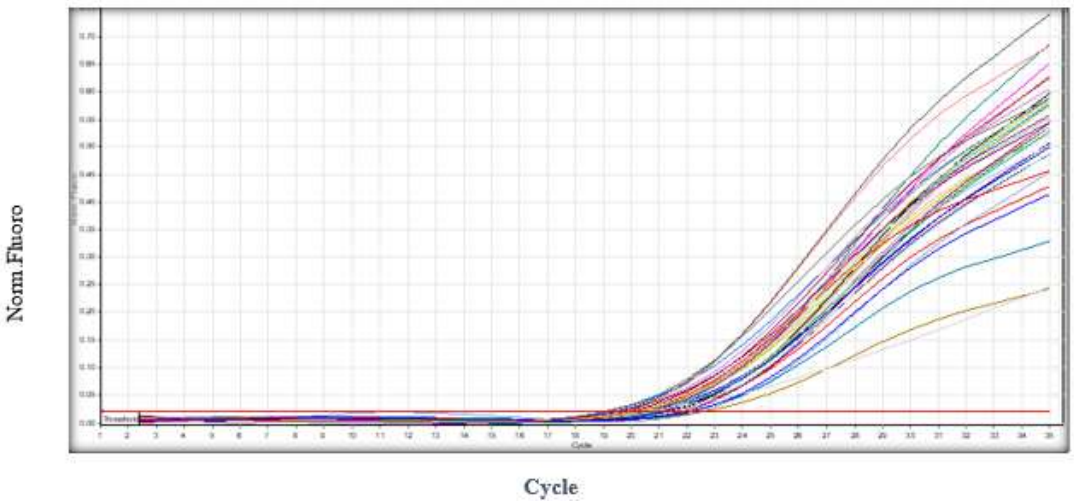

Figure5. *miR-1226* gene amplification was plotted using qPCR samples that covered all research groups. The CT values varied between 18 to 26. The photograph was taken directly from. Qiagen Rotor gene qPCR machine. Cycle=Number of cycle. Norm. Fluoro=Normal Fluorescence

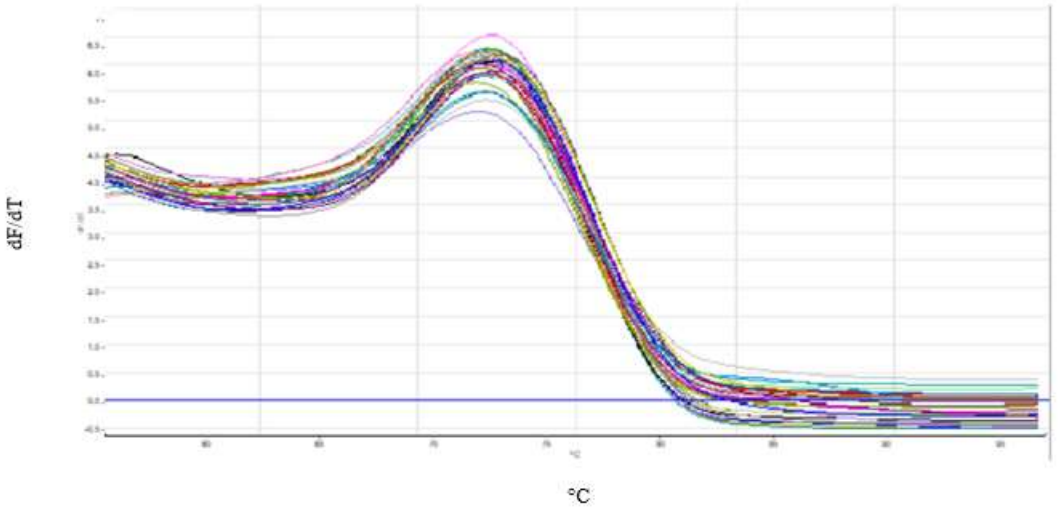

Figure 6. *miR-1226* gene dissociation curves using qPCR samples that covered all research groups. The images were captured using the Qiagen Rotor-Gene Q qPCR apparatus.°C=Temperature. dF/dT=Fluorescence melting peaks obtained by plotting the negative derivative of fluorescence over Temperature

Table 4. Fold of *miR1226* gene expression depending on the $2^{-\Delta\Delta Ct}$ method in infertile and fertile Females under the IVF program

Study groups		Mean ±SD							
		Ct of *miR1226*	Ct of *U6*	ΔCt	Ct of calibrator	ΔΔCt	$2^{-\Delta\Delta Ct}$	Experimental group/control group	Fold of gene expression
Infertile	Success (26)	21.34[a]	20.83	0.50	5.84	-5.33	40.25	40.25/3.60	11.19
	Failure (58)	20.94[a]	21.23	-0.29	5.84	-6.12	69.63	69.63/3.60	19.36
Total infertile (84)		21.14[a]	21.03	0.11	5.84	-5.73	52.94	52.94/3.60	14.72
Fertile	Success (21)	25.69[b]	19.83	5.86	5.84	0.03	0.98	0.98/3.60	0.27
	Failure (23)	22.99[c]	20.72	2.28	5.84	-3.56	11.79	11.79/3.60	3.28
Total Fertile (44)		24.29[d]	20.30	3.99	5.84	-1.85	3.60	3.60/3.60	1

Similar letters mean no significant differences, and different letters mean significant differences.

The fold change in *miR1226* was upregulated in the success 11.19, failure 19.36, total embryo implantation 14.72 in infertile females and failure 3.28 in fertile females under the IVF program. At the same time, it was down-regulated in the fertile success 0.27 group.The current study concluded that up-regulation of *miR-1226* gene expression is positively associated with failure of implantation even in fertile females under IVF program; these results suggested *miR-1226* might be involved in the infertility occurrence and development of failure embryo implantation by regulation of the expression of specific genes and can be used as a biomarker for the detection of failure embryo implantation.The results of the present study agree with previous studies that have reported that *miR-1226* is expressed in human mammary epithelial cells and their function in disease development [42,43]. Additionally, the functional role of *miR-1226* as a tumour suppressor is to prevent aberrant up-regulation of the MUC1 gene [44] The significant up-regulation of *miR-1226* may be related to the increasing chance of failure implantation in females under the IVF program; *miR-1226* up-regulation may prevent implantation by targeting mucin-16-gene expression. The total success and failure of embryo implantation in fertile females under the IVF programs were recorded highly significant from other groups. Maybe females in these groups have

many factors affected by the treatment of IVF program control *mir-1226* gene expression.An interesting finding in the present study is that the infertile failure group recorded the highest gene fold for all factors: *mucin-16* (98.36), *miR-210HG* (16.35), and *miR-1226* (19.36), the highest level of gene expression may be considered one of the causes of embryo implantation failure because increasing the thickness of the uterus endometrium may act as a barrier for attachments.

The correlation between mucin16 with mir-210-HG and mir-1226

The results of the correlation test between *mucin16*, *mir-210-HG*, and *mir-1226* genes in infertile and fertile females under IVF program are given in Table 5.

Table 5.The correlation between *mucin-16* with *miR210-HG* and *miR-1226* in infertile and fertile Females under the IVF program.

Study groups		*miR1210-HG* and *mucin16*				*miR1226* and *mucin16*			
		Fold change of *miR-210HG*	**Fold change of *mucin16***	**Pearson correlation (r)**	**P-value**	**Fold change of *miR-1226***	**Fold change of *mucin16***	**Pearson correlation (r)**	**P-value**
Infertile	Success(26)	5.61	23.43	-0.07	0.75	11.19	23.43	-0.28	0.16
	Failure (58)	16.35	98.36	0.45**	0.00	19.36	98.36	-0.09	0.51
Total infertile (84)		9.58	47.84	0.38**	0.00	14.72	47.84	-0.09	0.41
Fertile	Success(21)	0.43	0.84	0.26	0.25	0.27	0.84	0.19	0.41
	Failure (23)	2.18	1.19	-0.52*	0.01	3.28	1.19	-0.57**	0.01
Total Fertile (44)				-0.11	0.48			-0.22	0.14

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level

There was a significant positive correlation between *mucin16* and *miR-210-HG* in cases of failure and total embryo implantations in infertile females. At the same time, the association between *mucin16, miR-210-HG,* and miR-1226 showed a negative correlation in failure embryo implantation in fertile females undergoing IVF.

The results of this study may suggest that the expression of the *miR-210HG* and *miR-1226* genes are positively correlated with implantation failure in females undergoing IVF and that in infertile females, only up-regulation of *miR-210*gene expression is related to failure implantation by regulating the expression of mucin-16 gene. Still, in fertile females, up-regulation of *miR-210* with *miR-1226* is related to failure implantation by targeting the *mucin-16* gene expression.

Table 6. The Regression between *mucin16* with *miR210-HG* and *miR-1226* in infertile and fertile Females under the IVF program.

***Mucin-16* In Study groups**		**R**	**R-Square**	**F-value**	**F(Sig.)**	**Beta of miR-1226**	**Beta of miR-210-HG**	**VIF**
Infertile	Success (26)	No variables were entered into the equation.						
	Failure (58)	0.52	0.27	10.34	0.00	-0.26*	0.54**	1.12
Total infertile (84)		0.45	0.21	10.43	0.00	-0.26*	0.47**	1.15
Fertile	Success (21)	No variables were entered into the equation.						
	Failure (23)	0.57	0.32	9.83	0.00	-0. 57**		1.00
Total Fertile (44)		No variables were entered into the equation						

**Correlation is significant at the 0.01 level, *Correlation is essential at the 0.05 level

A stepwise multiple regression model investigated the correlation between *mucin-16* and, *mir-1226* and *mir-210-HG* in infertile and fertile females under the IVF program. The independent

variables were *miR-1226* and *miR-210*, while *mucin-16* was selected as the dependent variable.The regression analysis revealed a statistically significant correlation between *mucin-16* and *miR-1226* and *miR-210* in failure and total embryo implantation in infertile females and between *mucin-16* and *miR-1226* in failure embryo implantation in fertile females. This may be deduced from the measured t-value and its corresponding P-value. By consulting the F statistics and its corresponding P-value. Thus, it may be inferred that the model is sound and that a connection exists between *mucin-16* and the independent variables. A multicollinearity test was conducted to confirm the presence of the indicated correlation. The obtained VIF factor of the model (VIF<3) provides evidence that there is no multicollinearity issue.Thus, the results indicate the following equation:Infertile females -*Mucin-16* in failure embryo implantation = 0.26**miR-1226* + 0.54**miR-210 HG-Mucin-16* in total embryo implantation = 0.26* *miR-1226* + 0.47**miR-210 HG* Fertile females -*Mucin-16* in failure embryo implantation = 0.57* *miR-1226* The stepwise multiple linear regression model results in Table (6) indicate that successful embryo implantation does not depend on microRNAs. However, *miR-1226* and *miR-210-HG* control failure and total embryo implantation in infertile females by targeting *mucin-16* gene expression. In fertile females, *miR-210-HG* controls failed embryo implantation by targeting *mucin-16*. Finally, success and total implantation do not depend on microRNAs; maybe other factors effect on microRNAs; therefore, in the current study, the impact of microRNAs is not clear

Conclusion

The infertile groups that failed to get pregnant had higher levels of *mucin-16*, *miR-210*, and *miR-1226*.These factors may contribute to the increased likelihood of unsuccessful implantation in females participating in the IVF treatment. Targeting *mucin-16* gene expression, *miR-1226* and *miR-210-HG* are potential inhibitors of embryo implantation failure in infertile females. *miR-1226* regulates unsuccessful embryo implantation in fertile females by specifically targeting *mucin-16* gene expression. Elevated *mucin-16* levels significantly influence successful implantation in females participating in the IVF program.

Acknowledgement

Thank you to all of the patients who took part in the study as the medical and administrative personnel at the Al Farah Medical Center and the Rooh Al-Hayat Center for the Treatment of Infertility, who collected samples and information.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

No funding was received from an institution to conduct this study.

Ethics Statements

The approval number of the ethics committee was 105 in April / 2023 from the Health Ministry/ the Baghdad Health Department.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Author Asmaa M. Salih Almohaidi; data collection: Author Israa M.Majeed; analysis and interpretation of results: Author Asmaa M. Salih Almohaidi; draft manuscript preparation: Author Israa M. Majeed. All authors reviewed the results and approved the final version of the manuscript.

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ترتبط نتيجة الانغراس بتعبيرات ميوسين ١٦ والرنا الدقيق ١٢٢٦ و الرنا الدقيق ٢١٠ أثناء الاخصاب خارج الرحم للاناث العراقيات

اسراء محمود مجيد[١]، اسماء محمد صالح المهيدي[٢]

[١] قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.
[٢] قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.

الخلاصة

احدى العقبات الرئيسيه امام الحمل المبكر والمساعده على الانجاب هي فشل انغراس الاجنه . ان تقبل بطانة الرحم والتفاعلات بين الجنين والام هي العوامل التي تحدد عملية الانغراس , تنطوي عملية الانغراس على عدة جزيئات احدى هذه الجزيئات هو الرنا الدقيق الذي له دور مميز في عملية انغراس الاجنه حيث يعمل على تنظيم التعبيرالجيني. جين ميوسين -١٦ ينتج بروتينا ينتمي الى عائله الميوسين وهو ضروري لانشاء حاجز مخاطي وتعتبر الميوسينات المرتبطه بغشاء الرحم ضرورية لزرع الاجنه. الدراسه الحاليه قيمت ارتفاع مستوى تعبير الرنا الدقيق- ٢١٠ و١٢٢٦ مع تعبير جين ميوسين – ١٦ في الاناث العقيمات والخاضعات لبرنامج الاخصاب خارج الرحم وتاثيره على انغراس الاجنه عن طريق قياس زيادة التعبير الجيني . شملت هذه الدراسة ٢٦ عملية زرع ناجحه و ٥٨ عملية زرع فاشلة ضمن برنامج الاخصاب خارج الرحم و ٤٤ أنثى قادرة على الإنجاب كمجموعة سيطرة مقسمة إلى مجموعتين فرعيتين، ٢١ عملية زرع ناجحة و ٢٣ عملية زرع فاشلة.الاستنتاج الاول من الدراسه الحاليه حالات العقم الخاضعه لبرنامج التلقيح في المختبر مع حالات فشل انغراس الجنين في النساء الخصبات ترتبط بالارتفاع الكبيرلتعبير جين ميوسين١٦ في حين حالات نجاح انغراس الجنين في النساء الخصبات التي سجلت انخفاضا كبيرا في تعبير الجين .سجلت هذه النتائج ارتفاع التعبير الجيني للرنا الدقيق ٢١٠ و١٢٢٦ الذي قد يتحكم في انغراس الاجنه عن طريق تنظيم التعبير الجيني لميوسين ١٦ في الاناث العقيمات والخاضعات لبرنامج الاخصاب في المختبر .

الكلمات المفتاحية: التعبير الجيني ، الانغراس ، جين ميوسين-١٦، الاخصاب خارج الرحم ،الرنا الدقيق ٢١٠ ،الرنا الدقيق ١٢٢٦ .