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Mucin4 (MUC4) Gene and *Pri–miR146a* Expression in Iraqi Females under *In Vitro* Fertilization Program

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Abstract

MUC4, a type of reproductive mucus secreted by epithelial cells, can alter the uterine environment and weaken the epithelial lining, making it challenging for an embryo to implant. Furthermore, *MUC4* has been linked to mature endometriosis, a condition that affects the uterus. As a result, abnormal mucus production led to female Infertility. The current study evaluates the association between *mucin4, pri-miR146a,* with Infertility by measuring the fold change in gene expression to improve IVF program in Iraqi medical centres. This was achieved by assessing a new parameter in 128 infertility women under IVF. The study included 84 infertile females: - 26 successful implantations, 58 failure failed implantations under an *in vitro* fertilization program and 44 fertile females under IVF as control divided into two subgroups, 21 successful implantations and 23 failed implantations. Current work conclusion, the infertility, cases under the IVF program with fertile failure implantation cases related to significant down-regulation of mucin-4, except successful fertile cases recorded significant up-regulation. These results recorded up-regulation of *miR-146a-5p* gene expression and down-regulation of *miR146a-3p* expression. There is a significant negative correlation between *mucin4* with *miR-146a-5p* and *miR-146a-3p.* These relations may influence embryo implantation by regulating *mucin4* gene expression under the effect of negative correlation of both 5p and 3p in infertile females undergoing IVF programs.

Keywords: *Mucin4* gene, IVF, pri-*miR-146a, microRNA-146a-5p, microRNA-146a-3p.*

تعبير مورث ميوسين 4 والرنا الرقيق الاولي 146النوع a في الاناث العراقيات الخاضعات لبرنامج الاخصاب في المختبر

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الخلاصة

الميوسين الرابع هو نوع من المخاط التناسلي الذي تفرزه الخلايا الظهارية والذي يستطيع ان يغير بيئة الرحم ويضعف البطانة الظهاريه مما يجعل من الصعب انغراس الجنين. علاوة على ذلك فان الميوسين الرابع له علاقه بالتهاب بطانة الرحم في المراحل الناضجه وهذه الظروف تؤثر على الرحم . شملت الدراسه الحاليه تقييم الارتباط بين ميوسين 4 والحامض النووي الرقيق الاولي 146النوع a بواسطة قياس التغير في التعبير الجيني من اجل تحسين برنامج التلقيح الاصطناعي في المركز الطبي العراقي من خلال تقييم معلمه جديده

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عند 128امرأة من المصابات بالعقم ضمن برنامج الاخصاب في المختبر. منها 84 امراءة عقيمة ضمن هذه الدراسة وسجلت 26 عملية زرع ناجحه و 58 عملية زرع فاشلة ضمن برنامج الاخصاب خارج الرحم و 44 أنثى قادرة على الإنجاب كمجموعة ضابطه مقسمة إلى مجموعتين فرعيتين، 21 عملية زرع ناجحة و 23 عملية زرع فاشلة.الاستنتاج الاول من الدراسه الحاليه حالات العقم الخاضعه لبرنامج التلقيح في المختبر مع حالات فشل انغراس الجنين في النساء الخصبات ترتبط بالانخفاض الكبيرلتعبير جين ميوسين 4 ماعدا حالات نجاح انغراس الجنين في النساء الخصبات التي سجلت زياده كبيره في تعبير الجين.وجود علاقه سلبيه كبيره بين الميوسين الرابع مع الرنا الرقيق a–5p146 والرنا الرقيق a–3p146 .سجلت هذه النتائج ارتفاع التعبير الجيني للرنا الرقيق a–5p 146 وانخفاض التعبير الجيني للرنا الرقيق a–3p 146 قد يتحكم في زرع الاجنه عن طريق تنظيم التعبير الجيني للميوسين الرابع في الاناث المصابات بالعقم والخاضعات لبرنامج الاخصاب في المختبر.

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1. Introduction

The World Health Organization (WHO) has defined sterility as an illness. The World Health Organization - International Committee for Monitoring Assisted Reproductive Technologies (WHO-ICMART) dictionary states that "a disorder of the reproductive system is described by failure to obtain a clinical pregnancy after 12 months or more of regular unprotected sexual interaction [1]. According to the WHO-ICMART glossary, infertility is categorized into two forms: primary and secondary [2, 3]. Implantation is the process through which the embryo attaches to the inside of the uterus (Blastocyst failure to implant, which accounts for more than 50% of all unsuccessful pregnancies, is a significant cause of Infertility) [4-7]. *In vitro* fertilization (IVF) is a treatment option for female Infertility, particularly for women experiencing issues with their fallopian tubes or difficulties achieving fertilizing using an in vivo approach [8-10].

Mucins, a type of heavily sugar-coated protein, play a crucial role in shielding and lubricating the epithelial lining of reproductive tracts, providing protection and facilitating smooth functioning [11, 12]. Previously mucin expression was recorded in local studies of mucins and its relationship to Infertility was proven [13, 14]. The mucus of the cervix and endometrial, which play crucial functions in reproductive physiology, is produced by the epithelial cells of reproductive organs. Along with *MUC1* and *MUC16, mucin-4 (MUC4)* is the primary mucin present in the endometrial epithelium of several species [15]. Research has shown that *MUC4* expression is significantly higher in fertile females than infertile females and has confirmed *MUC4* as a novel target for infertility diagnosis [13]. Conversely, a study showed that the fold changes in *MUC1* were positive in the infertile group, while negative in the fertile and pregnant groups[14].

MicroRNAs (also known as miRNAs or microRs) are a class of 19–23 nucleotide non-coding RNAs essential for many biological processes by controlling the amounts of several proteins[16-19]. Two *miR-146* genes, *miR-146a* and *miR-146b*, are located on chromosomes 5 and 10, respectively, in the human genome. Each pre-miR produces two mature miRs: miR-*146a-5p* and *miR-146a-3p*.According to gene knockout studies, *miR-146a* deficiency causes chronic inflammation [20, 21]. The overexpression of *miR-146* has been linked to the development of various diseases in the Iraqi population, as reported in several studies [22, 23]. Despite advancements in understanding infertility, there remains a need to elucidate the molecular mechanisms underlying implantation failure and its association with mucin expression, hormonal states, and microRNA regulation. While previous studies have highlighted the role of mucins, particularly *MUC4*, in fertility and endometrial physiology, further investigation is required to comprehensively assess their utility as diagnostic markers and therapeutic targets. Additionally, the interplay between microRNAs, such as *miR-146a*,

and inflammatory pathways in the context of infertility warrants deeper exploration. The aim of study is to investigate the relationship between *MUC4* expression, hormonal states, and microRNA regulation in infertile females, with a focus on elucidating potential biomarkers and therapeutic avenues for improving IVF outcomes.

2. Material and Methods

2.1 Ethical approval

All procedures performed in this study involving the collection of blood samples and information from human participants were conducted by obtaining signed consent through the participation in research form. The study received ethical approval from the Health Ministry / the Baghdad Health Department, with the number of the ethics committee 105 in April / 2023

2.2 Blood Sampling

The data collection for this study, including blood sample collection and laboratory work, took place over a period of approximately 8 months, from November 2022 to July 2023. The patient group consists of females who have had no children or have had a problem conceiving in the last years, Infertile females (primary and secondary). Patients having chronic conditions such as diabetes mellitus, thyroid disease, cardiovascular disease, polycystic ovary (PCO), tubal factor and endometriosis were excluded. They were enrolled from public hospitals and Infertility centres and private IVF in Baghdad, Iraq. A total of 128 Iraqi females, fertile and infertile, were sorted instead of:-

-Group 1: included Infertile females under the IVF program, consisting of 26 infertile females who underwent successful IVF and 58 infertile females who underwent unsuccessful IVF.

-Group 2: included 44 fertile females under the IVF program and considered" as controls".

2.3 RNA Extraction

Total RNA was extracted directly from the whole blood sample using the Trans Zol Up Plus RNA Kit (Trans Gen, biotech. ER501-01), following the protocol provided by the manufacturer. The 2000c Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) was used to evaluate the concentration and purity of extracted RNA to determine the quality of samples. The samples ranged in RNA concentration range from 73-147ng/µl. The presence of an A260/A280 ratio of around 2.0 suggested that the RNA sample is 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 (0.1 µg / µl), GSP, 2xES Reaction Mix, EasyScript® RT/RI Enzyme Mix, gDNA Remover and RNase-free Water.

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 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 size bp	Tm °C	References
Mucin4 (Gene Expression)				
Forward	GCCCAAGCTACAGTGTGACTC	21	58	[24]
Reverse	ATGGTGCCGTTGTAATTTGTT	21		
GAPDH –Glyceraldehyde 3-phosphate dehydrogenase				
Forward	TGAGAAGTATGACAACAGCC	20	58	[25]
Reverse	TCCTTCCACGATACCAAAG	19		
pri-miR-146a (Gene Expression)				
Forward	TTAGGAGCTCGCTGGCTGGGACA	23	56	[26]
Reverse	CAGGATCTACTCTCTCCAGGTCCTCA	26		
U6 (Gene Expression)				
Forward	CTCGC TTCGGCAGCACA	17	56	[27]
Reverse	AACGCTTCACGAATTTGCGT	20		

2.4 Quantitative Real-Time PCR (qRT-PCR) runs

The Quantitative Real-Time PCR (qRT-PCR) was performed using the QIAGEN Rotor gene Q Real-time PCR System from Germany. To validate the expression of a target gene, a quantitative real-time qRT-PCR SYBR Green assay was utilized. The mRNA levels of endogenous control genes GAPDH and *miRU6 (*reference genes) were amplified and used to normalize the mRNA levels of the target genes.

An ideal reference gene has the following characteristics, it is expressed stably in different tissues and cells and its expression is not greatly affected by environmental, biological or abiotic stress or other factors.

The expression levels and fold changes of the Mucin4, *GAPDH, miR146a-5p,miR146a-3p* 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-4, GAPDH, miR146a-5p, miR146a-3p,* 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 optimized based on the thermal profile for the expression of *Mucin4, GAPDH, miR146a-5P, miR146a-3p* and *miRNAU6* genes. The protocol included an initial hold at 94°C for 30 seconds, annealing at 56-58°C for 15 seconds, and extension at 72°C for 20 seconds. A dissociation curve was measured in a range of temperature between 55-95c

2.5 Biostatistical analysis

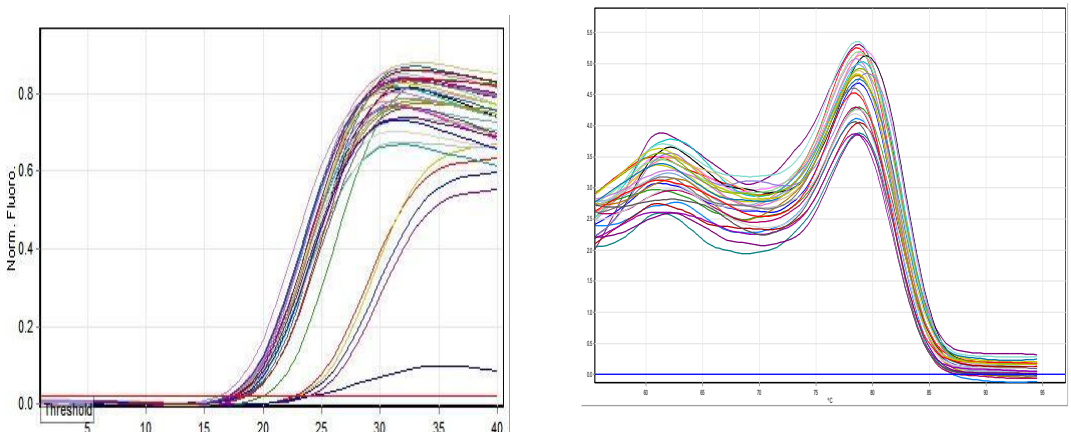
The fold gene of the quantified expression of the genes was calculated by the (2^-ΔΔct) method [28] SPSS version 25 was used for statistical analysis of the data. Duncan's multiple range test is a statistical test used to compare the means of multiple groups and the Pearson correlation coefficient (PCC) test is a correlation coefficient that measures linear correlation between two sets of data.

3. Results and discussion

3.1 Gene Expression for MUC

This study quantified the expression of the mucin4 gene across six groups: infertile females who were successful, failure and total infertile females, fertile success, failure and

total fertile females under the IVF program using the reference gene GAPDH. Figure 1 shows the CT of study samples and the melting curve. The amplification curve plot is C-shaped and the amplification concentration is between 1.5 and 2.0. The melting curve indicates that no primer dimer is present.



(a) **(b)**

Figure 1: Figure legend (a) *mucin4* dissociation curves by qPCR Samples included in the study group. (b) *MUC4* gene melting curves using qPCR samples. The image was captured using the Qiagen Rotor gene qPCR machine.

Table 2 displays the mean Ct of *MUC4* gene expression was found to be 18.69 for infertile success, 18.75 for failure, 18.73 for total infertile, 17.22 for fertile success, and 19.39 for fertile failure, while the mean Ct of *GAPDH* gene expression for the infertile success, failure at 20.39, 20.20, the total infertile females at 20.26, and the fertile success, failure at 20.12, 20.58.

The results of the present study reveal that *MUC4* expression was down-regulated in infertile individuals, regardless of treatment outcome, as well as in fertile individuals who failed to achieve success. In contrast, *MUC4* expression was up-regulated in fertile individuals who achieved success.

Table 2: Fold of *MUC4* gene expression Depending on $2^{-\Delta\Delta Ct}$ method in infertile and fertile Females under IVF program.

		Mean ± SD							
Study groups		**Mean Ct of *mucin4***	**Mean Ct of GAPDH**	**ΔCt**	**Mean Ct of calibrator**	**ΔΔCt**	**$2^{-\Delta\Delta Ct}$**	**Experimental group/control group**	**Fold of gene expression**
Infertile	Success (26)	18.69± 1.82 [b]	20.39±0.82	-1.71	4.92	-6.63	99.04	99.04/124.49	0.80
	Failure (58)	18.75±2.88 [b]	20.20±0.81	-1.46	4.92	-6.38	83.29	83.29/124.49	0.67
Total infertile(84)		18.73±2.59 [b]	20.26±0.81	-1.53	4.92	-6.45	87.43	87.43/124.49	0.70
Fertile	Success (21)	17.22±2.59 [a]	20.12±0.55	-2.89	4.92	-7.81	224.41	224.41/124.49	1.80
	Failure (23)	19.39±3.12 [b]	20.58±0.85	-1.18	4.92	-6.1	68.59	68.59/124.49	0.55
Total Fertile(44)		18.31±2.59 [b]	20.35±0.75	-2.04	4.92	-6.96	124.49	124.49/124.49	1

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

The current study found that *MUC4* gene expression was considerably higher in fertile success females and total fertile females undergoing the IVF program compared to fertile failure and total infertile (success and failure) females under the IVF program, as demonstrated in Table 2. The fold change of the *MUC4* gene for total infertile females (success, failure) under the IVF program is less than one, indicating that it is negatively linked with Infertility in situations of success and failure embryo implantation. *Mucin- 4 (MUC4)* and *mucin1 (MUC1)* are the most abundant mucin proteins produced in the endometrial epithelium, serving to protect and lubricate the epithelial surface of reproductive tracts [29, 30] and Muc-4 is highly expressed in the endometrial epithelium and plays a key function in the invasion of human cytotrophoblasts [29, 31, 32]. However, *MUC4* expression in normal endometrium, ectopic and ectopic endometrium, and endometriosis patients was studied quantitatively by immunohistochemistry, but there was a lack of *MUC 4* expression in the endometrium [33] -while Dharma Raj with his colleagues MUC4 expression was significantly higher in the ectopic endometriosis tissues. However, immunostaining for all three mucins demonstrates significant expression of *MUC1* and *MUC16* at the apical surfaces of endometrial epithelia, but little to no staining for *MUC4*[24]. Previous research by Miriza and her team found that the mucin4 fold change was down-regulation in Iraqi infertile females [13].

The current study may conclude that *mucin-4* gene expression is negatively linked within situations of embryo implantation, and it may be that failure of embryo implantation was related to decreased *mucin-4* gene expression.

The present study's results are consistent with the findings of Caraway and his team, who previously observed that embryo implantation is associated with a decrease in *mucin4* expression [34]. Several studies have shown that mucin4 could promote cell migration, change the endometrial environment, and create weak points in the epithelium, thus facilitating embryo implantation failure [33, 35].

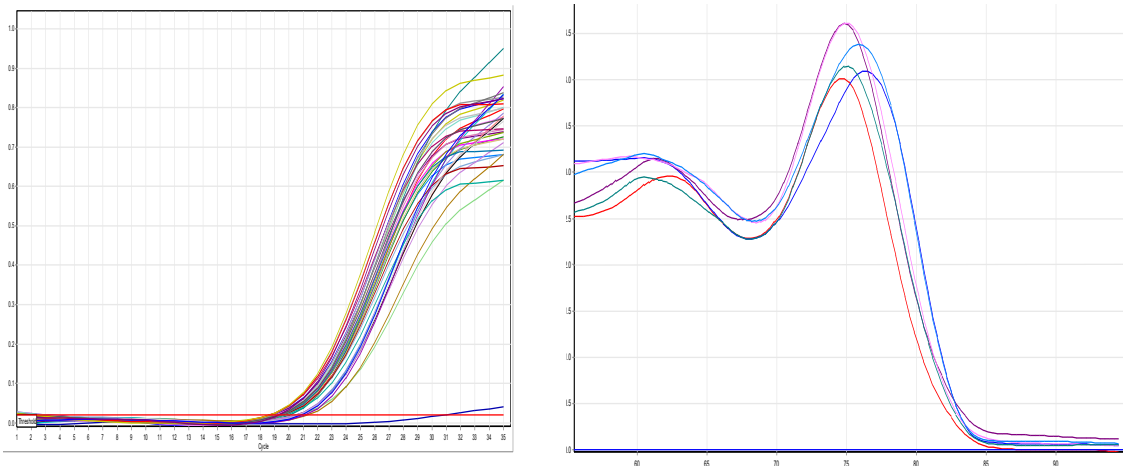
The Infertility in the present study and even failure implantation in fertile is related to significant down-regulation of *mucin-4.*

3.2 MicroRNAs expression level

The expression of miR146a-5P was assessed in six groups of females participating in the IVF program: infertile females Success, Failure and total infertile, fertile females, Success, Failure and total fertile females, using *U6* as the reference gene.

3.2.1 MiR146a-5p expression level

The mean Ct of *miR146a-5p* in blood samples from infertile females were 20.38 for successful cases, 21.44 for failures, and 21.11 for the total infertile group. In contrast, the mean Ct of fertile females success, failure, and total fertile females were 22.70, 22.01, and 22.36. The mean Ct of U6 genes for infertile females' success, failure, and total were 20.83, 21.23, and 21.10, respectively, while the mean Ct of U6 genes for fertile females success, failure, and total were 19.83, 20.72, and 20.28. Figure 2 depicts the CT of a research sample and the melting curve. Whereas the Amplification curve plots are C-shaped and the amplification concentration is between 1.5 and 2.0, the melting curve indicates that no primer dimer is present.



(a) **(b)**

Figure 2: Figure legend (a) *miR146a-5p* gene dissociation curves by qPCR Samples included in the study groups. (b) *miR146a-5p* gene melting curves using qPCR samples. The image was captured using the Qiagen Rotor gene qPCR machine.

The results of the present study are presented in Table 3. The fold change in *miR146a-5p* was positive in the infertile Success, Failure and total infertile females groups under IVF program.

Table 3: Fold of *miR146a-5p* gene expression Depending on $2^{-\Delta\Delta Ct}$ method in infertile and fertile Females under IVF program.

		Mean ± SD							
Study groups		**Mean Ct of *miR146a-5p***	**Mean Ct of *U6***	**ΔCt**	**Mean Ct 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 mean no significant differences and different letters mean substantial differences.

The findings show that the fold change of *miR146a-5p* in infertile females was favorable for both successful and failed embryo implantation. However, the fold change in failure was lower than the success. Still, the fold change in fertile failure is higher than fertile success, as shown in Table 3, which agrees that *MiR-146a* expression was decreased in repeated implantation failure (RIF) patients, this supports the findings of earlier research that *miR-*

146a-5P promotes the development of inflammation [4, 36, 37]. It is important to note that individuals with repeated implantation failure have elevated inflammation. Consequently, these patients' expression of specific microRNAs may alter

Figure -3 Figure legend (a) *miR146a-3p* gene dissociation curves by qPCR Samples included in the study groups. (b) *miR146a-3p* gene melting curves using qPCR samples. The image was captured using the Qiagen Rotor gene qPCR machine.

The present study indicated that miR146a-5p gene expression is positively linked with failure of implantation, even in fertile females. It may be that up-regulation of *miR146a-5p* gene expression impacted embryo implantation by the regulation expression of the mucin4 gene.

The current aligns with findings that *miR-146a-5p* was highly increased in endometriosis patients [40], *miR-146a-5p* showed dramatically increased expression during the implantation window period in the endometrium tissues of endometriosis infertile patients, potentially influencing embryo implantation by acting on several endometrial receptivity marker molecules [41], and in 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, have used these microRNAs to confirm the putative target genes of *miR-138-1-3p* in Repeated Implantation Failure embryo (RIFE). The collective downregulation of potential target genes for miR-138-1-3p suggests that the expression of these microRNAs may influence RIFE by contributing to the decreased expression of these genes [42, 43]. The significant up-regulation of *miR-146a-5p* may related to the increasing chance of failure implantation in females under the IVF program, *miR-146a-5p* up-regulation may prevent implantation.

3.2.2 MiR146a-3p expression level

Figure 3 shows the CT of a research sample and the melting curve. Whereas the Amplification curve plots are C-shaped and the amplification concentration is between 1.5 and 2.0, the melting curve indicates that no primer dimer is present.

Table 4 shows the results of *miR146a-3P* expression in infertile females. The fold change in infertile females' success and failure compared to total fertile control was positive for success and total infertile females and negatively harmful for failure infertile females. In contrast, the fold change in fertile female’s success and failure was negative and positive, respectively. Based on the result shown in Table 4, it can be concluded that down-regulation *miR146a-3p* expression may be associated with failure embryo implantation in infertility females in the IVF program.

Table 4 : Fold of *miR146a-3p* gene expression Depending on $2^{-\Delta\Delta Ct}$ method in infertile and fertile Females under IVF program.

Mean ± SD									
Study groups		**Mean Ct of *miR146a-3p***	**Mean Ct of *U6***	**ΔCt**	**Mean Ct of calibrator**	**ΔΔCt**	**$2^{-\Delta\Delta Ct}$**	**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)	25.09±2.40 [d]	21.23±1.20	3.86	6.31	-2.45	5.46	5.46 / 8.94	0.61
Total infertile(84)		24.19±2.73 [c]	21.11±1.24	3.09	6.31	-3.22	9.32	9.32 / 8.94	1.04
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 mean no significant differences, different letters mean significant differences.

The oocyte contains a high concentration of *miR-146a*, and its expression levels vary throughout oocyte maturation and pre-implantation embryo development [44]. Recent studies 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* was identified in premature ovarian failure. Furthermore, *microR146a-5p, microR146a-3p* and *microR485-5p* as novel biomarkers for ovarian function [46].

The total infertile and total fertile recorded highly significant significantly differences from other groups, which may be females in these groups have many factors affected by treatment of the IVF program leading to up-regulation or down-regulation of mir146a-3p expression.

3.3 The correlation between mucin4 with mir146a-5p and mir146a-3p.

Table 5 presents the correlation coefficient results between *mucin4* and *miR-146a-5p/miR-146a-3p* in infertile and fertile women undergoing IVF treatment. An association between *mucin4* and *mi146a-5p* is found in infertile success (P=0.00) and fertile failure (P=0.04). In contrast, a significant relationship between mucin4 and mi146a-3p is found in infertile success and failure, with a total of (P=0.00) and fertile success and failure of (P=0.01).

Table 5: the correlation between *mucin4* with *miR-146a-5p* and *miR-146a-3p* in infertile and fertile Females under the IVF program.

Study groups		***miR146a-5p* and *mucin4***				***miR146a-3p* and *mucin4***			
		Fold change of *miR146a-5p*	**Fold change of *mucin4***	**Pearson correlation (r)**	**P value**	**Fold change of *miR146a-3p***	**Fold change of *mucin4***	**Pearson correlation (r)**	**P value**
Infertile	Success (26)	5.82	0.80	-1.00	0.00**	3.41	0.80	-0.81	0.00**
	Failure (58)	3.66	0.67	-0.07	0.61	0.61	0.67	-0.39	0.00**
Total infertile(84)		4.23	0.70	-0.18	0.11	1.04	0.70	-0.41	0.00**
Fertile	Success (21)	0.57	1.80	-0.28	0.22	0.26	1.80	0.54	0.01*
	Failure (23)	1.73	0.55	-0.438	0.04*	3.84	0.55	-0.56	0.01*
Total Fertile(44)		1	1	-0.26	0.00**	1	1	-0.41**	0.00**

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

The result of the present study revealed a significant negative correlation between *mucin4* with *miR146a-5p* in infertile females with success and females with fertile failure females. While between mucin4 with *miR-146a-3p* was a significant negative correlation in infertile and fertile females under an *in vitro* fertilization program.

The current findings suggest that *miR146a-5p* gene expression is positively associated with implantation failure in females under IVF program. Specifically, the up-regulation of *miR146a-5p* gene expression, as well as down-regulation of *miR146a-3p*, is related to embryo implantation failure in infertility females undergoing IVF by targeting the *mucin4* gene. This is in agreement with previous studies showing that *miR-146a-5p* shows significantly enhanced expression and may affect embryo implantation by acting on a variety of endometrial receptivity marker molecules [47], *miR-146a-5p* was proven to impair trophoblast cell proliferation, invasiveness and migratory capacity through inhibiting *Wnt2* gene [42]. These factors could serve as regulatory elements for the endometrium to improve the result of IVF programme meanwhile the high ratio of failure implantation in the current procedure.

Conclusions

The current study found that infertility, as well as failed implantation in fertile individuals, was associated with a significant decrease in *mucin-4* expression. There is a significant negative correlation between *mucin4* with *miR-146a-5p* and *miR-146a-3p*. Significantly up-regulation of *miR-146a-5p* may related to the increasing chance of failure implantation in females under the IVF program, *miR146a-5p* up-regulation may prevent implantation and down-regulation of *miR146a-3p* expression may be linked to embryo implantation failure in infertility females undergoing IVF. Moreover, a significant correlation between *muc4* with *miR-146a-5p* and *miR-146a-3p* consequently may refer to the regulated effect of *miR-146a-5p* and *miR-146a-3p* on the expression of *muc4.*

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Disclosure statement

There are no competing interests to declare.

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