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# IVF outcome under the effect of *mucin* -1 gene expression and some related *microRNAs*

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## Abstract

**Background:** Embryo implantation is a complex procedure, including interactions between the blastocyst (an early stage of embryo development) and the uterus's luminal epithelium (the inner lining) during its receptive phase. *Mucin 1* (MUC1) is a glycoprotein that is an essential part of the cell membrane. It is mainly found on the outer surface of secretory epithelial cells and the glandular epithelium in various organs such as the uterus. The absence of MUC1 on the surface of uterine epithelial cells is considered essential for the successful attachment of an embryo. The endogenous miRNAs, non-protein coding, have a length of 21-24 nucleotides. Research has demonstrated that miRNAs play a role in several biological processes by controlling gene expression after transcription. **Aim:** To determine the gene expression to evaluate the correlation between mucin1 and certain microRNAs, including *miR146a-5p, miR146a-3p,* and *miR485-5p,* in embryo implantation. So, examine the influence of different types of microRNAs on the expression of the *mucin-1* gene in infertile Iraqi females undergoing an in vitro fertilization program. **Methods:** This study involved 26 instances of successful implantations and 58 instances of unsuccessful implantations within an in vitro fertilization program. A total of 44 fertile females were selected as controls and categorized into two subgroups: 21 cases with successful implantations and 23 cases with unsuccessful implantations. **Results:** The observed findings indicate an increase in the expression of both the *miR146a-5p* and *miR485-5P* genes, with a decrease in the expression of *miR146a-3p*. These changes in gene expression may have a role in controlling embryo implantation in infertile females undergoing IVF programs by regulating the expression of the *mucin1* gene. **Conclusion:** The current study's findings indicate that the expression of the *MUC1* gene is directly regulated by *miR146a-5p* and *miR485-5p.* There is a significant positive association depending on the correlation between *mucin1* and *miR485-5P* and a significant negative correlation between *mucin1* and *miR146a-5p* and *miR146a-3p*. Implantation success in infertile females is related to the usual amount of *mucin-1* expression. Implantation failure in infertile females undergoing IVF is linked to a significant increase in *mucin-1* expression.

**Keywords:** Gene expression, implantation, *mucin1gene*, IVF, *microRNA-146a-5p*, *microRNA-146a-3p, microRNA-485-5p*
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## Introduction

Infertility is a condition of the reproductive system that prevents women from becoming pregnant after performing frequent, unprotected intercourse for twelve months or more. According to the Centers for Disease Control and Prevention, Infertility in women has three primary underlying causes: ovulatory dysfunction, transport anomalies, and implantation issues (1). A previous study conducted in Baghdad city found that the leading causes of infertility in Iraqi Arab females are PCO (46%), ovarian reverse 20%, 10% for each fallopian disorder and unexplained infertility, 8% uterine cysts, 4% hypothyroidism, and 2% endometrioses (2). Other Iraqi studies documented a notable decrease in dopamine levels alongside a highly significant increase in prolactin hormone (PRL) and luteinizing hormone (LH) levels (3). Additionally, the expression of the BMP15 gene showed a significant decrease, correlated with an increase in FSH hormone levels in infertile women (4).In vitro fertilization and embryo transfer (IVF-ET) technology is a crucial alternative for couples experiencing infertility. Embryo cultivation in a laboratory setting and techniques and procedures for ovulation induction have gradually evolved. The implantation rate remains between 25 and 40%, limiting the success of IVF-ET (5). Human pregnancy

success depends on various unique processes, such as embryo implantation, decasualization, placentation, and parturition. Each of these events is required to progress to the next stage of pregnancy (6). Molecular and physiological mechanisms regulate implantation. Opposition, adhesion /attachment, invasion, and immune modulation are all required for human implantation (7). The embryo is also exposed to a glycocalyx linked with the luminal epithelium, which includes several adhesion molecules. Mucin-1 is one of them (8).

Mucins are high-molecular-weight glycoproteins that lubricate the epithelial surfaces of the respiratory, gastrointestinal, and reproductive tracts (9). The mucins released by the epithelial cells of reproductive organs produce the cervix and endometrial mucus, crucial components of reproductive physiology. Moreover, mucins function as transcriptional and post-transcriptional regulators and mediators of inflammatory and immunological responses (10, 11).

MicroRNAs, short non-coding RNAs with 20 to 24 nucleotides, play a role in controlling gene expression after transcription because they affect the stability and translation of messenger RNAs. One of these, miRNA-485-5p, has demonstrated that the quantity of miRNA485-5p present affects the growth and prognosis of cellular tumors (12).The miRNA485-5p expression is a biomarker for human illnesses (13-15). The miRs that have been extensively researched include the genome of humans, which has two miR146 genes, miR146a, and miR146b, found on chromosomes 5 and 10, respectively. Every pre-miR generates a pair of mature miRs, specifically miR146a-5p and miR146a-3p. Gene knockout experiments have shown that a lack of miR-146a leads to long-term inflammation (16, 17). Additionally, research has indicated that MiR-146 plays a role in controlling the movement and penetration of trophoblasts during the early stages of pregnancy (18). Another study indicates that miR-146-3p is associated with embryo implantation (19). Previous research has shown that patients with periodontitis and rheumatoid arthritis have greater levels of miR-146a expression, which is associated with a more severe form of inflammation (20, 21). Previous Iraqi studies demonstrated the role of mucin in infertility (10, 11, 22, 23); in another local Iraqi study, there is a relation between hormonal status and mucin in Iraqi infertile females (2). This study aims to explore the relationship between several microRNAs and mucin-1 gene expression.

## Materials and Methods

### Patients and samples

This study's blood sample collection and practical activities spanned from November 2022 to July 2023. The patient category comprises infertile females who have either never had children or have experienced difficulties conceiving over the past several years and patients with chronic illnesses such as diabetes mellitus, thyroid disease, cardiovascular disease, and polycystic ovary (PCO). Both tubal factors and endometriosis were ruled out. The control group consists of fertile females with the ability to have children who have they were enrolled in public hospitals and Infertility centers and private IVF in Baghdad, Iraq. 128 Iraqi females under the IVF program were divided into two major groups' infertile and fertile females. Then, each group was divided into two minor groups.

-Group-1- (26) infertile females who underwent successful IVF.

-Group-2- (58) infertile females who underwent unsuccessful IVF.

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

-Group -4- (23) fertile females who underwent unsuccessful IVF as control.

The study received ethical approval from the Health Ministry / the Baghdad Health Department, with the number of the ethics committee 105 in April / 2023

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. To determine sample quality, the

concentration and purity of extracted RNA were measured using a 2000c Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). The samples had RNA concentrations ranging from 73 to 147 ng/µl. An A260/A280 ratio of approximately 2.0 indicated that the RNA sample was pure.

Total RNA was turned into complementary DNA (cDNA) using the Easy Script® One-Step gDNA Removal and complementary DNA Synthesis Super Mix Kit. According to the manufacturer's instructions, the operation was performed in a reaction volume of 20 µl. (4 µl) of total RNA had to be reversely transcribed. The components of this kit are mRNA/miRNA, Anchored Oligo(dT) 18 Primer (0.5µg/µµl), Random Primer (0.1µg/µµl), GSP, 2xES Reaction Mix, EasyScript® RT/RI Enzyme Mix, gDNA Remover, and RNase-free Water. Ten minutes at 25°C was used to incubate a randomly selected primer. The qPCR process involved incubating GSP with an anchored oligo (dT) 18 primer at 42°C for 15 minutes. The enzymes were inactive after being incubated at 85°C for 5 seconds. Alpha DNA Company Ltd. (Canada) synthesized and lyophilized the primers. The primer sequences utilized in the assays for this study are presented in Table 1.

**Table 1. Primers used in the current study**

| Primer | Sequence (5′→3′ direction) | primer bp | Tm °C | References |
|---|---|---|---|---|
| *Mucin1* (Gene Expression) | | | | |
| Forward | 5 AGAGAAGTTCAGTGCCCAGC 3 | 20 | 58°C | (24) |
| Reverse | 5 TGACATCCTGTCCCTGAGTG 3 | 20 | | |
| *GAPDH* –Glyceraldehyde 3-phosphate dehydrogenase | | | | |
| Forward | 5TGAGAAGTATGACAACAGCC3 | 20 | 58°C | (25) |
| Reverse | 5TCCTTCCACGATACCAAAG3 | 19 | | |
| ***miRNA -485-5p*** (Gene Expression) | | | | |
| Forward | 5 GGCTGGCCGTGATGAATTC 3 | 19 | 56°C | (26) |
| Reverse | 5 GCGAG CACAGAATTAATACGAC 3 | 22 | | |
| ***pri-miR-146a*** (Gene Expression) | | | | |
| Forward | 5 TTAGGAGCTCGCTGGCTGGGACA 3 | 23 | 56°C | (27) |
| Reverse | 5 CAGGATCTACTCTCTCCAGGTCCTCA 3 | 26 | | |
| *U6* (Gene Expression) | | | | |
| Forward | 5CTCGC TTCGGCAGCACA3 | 17 | 56°C | (28) |
| Reverse | 5AACGCTTCACGAATTTGCGT3 | 20 | | |

## Quantitative Real-Time PCR runs.

The Quantitative Real-Time PCR (qRT-PCR) was performed using the QIAGEN Rotor gene Q Real-time PCR System from Germany. The expression levels and fold changes of the Mucin1, GAPDH, miR146a-5p, miR-146a-3p, miR485-5P, and miRU6 genes were assessed. The Trans-Start® Top Green qPCR Super Mix kit measured the threshold cycle (Ct). Each reaction was conducted in duplicate. The required volume of each component was ascertained using Table 2. Based on the thermal profile displayed in Table 3 and Table 4, the cycling program was set for the following optimal cycle.

**Table 2. The components of qRT-PCR were employed in the Mucin-1, GAPDH, miR146a-5p , miR146a-3p, miR485-5P and miR-U6 genes expression experiments**

| components | 20 microliters ( µl) |
|---|---|
| 2xTransStart®Top Green Quantitative PCR Super Mix | 10 |
| Nuclease-free water | 6 |
| Forward Primer (10 µM). | 1 |
| Reverse Primer (10 µM). | 1 |
| cDNA | 2 |

**Table 3. The thermal profile of Mucin1 and GAPDH gene expression**

| Steps | Temperature ºC | Time (sec.) | Number of Cycles |
|---|---|---|---|
| Enzyme activation step | 94 | 30 | 1 |
| Denaturation step | 94 | 5 | 35 |
| Annealing step | 58 | 15 | |
| Extension step | 72 | 20 | |
| Dissociation step | 55 -95 | | 1 |

**Table 4. The thermal profile of miR146a-5P, miR146a-3p, miR485-5p and miRNA U6 genes expression**

| Steps | Temperature ºC | Time (sec.) | Number of Cycles |
|---|---|---|---|
| Enzyme activation step | 94 | 30 | 1 |
| Denaturation step | 94 | 5 | 40 |
| Annealing step | 56 | 15 | |
| Extension step | 72 | 20 | |
| Dissociation step | 55 -95 | | 1 |

## Statistical analysis

The in vitro trials were conducted in duplicate. The fold gene expression was determined using the (2^-ΔΔct) method (29). The findings were reported as the mean value plus or minus the standard deviation (SD). Duncan's multiple-range test was utilized for statistical comparisons. A Pearson correlation analysis and stepwise multiple regression were performed to evaluate the association between the expression of miR146a-5p, miR146a-3p, and miR485-5P. The data from all human subjects were analyzed using SPSS-25 (SPSS Inc., Chicago, IL, USA), with significance level of $P < 0.05$.

-Results

The expression of the mucin1 gene was measured in six groups: successful and failed embryos. Implantation in infertile females and successful and failed embryo implantation in fertile females undergoing IVF, using the reference gene GAPDH.

Figure1- shows the relation between relative fluorescence and cycle number. Amplification plots are obtained by graphing the fluorescence signal of each sample versus the cycle number. Thus, amplification plots depict the build-up of the product during the real-time PCR experiment. The plots are generated using a series of diluted samples of the target DNA sequence, and Figure 2 shows melting curve analysis may identify non-specific products, such as primer-dimer. These additional peaks, located to the left of the peak representing the amplified product, indicate the presence of such non-specific products in the melt curve.

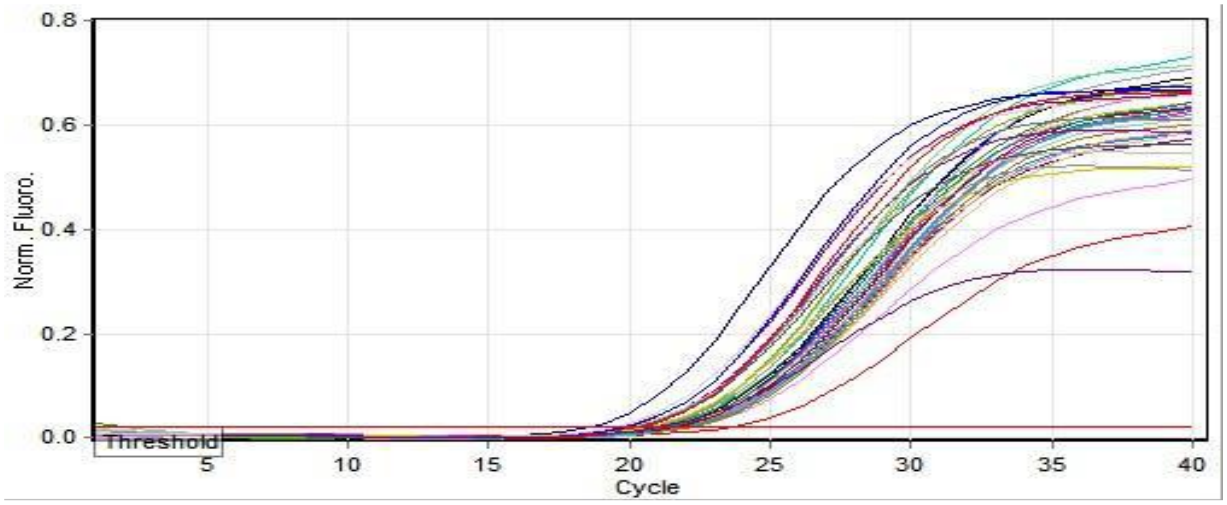


**Figure 1. The amplification of the Mucin-1 gene was analyzed using qPCR samples encompassing all research groups. The CT values ranged from 15 to 23. The image was captured directly from the Qiagen Rotor-Gene qPCR apparatus.**

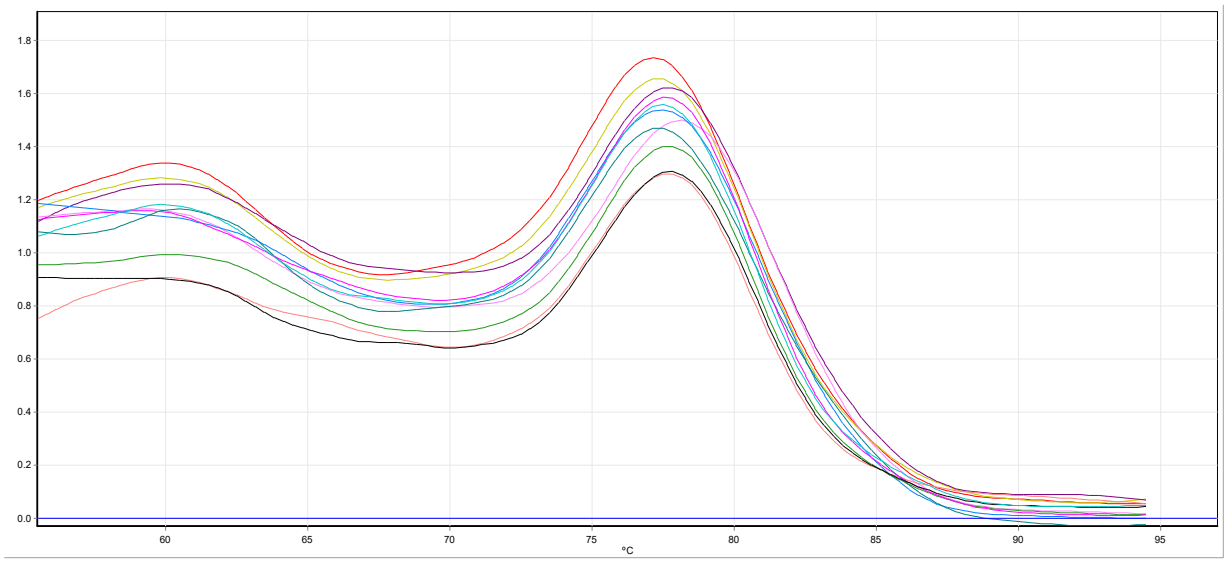


**Figure 2. Mucin1 gene dissociation curves using qPCR samples that covered all research groups. The pictures were obtained using the Qiagen Rotor-Gene Q qPCR system.**

Table 5 displays the findings of the present investigation, revealing that the fold change in MUC1 gene expression was 0.44, 0.79, and 0.8 2, indicating a down-regulation in successful and total embryo implantation in infertile females and failed embryo implantation in fertile females. Comparatively, the fold change was 1.01 and 1.24, indicating up-regulation in infertile females with failed embryo implantation and fertile females with successful embryo implantation, respectively.

**Table 5. Fold of *MUC1* gene expression depending on the 2-$^{\Delta\Delta Ct}$ method in infertile and fertile females under the IVF program**

| Study groups | | Mean ±SD | | | | | | Experimental group/control group | Fold of gene expression |
|---|---|---|---|---|---|---|---|---|---|
| | | Ct of *mucin-1* | Ct of *GAPDH* | ΔCt | ΔCt of calibrator | ΔΔCt | $2^{-\Delta\Delta Ct}$ | | |
| Infertile | Success (26) | 20.58±1.73 c | 20.39±0.82 | 0.18 | 3.34 | -3.16 | 8.94 | 8.94 /20.11 | 0.44 |
| | Failure (58) | 19.19±1.76 a b | 20.20±0.81 | -1.01 | 3.34 | -4.35 | 20.39 | 20.39 /20.11 | 1.01 |
| Total infertile (84) | | 19.62±1.86 a b | 20.26±0.81 | -0.65 | 3.34 | -3.99 | 15.89 | 15.89 / 20.11 | 0.79 |
| Fertile | Success (21) | 18.81±1.84 a | 20.11±0.55 | -1.30 | 3.34 | -4.64 | 24.93 | 24.93 / 20.11 | 1.24 |
| | Failure (23) | 19.87±2.29 a b | 20.58±0.85 | -0.71 | 3.34 | -4.05 | 16.56 | 16.56 / 20.11 | 0.82 |
| Total Fertile (44) | | 19.37±2.13 a b | 20.36±0.75 | -0.99 | 3.34 | -4.33 | 20.11 | 20.11/20.11 | 1 |

Similar letters mean no significant differences and dissimilar letters mean substantial differences.

The expression of miR146a-5P was assessed in six groups, including successful and failed embryo implantation in infertile and fertile females participating in an in vitro Fertilization (IVF) program. The reference gene U6 was used for analysis. Figure 3 illustrates the relationship between relative fluorescence and cycle number. Amplification plots are generated by plotting the fluorescent signal of each sample against the cycle number. Thus, amplification plots depict the build-up of the product during the real-time PCR experiment. The plots are generated using a series of diluted samples of the target DNA sequence. Figure 4 shows that melting curve analysis may identify non-specific products like primer-dimer. These additional peaks, located to the left of the peak representing the amplified product, indicate the presence of such non-specific products in the melt curve.

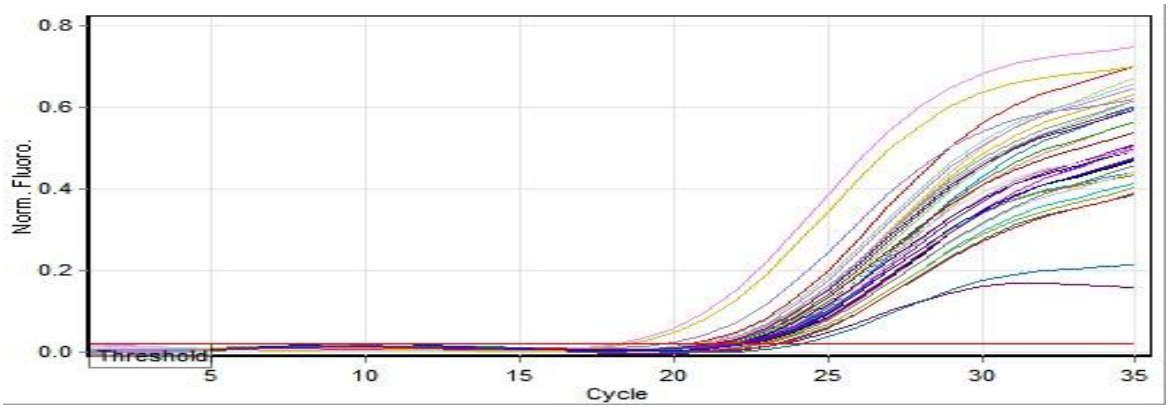


**Figure 3. miR146a-5p gene amplification was plotted using qPCR samples from all research groups. The Ct values varied between 17 to 24. The picture was taken from the Qiagen Rotor gene qPCR machine**

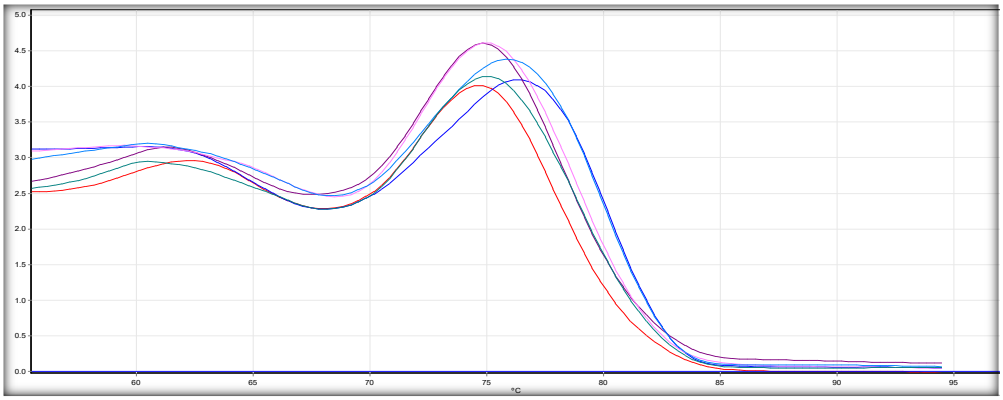

**Figure 4. miR146a-5p gene dissociation curves including qPCR samples from all research groups. The picture was obtained using the Qiagen Rotor-Gene Q qPCR system.**

The findings of the current investigation are displayed in Table 6. The fold change of miR146a-5p in success, failure, and total implantation in infertile females was 5.82, 3.66, and 4.23, while the fold changes of miR146a-5p in Success, and failure, implantation in fertile females were 0.57 and 1.73 indicating up regulated in all implantation cases in infertile females and cases of failed embryo implantation in fertile females undergoing IVF.

**Table 6. Fold of miR146a-5p gene expression Depending on 2-ΔΔCt method in infertile and fertile Females under the IVF program**

| Study groups | | Mean ±SD | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Ct of *miR146a-5p*** | **Ct of *U6*** | **ΔCt** | **ΔCt of calibrator** | **ΔΔCt** | **$2^{-\Delta\Delta Ct}$** | **Experimental group/control group** | **Fold of gene expression** |
| Infertile | Success (26) | **20.38±0.91** [a] | 20.83±1.32 | -0.46 | 3.66 | -4.12 | 17.39 | 17.39 /**2.99** | 5.82 |
| | Failure (58) | **21.44±1.87** [b c] | 21.23±1.20 | 0.21 | 3.66 | -3.45 | 10.93 | 10.93 /**2.99** | 3.66 |
| Total infertile (84) | | **21.11±1.69** [b] | 21.10±1.24 | 0.01 | 3.66 | -3.65 | 12.55 | 12.55 /**2.99** | 4.23 |
| Fertile | Success (21) | **22.70±1.36** [d] | 19.83±0.47 | 2.87 | 3.66 | -0.79 | 1.73 | 1.73 / **2.99** | 0.57 |
| | Failure (23) | **22.01±1.56** [c d] | 20.72±1.15 | 1.29 | 3.66 | -2.37 | 5.17 | 5.17 / **2.99** | 1.73 |
| Total Fertile (44) | | **22.36 ±1.49** [d] | **20.28±0.99** | **2.08** | 3.66 | **-1.58** | **2.99** | **2.99 / 2.99** | **1** |

Similar letters indicate no significant differences, but different letters indicate large differences.

Figure 5 shows the relation between relative fluorescence and cycle numbers. Amplification plots are generated by plotting the fluorescent signal of each sample against the cycle number. Thus, amplification plots depict the build-up of the product during the real-time PCR experiment. The plots are generated using a series of diluted samples of the target DNA sequence, and Figure 6 shows melting curve analysis may identify non-specific products, such as primer-dimer. These additional peaks, located to the left of

the peak representing the amplified product, indicate the presence of such non-specific products in the melt curve.

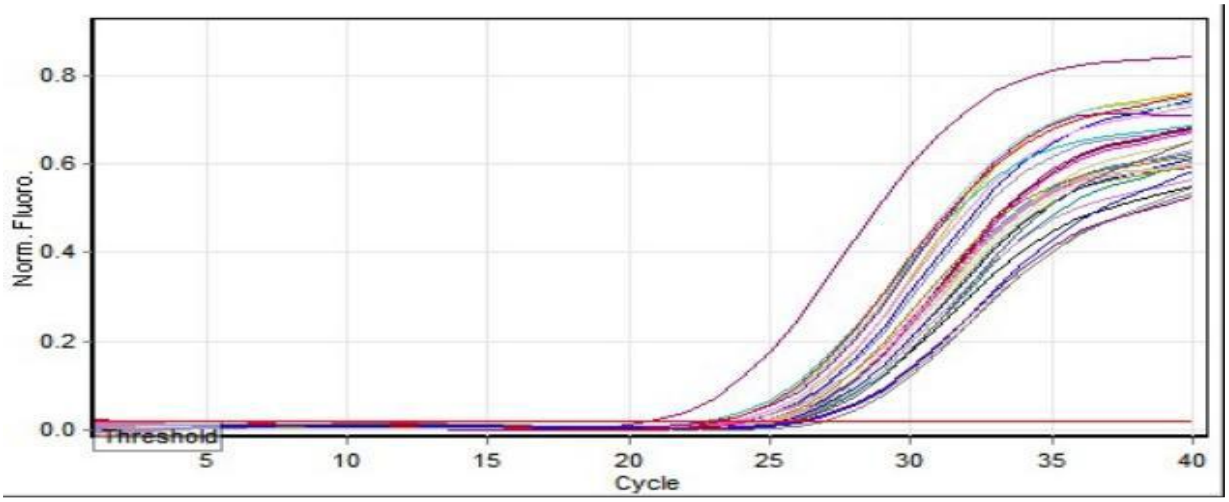


**Figure 5. miR146a-3p gene amplification was plotted using qPCR samples from all research groups. The Ct values ranged from 18 to 26. The image was captured directly from the Qiagen Rotor-gene qPCR apparatus.**

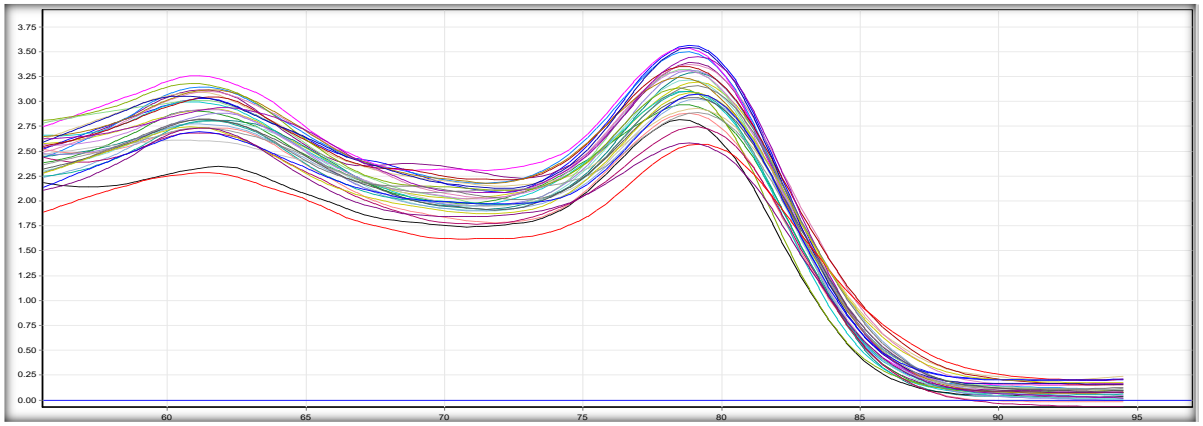

**Figure 6. miR146a-3p gene dissociation curves using qPCR samples that covered all research groups. The picture was obtained with the Qiagen Rotor-Gene Q qPCR apparatus.**

The findings of the current investigation are displayed in Table 7. The fold change of *miR146a-3p* in success, failure, and total implantation in infertile females was 3.41, 1.22, and 1.84, while the value of fold change in success, failure, and total implantation in fertile females was 0.26, 3.84 and 1 indicating up-regulated in all cases of embryo implantation in infertile females and the case of failed embryo implantation in fertile females undergoing IVF.

**Table 7. Fold of *miR146a-3p 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 *miR146a-3p* | Ct of *U6* | ΔCt | ΔCt of calibrator | ΔΔCt | 2^- ΔΔCt | Experimental group/control group | Fold of gene expression |
|---|---|---|---|---|---|---|---|---|---|
| Infertile | Success (26) | 22.22±2.38 [a] | 20.83±1.32 | 1.38 | 6.31 | -4.93 | 30.48 | 30.48 / 8.94 | 3.41 |
| | Failure (58) | 23.99±2.19 [d] | 21.13±1.29 | 3.87 | 6.31 | -3.44 | 10.89 | 10.89/ 8.94 | 1.22 |
| Total infertile (84) | | 23.28±2.42 [c] | 21.01±1.30 | 2.27 | 6.31 | -4.04 | 16.45 | 16.45/ 8.94 | 1.84 |
| Fertile | Success (21) | 24.92±1.02 [d] | 19.83±0.47 | 5.09 | 6.31 | -1.22 | 2.33 | 2.33 / 8.94 | 0.26 |
| | Failure (23) | 21.93±3.69 [a] | 20.72±1.15 | 1.21 | 6.31 | -5.1 | 34.29 | 34.29 / 8.94 | 3.84 |
| Total Fertile (44) | | 23.43±3.12 [b] | 20.28±0.99 | 3.15 | 6.31 | -3.16 | 8.94 | 8.94 /8.94 | 1 |

Similar letters indicate no significant differences, but different letters indicate large differences.

The fold change of miR485a-5p in infertile females, relative to the total embryo implantation in fertile females (control), showed up-regulation for success, failure, and total embryo implantation. Conversely, the fold change in successful and unsuccessful implantation in fertile females was down-regulation and up-regulation, respectively.

Figure 7 illustrates the relationship between relative fluorescence and cycle number. Amplification plots are generated by plotting the fluorescent signal of each sample against the cycle number. Thus, amplification plots depict the build-up of the product during the real-time PCR experiment. The plots are generated using a series of diluted samples of the target DNA sequence. Figure 8 shows that melting curve analysis may identify non-specific products, such as primer-dimer. These additional peaks, located to the left of the peak representing the amplified product, indicate the presence of such non-specific products in the melt curve.

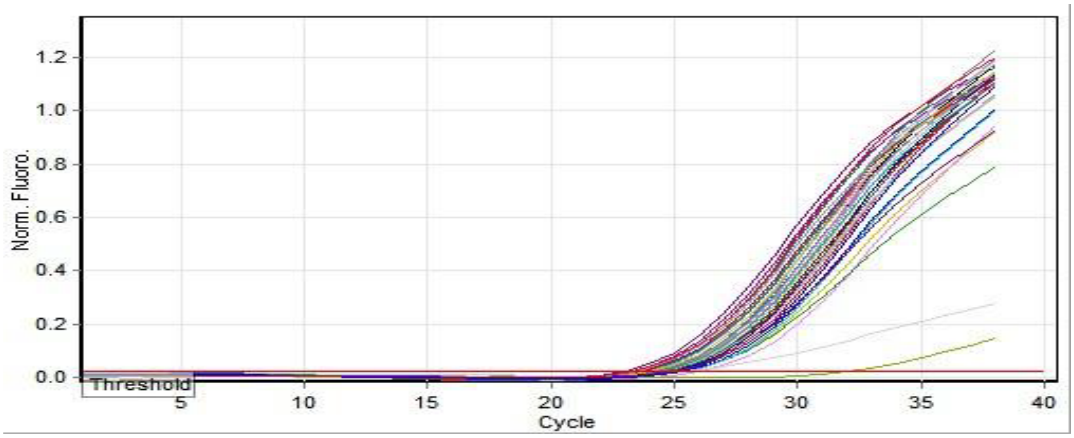


**Figure7. miR485a-5p gene amplification was displayed using qPCR samples from all research groups. The Ct values ranged between 19 and 32. The picture came straight from the Qiagen Rotor gene QPCR apparatus.**

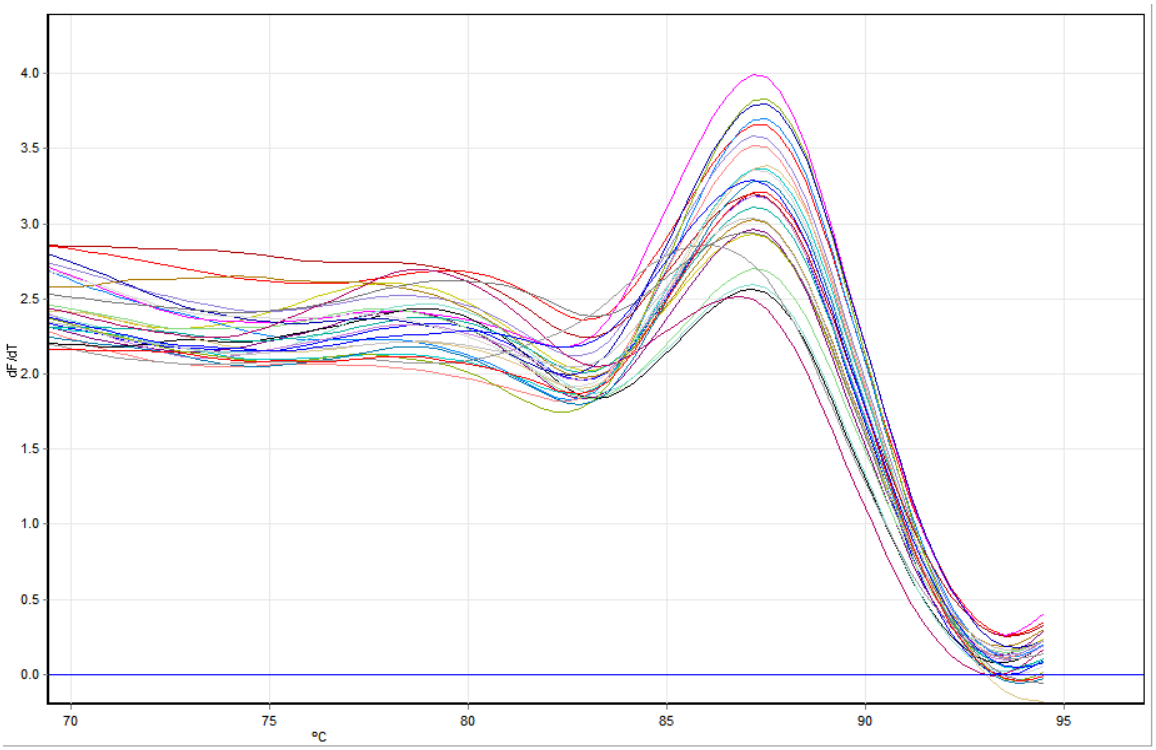


**Figure 8. *miR485a-5p* gene dissociation curves using qPCR samples that covered all research groups. The images were captured using the Qiagen Rotor-Gene Q qPCR apparatus.**

The current study indicates that the fold change of miR485-5p was up-regulated in the infertile female's success 1.75, failure 3.41 and 2.77 total embryo implantation and 2.91 in failure implantation in fertile females under the IVF program. At the same time, there was a down-regulation of 0.31 in the successful implantation in fertile females.

**Table 8. Fold of *miR485-5P* gene expression depending on the $2^{-\Delta\Delta Ct}$ method in infertile and fertile Females under the IVF program**

| | | Mean ±SD | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Study groups** | | **Ct of *miR485-5p*** | **Ct of *U6*** | **ΔCt** | **ΔCt of calibrator** | **ΔΔCt** | **$2^{-\Delta\Delta Ct}$** | **Experimental group/control group** | **Fold of gene expression** |
| Infertile | Success (26) | 24.05±2.13 [a b] | 20.83±1.32 | 3.21 | 5.8 | -2.59 | 6.02 | 6.02 / 3.43 | 1.75 |
| | Failure (58) | 23.48±1.13 [a] | 21.23±1.20 | 2.25 | 5.8 | -3.55 | 11.71 | 11.71 / 3.43 | 3.41 |
| Total infertile (84) | | 23.66 ±1.52[a] | 21.11±1.24 | 2.55 | 5.8 | -3.25 | 9.51 | 9.51 / 3.43 | 2.77 |
| Fertile | Success (21) | 25.55 ±1.09[c] | 19.83±0.47 | 5.71 | 5.8 | -0.09 | 1.06 | 1.06 / 3.43 | 0.31 |
| | Failure (23) | 23.21±2.65 [a] | 20.72±1.15 | 2.48 | 5.8 | -3.32 | 9.99 | 9.99 / 3.43 | 2.91 |
| Total Fertile (44) | | 24.32 ± 2.35[a b] | 20.29±0.99 | 4.02 | 5.8 | -1.78 | 3.43 | 3.43 / 3.43 | 1 |

Similar letters indicate no significant differences, but different letters indicate large differences.

The association coefficient between mucin1 gene and miR-146a-5p, miR-146a-3p, and miR-485-5p in infertile and fertile females participating in the IVF program is presented in Table 9. A negative correlation is reported in success and failure embryo implantation in infertile and failure implantation in fertile females under the IVF program and recorded a significant negative correlation (P=0.00) between mucin1 gene with miR-146a-3p in successful embryo implantation in infertile females. In contrast, the association between mucin1 gene and miR-485-5p significantly correlated with success and failure implantation in infertile females (P<0.01).

**Table 9. The correlation between *mucin1* with *miR146a-5p, miR146a-3p and miR485-5p* in infertile and fertile Females under IVF grogram**

| **miRNAs** | | **Study groups** | | | |
|---|---|---|---|---|---|
| | | **Infertile** | | **Fertile** | |
| | | Success (26) | Failure(58) | Success (21) | Failure (23) |
| *miR-146a-5p* and *Mucin-1* | Pearson correlation (r) | -0.65 | -0.28 | -0.12 | -0.43 |
| | P-value | 0.00** | 0.03* | 0.62 | 0.04* |
| *miR-146a-3p* and *Mucin-1* | Pearson correlation (r) | -0.89 | -0.16 | 0.28 | -0.37 |
| | P-value | 0.00** | 0.11 | 0.22 | 0.09 |
| *miR-485-5p* and *Mucin-1* | Pearson correlation(r) | 0.58 | 0.45 | 0.35 | 0.39 |
| | P-value | 0.00** | 0.00** | 0.11 | 0.07 |
| **Correlation is significant at the 0.01 level *Correlation is significant at the 0.05 level | | | | | |

A stepwise multiple regression model investigated the significant correlation between mucin-1 with miR146a-5p, and miR485-5p in infertile and fertile females under the IVF program. The independent variables were miR146a-5p, miR146a-3p, and miR485-5p, while mucin-1 was selected as the dependent variable.

**Table 10. The Regression results between *mucin1* with *miR485-5p, miR146a-5p*, and *miR146a-3p* in infertile and fertile Females under the IVF grogram**

| Dependent variable (*mucin-1*) in Study groups | | R | R-Square | F-value | F(Sig.) | Independent variables | | | | | | | variance inflation factor (VIF ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta of *miR -146a-3p* | Beta of *miR -146a-5p* | t-value | p-value | Beta of *miR -485-5P* | t-value | p-value | |
| Infertile | Success (26) | 0.74 | 0.55 | 13.82 | 0.00** | | -0.50 | -3.30 | 0.00** | 0.38 | 2.49 | 0.02* | 1.2 |
| | Failure (58) | 0.52 | 0.27 | 10.08 | 0.00** | | -0.26 | -0.26 | 0.02* | 0.44 | 3.77 | 0.00** | 1.0 |
| Total infertile (84) | | 0.60 | 0.36 | 22.48 | 0.00** | | -0.33 | -3.62 | 0.00** | 0.45 | 4.97 | 0.00** | 1.0 |
| Fertile | Success (21) | | | | | | | | | | | | |
| | Failure (23) | 0.43 | 0.18 | 4.63 | 0.04* | | -0.43 | -2.15 | 0.04* | | | | 1.0 |
| Total Fertile (44) | | 0.35 | 0.12 | 5.70 | 0.02* | | -0.35 | -2.39 | 0.02* | | | | 1.0 |

(**is significant at the 0.01 level, *is significant at the 0.05 level, Beta mean Standardized Coefficients)

The regression analysis revealed a statistically significant correlation between mucin-1 with miR146a-5p and miR485-5p in success, failure, and total embryo implantation in infertile females and between mucin-1 with miR146a-5p in failure and total embryo implantation in fertile females. This study 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-1 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-1 in Success embryo implantation = -0.50* miR-146a-5p + 0.38*miR--485-5P

-Mucin-1 in Failure embryo implantation = -0.26* miR-146a-5p + 0.44* miR--485-5P

-Mucin-1 in total embryo implantation = -0.33* miR-146a-5p + 0.45* miR-485-5P

Fertile females

-Mucin-1 in failure embryo implantation =-0.43* miR-146a-5p

--Mucin-1 in total embryo implantation = -0.35* miR-146a-5p

The stepwise multiple linear regression model results in Table 10 indicate that miR146a-5p and miR485-5p control success, failure, and total embryo implantation in infertile females by targeting mucin-1 gene expression. In fertile females, successful embryo implantation does not depend on microRNAs, while miR146a-5p controls failed and total embryo implantation by targeting mucin-1.

Finally, there is no statistical relationship between miR146a-3p and mucin-1 gene expression in success, failure, and total embryo implantation in infertile and fertile females under IVF, and successful embryo implantation in fertile females does not depend on microRNAs; maybe infertility does not depend on microRNA maybe there are many factors inter connected with especially

infertility consider as a medical problem like any other multifactorial disease there are many environmental factors and genetics factors could effect on it.

Discussion

The current investigation has uncovered that the expression of the MUC1 gene is significantly elevated in infertile females who have failed embryo implantation compared to infertile females who have successful implantation. It is strongly associated with the failure of implantation in cases of infertility. The results of this study may be connected to the conclusion that MUC1 is significantly expressed in cases of implantation failure in infertile females undergoing the IVF procedure. The negative correlation between successful implantation and MUC1 expression in the endometrium at the location of embryo attachment may result from many variables. They might be associated with the epigenetic impact of MUC1 on the post-translational process. MUC-1 serves the purpose of inhibiting the attachment of the blastocyst to regions with unfavorable conditions for implantation. This protein's production intensifies before implantation to hinder the embryo's attachment in an incorrect position (30). The increased endometrial expression of MUC1 in females who have experienced unsuccessful implantation and the elevated occurrence of minor MUC1 alleles in this infertile group indicate a connection between MUC1 and infertility (31). The present study corroborates previous research conducted in Baghdad city and reported by Saeed and her team (9), which demonstrated a negative impact on the fold change of MUC1 in infertile females. However, it contradicts another study reported by Mirza and colleagues (8), which found an increase in gene expression for mucin-1 in infertile females. Furthermore, the present findings align with a prior investigation conducted in Iraq, which examined genetic determinants and underscored the correlation between genetic variation and the outcome of embryo implantation (32-36). The significant disparity in the success of embryo implantation between fertile and infertile females may be attributed to various unexplored factors or the presence of specific factors in all females in these groups that contribute to the success of the IVF procedure.

The results of the fold change of miR-146a-5P in infertile females regarding the success and failure of embryo implantation were positive. In contrast, the fold change in failed implantation was lower compared to successful implantation. However, the fold change in failed implantation was more pronounced compared to successful implantation. This study is consistent with the observation that the expression of miR-146a was reduced in patients who experienced recurrent implantation failure (37), which aligns with the findings of previous investigations. miR-146a-5P has a role in the progression of inflammation (38), and it has been observed that people experiencing recurrent implantation failure exhibit increased inflammation. As a result, the expression of some microRNAs in these individuals may change (39). Consistent with previous research, the present study confirms that miR-146a-5p expression is significantly elevated in patients with endometriosis (40). The central biological role of miR-146a-5p is to inhibit the process of translating messenger targets, usually RNAs (mRNA), by attaching to the highly complementary 3' untranslated region.

Furthermore, microRNAs possess highly persistent biological properties. Hence, the durability and permanence of microRNAs in body fluids have rendered them appealing candidates as biomarkers for many illnesses, such as cancer and pregnancy problems (41). Research has demonstrated that miR-146a-5p plays significant roles in several processes related to embryonic development, organ formation, fetal development, Pluripotent embryonic stem cells, cell differentiation, proliferation, apoptosis, organ development, and growth regulation (42, 43). The notable increase in miR-146a-5p expression may be associated with a higher likelihood of implantation failure in females undergoing the IVF procedure. Up-regulation of mir146a-5p

may serve as a preventive mechanism against implantation.

The findings presented in Table 7 of this study indicate that there is an association between the up regulation of the miR146a-3p gene and the failure of embryo implantation in infertile females undergoing the IVF program. The oocyte contains a high level of miR146a expression. The miR146a expression levels fluctuate throughout oocyte maturation and pre-implantation embryonic development (44) . Recent research found that miR-146a was strongly expressed in primary ovarian insufficiency (POI) patients' plasma and suggested that miR-146a is involved in the death of ovarian granulose cells in POI patients (45) , up-regulation of miR-146a-3p and down-regulation of miR-22-3p, let-7c, and miR-144 were identified in premature ovarian failure, and microR146a-5p, microR146a-3p, and microR485-5p are novel biomarkers for ovarian function (46) . The significant up-regulation of miR-146a-3p may be related to failure implantation in females under the IVF program; mir146a-3p up-regulation may prevent implantation.

The infertile and fertile groups exhibited statistically significant differences compared to the other groups. Perhaps the expression of miR-146a-3p is influenced by many factors related to regulating the IVF program in females in these groups.

The current study indicates that the fold change of miR485-5p was up-regulated in the infertile female's success 1.75, failure 3.41 and 2.77 total embryo implantation and 2.91 in failure implantation in fertile females under the IVF program. At the same time, there was a down-regulation of 0.31 in the successful implantation in fertile females.

The current study found that the miR485-5p gene is highly expressed in infertility and is linked to failure embryo implantation. Prior research has demonstrated that miR-485-5p plays significant roles in regulating various cellular processes, such as cell proliferation, differentiation, apoptosis, and mobility. It has been observed that the deregulation of miR-485-5p is associated with inflammation (47) . miR-485-5p has many effects on biological processes in female bodies, which may be related to the success of the IVF program.

The recent study shows that miR485-5p gene expression is a significant linked with infertility and that up-regulation of miR1485-5p gene expression may impact embryo implantation by regulating mucin1 gene expression. The result of fold change in the present study agrees with the study mentioned; the mciroRNA (circHECTD1) silenced cell expressions of proteins such as MUC1 were down-regulated, whereas the Bcl-2-associated X-protein (BAX) level was up-regulated. The downregulation or up-regulation of these proteins caused by circHECTD1.knock down was reversed by inhibiting miR-485-5p. The findings support the theory that circHECTD1 sponges miR-485-5p to regulate MUC1 (26) and earlier research. MiR-485-5p can suppress MUC1 expression. MiR-485-5p binds to the 3′-UTR region of MUC1 and inhibits or silences its expression (26, 48), and indicates that miR-485-5p targeted MUC1 directly (26, 49). The success group in the fertile females has numerous elements not previously examined influenced by the treatment of IVF program control on regulating the miR-485-5p expression.

The analysis exposes a significant negative correlation between mucin1 and miR146a-5p and miR-146a-3p in infertile females. Conversely, a significant positive relationship was seen between mucin1 gene and miR485-5p.

The present study can conclude that it is possible that the expression of the miR146a-5p gene affects embryo implantation by regulating the expression of the mucin-1 gene, and there is a significant positive correlation between the expression of the mucin-1 and miR-485-5p gene and implantation failure in infertile females. The up-regulation of miR146a-5p gene expression may affect embryo implantation by influencing the expression of the mucin-1 gene. Consistent with previous research, infertile females with endometriosis had significantly elevated levels of miR-146a-5p

in their endometrial tissues during the implantation window period. These chemicals can influence the process of embryo implantation by impacting several molecules that serve as markers for endometrial receptivity (50). The current work goes with earlier research that identified six up-regulated microRNAs (miR-138-1-3p, miR-29b-1-5p, miR-363-3p, miR-34b-3p, miR-146a-5p, and miR-363) in the body, and used these microRNAs to confirm the putative target genes of miR-138-1-3p in the Repeated Implantation Failure Embryo (RIFE). Collective down-regulation of miR-138-1-3p's potential target genes shows that over-expression of these microRNAs may impact RIFE's lowered expression of these genes (51).

The present study agrees with the study that mentioned that when miR-485-5p was blocked, the levels of proteins like MUC1 went down in cells that did not have circRNA (circHECTD1), but the levels of Bcl-2-associated X-protein (BAX) went up. These proteins did not change when circHECTD1 was turned on or off . The findings support the theory that circHECTD1 sponges miR-485-5p to regulate MUC1 and earlier research. miR-485-5p can suppress MUC1 expression. miR-485-5p binds to the 3′-UTR regions of MUC1 and inhibits or silences its expression (49), which indicates that miR-485-5p targets MUC1 directly (26, 48). Additionally, a study has reported that mucin-1 acts as a downstream gene of miR-485-5p (46).

The results of the stepwise multiple linear regression models indicate that miR-146a-5p and miR-485-5p have a significant influence on success, failure, and total implantation in infertile females. However, only miR-146a-5p controls the failure and total implantation in fertile females by targeting the expression of the mucin-1 gene expression, and the success of embryo implantation in fertile females occurs without regulation by any microRNAs. This agrees with previous studies, which indicated that miR-146a-5p shows significantly enhanced expression and may affect embryo implantation by acting on various endometrial receptivity marker molecules(40).

By blocking the Wnt2 gene, miR-146a-5p was shown to limit the growth, invasion, and migration of trophoblast cells (41), and the expression of the miR146a-3p gene may affect embryo implantation without regulating the expression of the mucin-1 gene.

## Conclusion

The elevated expression of the MUC1 gene may correlate with the infertility of females. Undergoing in vitro fertilization (IVF) to implant embryos successfully. The up-expression of the miR146a-5p and miR485-5p genes may regulate embryo implantation by controlling the expression of the mucin1 gene. This is because there is a significant negative association between mucin1 and miR146a-5p and a significant positive correlation between mucin1 and microR485-5p.

miR-146a-3p up-regulation may prevent implantation without effect on mucin-1 gene expression.

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