INTERNATIONAL JOURNAL OF
MEDICAL BIOCHEMISTRY

DOI: 10.14744/ijmb.2025.92668
Int J Med Biochem 2025;8(2):107–115

Research Article

MicroRNAs and pro-inflammatory cytokines as candidate biomarkers for male infertility

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Abstract

Objectives: The effect of infertility does not exert a futuristic effect on society only but puts emotional and psychological stress on couples no matter who may show this problem. Thus, searching for obvious and hidden reasons to treat this problem took significant leaps to overcome it and provide couples with means of treatment. This article aims to investigate the role of specific microRNAs (miRNAs) as miR-429 and miR-425 and pro-inflammatory cytokines as interleukin-1 alpha (IL-1α) and tumor necrosis factor-alpha (TNF-α) on male fertility.

Methods: 100 semen samples were collected from healthy men with offspring and another 100 samples were collected from men suffering from fertility impairment for Semin fluid analysis (SFA). Cytokines levels in the serum were measured using sandwich ELISA technique, whereas DNA samples were obtained from both categories of participants from blood.

Results: Results showed that infertile patients showed high level of both tumor necrosis factor (TNF-α) and interleukin (IL-1A) which affected semen quality, motility, and to fertilize mature oocytes. In addition, high levels of miRNA 425, and 429 were detected in patients compared to control. We found specific type of single nucleotide polymorphism (SNPs) that reduced the ΔG in miRNAs within patients giving them the chance to be circulated for longer half time than the control which modified the RNA decay mechanism.

Conclusion: Immunological and epigenetic factors can play a crucial role in infertility manifestation in male. Since immunological factors are widely studied and been taken in concern in fertility clinics, epigenetic factors may be the key to overcome such clinical case and need to take in concern to provide a proper medical care.

Keywords: IL-1α, male infertility, miRNA-425, miRNA-429, TNF-α

How to cite this article: Samawi FT, Aqeeli GJ, Algafari RN, Alsaidi MA, Al-Saedi MK, Jasim SA, Ramadhan RS. MicroRNAs and pro-inflammatory cytokines as candidate biomarkers for male infertility. Int J Med Biochem 2025;8(2):107–115.

The case of infertility inflects a socially striking sensitivity than for other health conditions. Married couples mostly live in denial and lack the ability and medical knowledge to define themselves as infertile in the majority of cases regardless medical reports they are provided with by medical practitioners. Even more, medical models react with medical conditions as a phenomenon affecting the individual, while infertility is treated as condition affect the entire society in the

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Submitted: October 24, 2024 **Revised:** January 24, 2025 **Accepted:** January 25, 2025 **Available Online:** March 06, 2025

industrial nations and could lead to functional impairment [1]. Developing of infertility is affected by various previously infected diseases and risk factors. Male factors occupy half of reasons in 13–18% infertility cases [2]. The failure to conceive a fetus after more than a year of regular unprotected sexual intercourse with the same partner is a feature of this condition [3]. Defective sperm function is the most known causes in male infertility. Abnormal semen parameters like low sperm concentration, impaired motility, and deformed morphology may play a crucial factor in infertility [4]. The infertility affects about 10% of women and 7% of men may produce a burden that may be announced high by WHO when subfertility / infertility reaches this percentage [5, 6]. Infertility may be categorized in in four reasons or factors: male factors, female factors, both male and female factors, in addition to unknown etiological factors [7]. A quantitative and signification idiopathic proportion of azoospermia, cryptozoospermia, and oligoasthenozoospermia may accompany male infertility and/or qualitative (asthenospermia, teratozoospermia, and necrospermia) abnormalities [8]. Male impairment or reduced fertility can be considered as a complex with multifactorial disease etiologies where genetic and epigenetic factors may play a key role and contribute to its manifestation [9]. Many genes were found to play a crucial role in controlling spermatogenesis, but the biological function of the majority of these genes in the control of the spermatogenesis process is still to be understood [10]. Among epigenetic factors affecting infertility, the single stranded miRNAs with length of 22 – 24 nucleotides were found to play a regulatory function in gene expression by forming semi – complementary structure in the untranslated 3' region related to mRNA [11]. About 1982 miRNAs playing a crucial role in cell cycle, cell differentiation, cell metabolism, and many biological processes, cell proliferation and apoptosis, male and female gametogenesis and embryo development were identified [12]. The body circulating extracellular miRNAs are categorized by stability and can be detected in body fluid samples of patients. The quantity of these miRNA may reflect the medical condition and disease status when compared to normal values [13]. The miR-429 is a member of miRNA-200 family, is highly expressed in in epithelial and mesenchymal tissues. Lack of regulation in these tissues due to lack of function of this type of miRNA may result in malignant tumors like oral squamous cell carcinoma [14]. The main function of miRNA-429 and its family as reported to inhibit inflammatory signaling and suppress multiple gene expression and production of IL8 [15]. They are an accumulative data regarding the importance and role of miRNA-429 in pathological and physiological events has and its possible role in oral inflammatory processes remains to be elucidated [16]. In addition, miRNA-425 was found to be associated with different diseases and biological processes like anti-angiogenesis [17], tumorigenesis [18] and inflammatory cytokine production [19]. In a separated report [20], miR-425 contributes to tumor development by inhibiting the tumor suppressor catenin α-3 in hepatocellular carcinoma. miR-425 is also reportedly upregulated in gastric cancer [21].

The purpose behind this study is to establish the relationship among miRNA-429 which considered as a tumor suppression factor and highly related to the function of immune system, and miRNA-425 which plays a crucial role in healthy proliferation of ovaries and fertility through immunological, genetic and epigenetic analysis.

Materials and Methods

Ethical approval

This study was approved by the Ethic Committee in Biotechnology Research Center under Ref. no. M. B 52 in 2/1/2022.

Consent of participation

All participants were asked to sign a participation form, and a questionnaire in which necessary information were recorded. No personal photos, videos, clinical tests, and names help to identify participants was published. The Helsinki declaration was followed during collecting personal data from patients.

Exclusion criteria

Men with chronic disease e.g., Cardiovascular, Diabetes Mellitus and Hypertension Varicocele (according to the clinical examination and ultrasonic waves), were excluded from this study.

Inclusion criteria

Males with fertility impairment showing no signs of chronic diseases, and previously had offspring were included in this study.

Sample collection

Semen samples were collected from 100 healthy men (normozoospermia) with a normal reproductive history and physical examination. All controls had children within 1 year, whereas 100 semen samples were collected from men with fertility impairment. Patients and control fall in the same age group 18 – 55 years.

Semen quality analysis

After two to five days of sexual abstinence, sperm samples were collected and incubated at 370C. After 30 minutes of incubation, liquefaction was observed and semen was analyzed according to World Health Organization recommendations, 2010, which included volume, sperm concentration, motility, and morphology.

Determination of IL-1A and TNF-α levels using Enzyme-Linked Immunosorbent Assay kit (ELISA)

The serum levels of human IL-1A and TNF- were determined using the human IL-1A and TNF-ELISA Kit (sandwich ELISA technique, Abcam, USA) according to manufacturer protocol. Resulting data were recorded and statistically analyzed.

Isolation of miRNAs

miRNAs were isolated from spermatozoa by using special EasyPure® miRNA Kit from TransGen biotech (China) as instructed by the manufacturer.

Isolation of total RNA

Trizol (TRIzol LS Reagent), was used to extract total RNA from sperm pellets, as directed by the manufacturer. Spectrophotometry was utilized to assess the purity and concentration of total RNA using the Nanodrop 2000 (Thermo Scientific). The isolated RNA samples were kept at -800C until use.

First-strand synthesis

The first strand was generated using EasyScript® One-Step cDNA synthesis kit (China). A volume of 5 µ of miRNA was mixed with kit components to final volume of 20 µl as instructed by the manufacturer. qPCR program was 940C for 5 sec, 600C for 15sec. in 40 cycles. The same protocol was used to generate cDNA from total RNA.

Primers used in Quantitative PCR (qRT–PCR)

Primers used during qRT – PCR amplification are listed below:

Genes	Forward primer (5´--->3´)	Reverse primer (3´ --->5´)	TM
rnub6B	AGAGAAGATTAGCATGGCCCCT	GCGAGCACAGAATTAATACGAC	62
mir-425	AATGACACGATCACTCCCGTTGA	GCGAGCACAGAATTAATACGAC	62
mir-429	TAATACTGTCTGGTAAAACCGT	GCGAGCACAGAATTAATACGAC	58
gapdh	GAAATCCCATCACCATCTTCCAGG	GAGCCCCAGCCTTCTCCATG	64
il-1A	TGTATGTGACTGCCCAAGATGAAG	AGAGGAGGTTGGTCTCACTACC	66
tnf-α	CTCCAGGCGGTGCTTGTTC	GGCTACAGGCTTGTCACTCG	58

Quantitative real-time PCR

Three microliters of cDNA were employed as templates in 20-mL reaction volume for quantitative real-time (qRT) PCR assessment of chosen genes. The primers utilized to evaluate gene expression are detailed in Table 1. including a thirty-second activation stage at 940C, followed by 40 cycles of five seconds at 940C (denaturation step), thirty seconds at 600C (annealing step), and ten seconds at 720C (extension step). The melting curves of the final dissociation curves were then generated. The Cepheid (smart cycler) Real-Time PCR Detection System was used for all of the tests, and the data (Ct values) were compared to internal controls RNU6B (U6) and GADPH. When housekeeping genes are employed in molecular studies, it is expected that the cells or tissue being analyzed will maintain constant levels of expression. These controls were found to be a very reliable method for qRT-PCR normalization when utilized in clinical research.

Analysis of gene expression

The levels of expression of the four chosen genes were normalized relative to endogenous controls. Using the comparative ΔCt technique, the relative miRNA and mRNA levels in the examined samples from fertile and infertile males were estimated individually. The threshold cycle (Ct) in a qRT-PCR is the number of cycles when a fluorescence curve initiated inside an interaction crosses the threshold.

The ΔCt has been computed by deducting the Ct amount of RNU6B and GAPDH from the ΔCt amount of target miRNA and mRNA, respectively: ΔCt (Ct of target miRNA-Ct RNU6B), ΔCt (Ct mRNA of interest-Ct GADPH). The ΔΔCt of the specimen from males with suspected infertility was then subtracted from the ΔCt of the healthy samples: ΔΔCt=(ΔCt1-ΔCt calibrator). This approach compares how a nucleic acid sequence is expressed in a test specimen to how it is expressed in a control sample containing the same sequence. The equation ΔΔCt was used to figure out where the fold-change cutoff was for each gene.

Statistical analysis

The Statistical Packages of Social Sciences (SPSS 2018) was used for statistical analysis. The parameters measured during the study were input to calculate the least significant difference (LSD) to signify the difference among tested groups.

Results

Basic seminal parameters distribution of the sterile and healthy men groups

Certain macroscopic and microscopic semen parameters showed some differences between the infertile and healthy control groups as displayed in Table 1.

Correlation between semen quality and TNFα

This current study investigated the correlation between TNFα and semen quality in infertile patient of males. Results obtained are illustrated in Table 2.

Relation between ILA1 and infertility

Levels of IL1A that produces interleukin 1 alpha was determined in serum of both patients and control. Results indicated that IL-1A were significantly higher compared to that

Table 1. Semen microscopical analysis for all groups

Parameters	Fertile healthy group (no=100)	Infertile group (no=100)	p‡
Volume (ml)	2.81±1.01[a]	3.22±1.21[a]	NS
Sperm concentration (million/ml)	77.64±59.22[a]	47.93±36.12[b]	**0.001**
Total motility (%)	71.36±8.38[a]	43.33±19.45[b]	**0.001**
Morphologically normal sperm (%)	37.43±15.66[a]	12.97±2.26[b]	**0.001**

Data were prepared as mean±SD; T-TEST was employed for statistical analysis. [a, b]: Indicates if there is a significant difference between compared data. NS: Not statistically significant; SD: Standard deviation.

Table 2. The correlation between TNF-α and semen quality in patients under study

Variable of semen analysis	n	% (n) of samples with elevated TNF-α conc. (≥20 pg/mL)[a]	p[b]	% (n) of samples with very high TNF-α conc. (≥40 pg/mL)[c]	p[b]	Median (range), pg/mL	p[d]
Sperm count							
<20×10⁶/ml	6	66.7 (4)	NS	33.3 (2)	NS	28.7 (9.2–74.5)	NS
≥20×10⁶/ml	94	46.5 (66)		25.4 (36)		17.7 (0.1–144.4)	
<40×10⁶/ml	26	57.7 (15)	NS	15.4 (4)	NS	25.0 (5.0–71.5)	NS
≥40×10⁶/ml	74	45.1 (55)		27.9 (34)		16.7 (0.1–144.4)	
Progressive motility							
<40%	88	44.7 (42)	NS	23.4 (22)	NS	16.9 (0.3–136.2)	NS
≥40%	12	51.9 (28)		29.6 (16)		20.9 (1.5–142.7)	
Total sperm count per ejaculate							
<96×10⁶[a]	24	50.0 (12)	NS	25.0 (6)	NS	19.9 (5.2–86.0)	NS
≥96×106	55	46.8 (58)		25 (32)		18.7 (0.1–144.4)	
<200×10⁶[c]	10	41.1 (23)	NS	17.9 (10)	NS	15.2 (1–144.4)	NS
≥200×10⁶	11	51.1 (47)		30.4 (28)		20.7 (0.1–142.7)	
Ejaculate volume							
<4.5 mL	92	42.4 (39)	NS	22.8 (21)	NS	15.3 (0.1–144.4)	NS
≥4.5 mL	8	55.4 (31)		30.4 (17)		25.3 (1.5–142.7)	

a, b, are not significant from each other in the statistical sense. c, and d are not significantly different. TNF-α: Tumor necrosis factor-α; NS: Not statistically significant.

in the control group (p=0.025, p<0.001). Table 3 illustrates the results obtained in this study.

Quantification of IL-A1 and TNFα expression using real-time PCR

The current study results revealed that there were significant differences in IL-A1 gene expression between patients and control. Results are shown in Table 4.

Measurement of miRNA – 429 and miRNA – 425 in study groups

The level of gene expression of miRNA – 429 and miRNA – 425 was measured in study groups. Results obtained are shown in Table 5.

Person correlation between varies study parameters

This current study investigated the Pearson correlation between some important factors related to Iraqi infertile patient groups males, correlation coefficient analysis was done including TNF alpha and IL-1 expression fold (2-ΔΔCt), TNF alpha and IL-1 serum level and finally (miR-429 and miR-425) expression fold, the results are shown in Table 6.

Molecular analysis of miRNA – 425 gene

DNA sequence of miRNA – 425 gene was performed for both control and patients and aligned to identify difference among them. The alignment data in shown in Appendix 1.

From alignment and sequence obtained, the similarity matrix was generated as shown in Figure 1.

Table 3. Comparison of IL-1A between patients and healthy group (mean±SD)

Item (pg/ml)	POI group (20)	Control group (22)	p
Serum IL-1α	1.07±1.61	0.48±0.53	0.025*
Follicular fluid IL-1α	3.65±4.16	1.67±1.03	<0.001*

*: p<0.05. IL-1A: Interleukin; SD: Standard deviation, POI: Patients of Interest.

Molecular analysis of miRNA – 429

An equal no. of patients and control DNA sequences were analyzed for DNA alignment, genomic location of the query, and percent identity matrix as shown in Appendix 2, and Figure 2.

Discussion

Socially, the literature on fertility is increasingly elaborating the cultural context of living this experience among individuals [22]. Accessing the medical record for survey and providing medical care and studying social attitudes may not provide a complete picture of who may or may not receive such care. Referencing a cross–sectional study population–based sample women showed that self – the definition of being infertile may provide the key to treatment [23]. However, the factors for infertility are a lot and some of them are either immunologically of genetically determined. Infection with microbes will trigger both Tumor necrosis factor alpha (TNF-α) and interleukin 1 (IL-1) as the key mediators of acute inflammatory reactions. TNF-α shows a wide variety of biologic actions which might interfere

Table 4. Levels of IL1A and TNF-α expression in patients and normal measured using qRT–PCR

IL1A						
Group	**Mean of Ct IL1 alpha**	**Mean of Ct gapdh**	**ΔCt (mean of Ct IL1 alpha-mean of Ct gapdh**	**2-ΔCt**	**Experimental group/ control group**	**Fold of gene expression**
Group 1 (patient)	20.755	22.11	-1.355	2.558	2.558/0.454	5.637
Group 2 (control)	23.25	22.11	1.140	0.454	0.454/0.454	1.000
TNF-α						
Group	**Mean of Ct TNF alpha**	**Mean of Ct gapdh**	**ΔCt (mean of Ct TNF alpha-mean of Ct gapdh**	**2-ΔCt**	**Experimental group/ control group**	**Fold of gene expression**
Group 1 (patient)	22.838	22.11	0.728	0.604	0.604/0.110	5.510
Group 2 (control)	25.3	22.11	3.190	0.110	0.110/0.110	1.000

IL-1A: Interleukin; TNF-α: Tumor necrosis factor-α; qRT–PCR: Quantitative real-time–PCR.

Table 5. Level of expression of miRNA 429 and miRNA – 425 compared to control group

miRNA 429						
Group	**Mean of Ct miRNA 425**	**Mean of Ct RNU6B mean of Ct RNU6B**	**ΔCt (mean of Ct miRNA 425-**	**2-ΔCt**	**Experimental group/ control group**	**Fold of gene expression**
Infertile group	23.23	22.18	1.05	0.482	0.482/0.151	3.19
miRNA – 425						
Group	**Mean of Ct miRNA 425**	**Mean of Ct RNU6B mean of Ct RNU6B**	**ΔCt (mean of Ct miRNA 425-**	**2-ΔCt**	**Experimental group/ control group**	**Fold of gene expression**
Infertile group	21.6	22.18	-0.58	1.494	1.494/0.503	2.96
Fertile (control) group 2	23.1	22.11	0.99	0.503	0.503/0.503	1
Fertile (control) group	24.83	22.11	2.72	0.151	0.151/0.151	1

Table 6. Person correlation among immunological and epigenetic factors measured in this study

	TNF alpha fold	**IL1 A fold**	**TNF alpha serum level**	**IL1 A serum level**	**miRNA429**	**miRNA425**
TNF alpha fold	1	0.032	0.033	0.071	-0.146	0.053
IL1 A fold	0.032	1	-0.088	0.022	-0.153	0.356
TNF alpha serum level	0.033	-0.088	1	-0.018	-0.263	0.103
IL1 A serum level	0.071	0.022	-0.018	1	-0.308	0.457
miRNA429	-0.146	-0.153	-0.263	-0.308	1	0.060
miRNA425	0.053	0.356	0.103	0.457	0.060	1

IL-1A: Interleukin; TNF-α: Tumor necrosis factor-α.

Figure 1. Percent identity matrix of patients and control. The letter C refers to control (healthy) subjects, whereas P refers to patients.

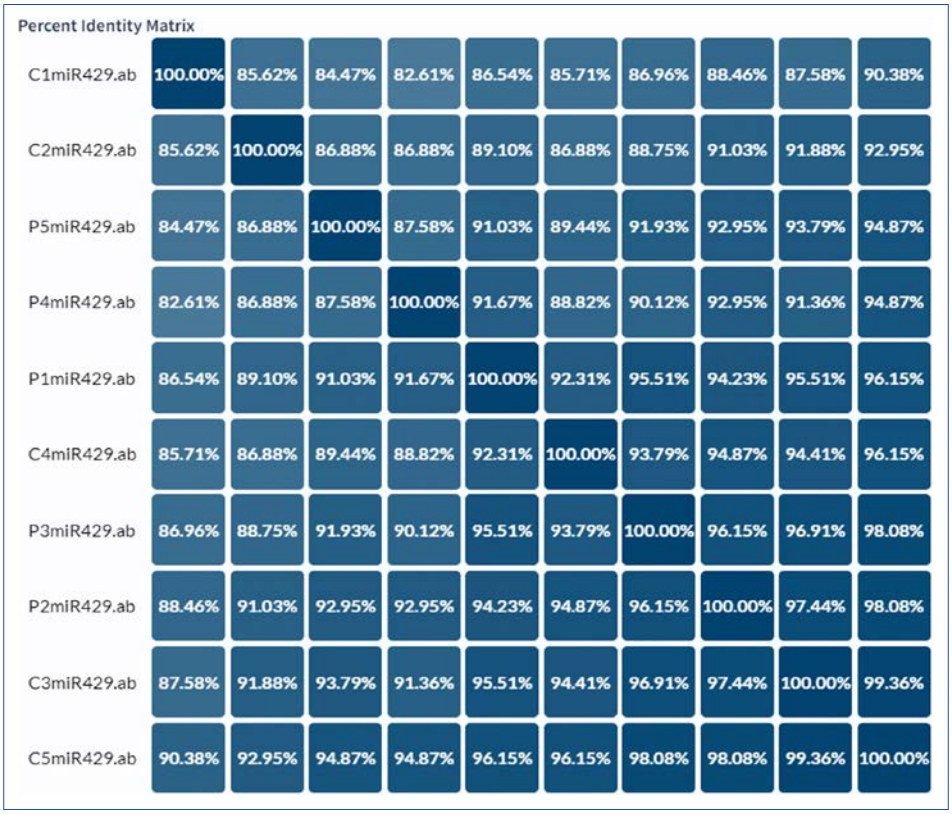

Figure 2. Percent identity matrix of patients and control for miRNA – 429. The letter C refers to control (healthy) subjects, whereas P refers to patients.

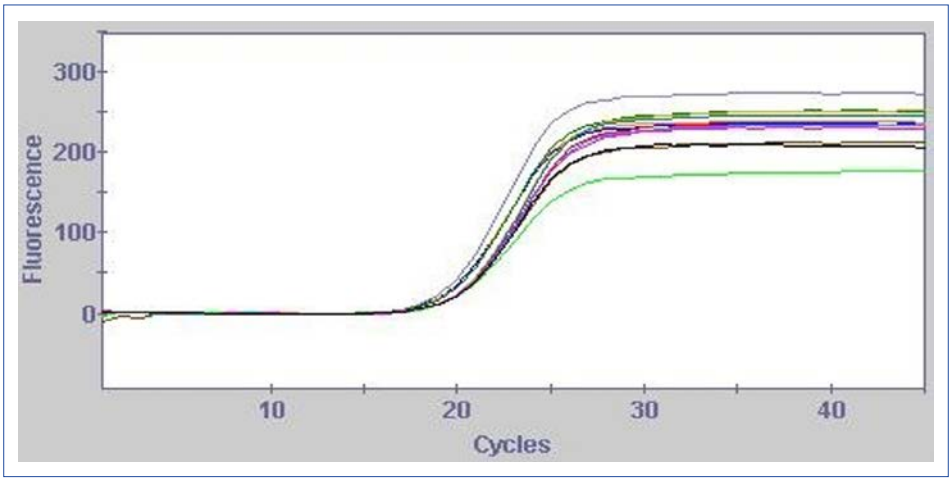

Figure 3. qRT–PCR of miRNA–425, and miRNA–429 in patient group. The figure shows amplification started at Ct 20 indicating high concentration of both miRNAs in blood.
qRT–PCR: Quantitative real-time–PCR.

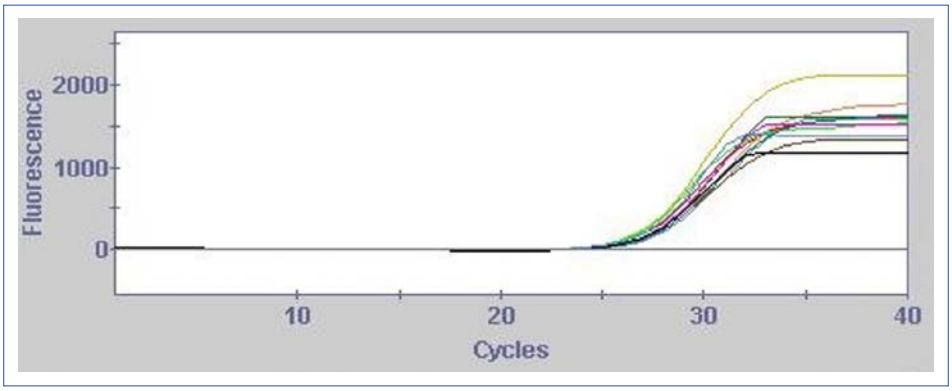

Figure 4. qRT–PCR of miRNA–425, and miRNA–429 in control group. The figure shows amplification started at Ct 30 indicating normal concentration of both miRNAs in blood.

with reproductive functions, like induction of the immune-cascade and chemotactic activity on neutrophils, cytolytic and cytostatic effects on tumor cells, induction of fibroblastic growth, stimulation of collagenase, prostaglandin synthesis, and potential influence on sperm motility and functional capacity. In Tables 2, 3, and 4 the correlation between TNFα and semen characteristics was determined. We found a decrease in semen criteria with elevation of TNFα concentration in blood that included semen quality, motility, and size of ejaculate. This may be attributed to that macrophages in testicles may produce cytokine-guided paracrine regulatory influence on Leydig cell function as an example of immune-endocrine interactions in the male reproductive system [24]. Interleukin 1 also is an important mediator of immunologic and pathologic responses to stress, infection, and antigenic challenge. In Table 6, it acts synergistically with other factors in the activation and differentiation of B- cells to immunoglobulin secreting cells, and it stimulates the activation and differentiation of natural killer (NK) cells, fibroblasts, and thymocytes. It acts anti-proliferatively, increases the cytotoxicity of macrophages. It has a variety of effects in the brain, such as induction of fever as an endogenous pyrogen, alteration of slow-wave sleep, and an important role in modulating reproductive functions through stimulation of corticotropin-releasing factor and ACTH secretion and further influence on the hypothalamic-pituitary-gonadal axis [25].

Since both miRNA- 425, and miRNA – 429 are both subject of interest and are major affecters in levels of TNFα and IL 1A, their expression rate was measured in both groups under study. An elevation was measured in patient group that reached 5 folds compared to control group as an indicator for increased need of the body for the effect of these miRNAs to take place in reduction of inflammation. Figure 3, we noticed that Ct value began at an early stage (Ct20) whereas the reaction began at Ct 25 in control group as shown in Figure 4.

The study extended further toward sequencing of both miRNA – 425 and miRNA – 429 genes form which we were able to identify variations that affected the role of the gene. In patients with infertility problem, the high level of both miRNAs may be attributed to epigenetic factors appeared in two points: first multiple SNPs were identified in patients that are listed in details in Table 7, and Table 8 respectively.

Table 7. DNA SNPs identified in miRNA – 425 gene

Variant	Class	Location	Alleles	Ambiguity code	Source	Consequence
rs769996937	SNP	3:49020120	C/G	S	dbSNP	Intron variant
rs1265172024	SNP	3:49020128	A/C	M	dbSNP	Intron variant
rs1250721195	SNP	3:49020144	C/G	S	dbSNP	Intron variant
rs759930741	indel	3:49020146-49020179	CCGAAAGAGCACTG	Non	dbSNP	Mature miRNA variant
rs759930741	indel	3:49020146-49020179	CCGAAAGAGCACTG	Non	dbSNP	Mature miRNA variant
rs368663793	SNP	3:49020225	A/G	R	dbSNP	Non coding transcript exon variant
rs1302210268	SNP	3:49020229	G/A	R	dbSNP	Non coding transcript exon variant
rs745809471	SNP	3:49020235	C/T	Y	dbSNP	Intron variant

SNPs: Single nucleotide polymorphism.

Table 8. DNA SNPs identified in miRNA – 429 gene

Variant	Class	Location	Alleles	Ambiguity code	Source	Consequence
rs375624023	SNP	1:1169013	G/A/C	V	dbSNP	non coding transcript exon variant
rs749785386	SNP	1:1169015	T/C	Y	dbSNP	non coding transcript exon variant
rs370935426	SNP	1:1169058	T/C	Y	dbSNP	mature miRNA variant
rs375039921	SNP	1:1169068	T/C	Y	dbSNP	mature miRNA variant
rs368678282	SNP	1:1169075	G/A	R	dbSNP	mature miRNA variant

Second, variation in folds of normal and patients miRNAs is associated with RNA decay mechanism that is mainly depend upon the free energy of RNAs and configuration. In this case the configuration and free energy of both miRNAs were measured (Fig. 5).

Results for thermodynamic ensemble prediction is that the free energy of the thermodynamic ensemble is -87.66 kcal/mol. The frequency of the MFE structure in the ensemble is 0.01%.

The ensemble diversity is 50.04 for healthy subjects. Whereas results for thermodynamic ensemble prediction for patients were that the free energy of the thermodynamic ensemble is - 84.29 kcal/mol and the frequency of the MFE structure in the ensemble is 0.04 %. The ensemble diversity is 55.55.

With such data it can be said that the mechanism for RNA decay is different between the studied group estimating a longer half-

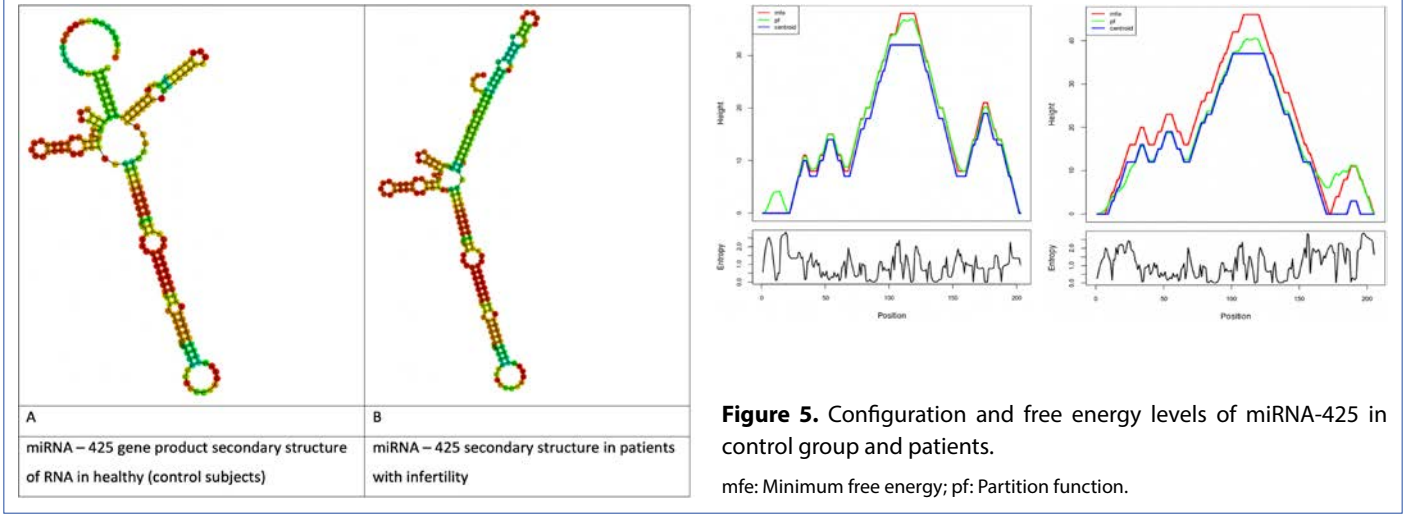

Figure 5. Configuration and free energy levels of miRNA-425 in control group and patients.

mfe: Minimum free energy; pf: Partition function.

life of miRNAs in patients than healthy which increases the concentration of these molecules in blood initiating a clinical case.

Conclusion

In patients with infertility problem, TNFα was found to in high levels accompanied with IL 1A indicating a case of inflammation affected semen quality, but with high levels of miRNA 425 and 529, symptoms may be mimicked showing no signs of infection, clinical case, and drive patients to seek other ways of treatment. Thus, it is highly recommended that such immunological factors and epigenetic factors should be measured to provide a proper way of treatment.

Appendix Files: https://jag.journalagent.com/ijmb/abs_files/IJMB-92668/IJMB-92668_(3)_Appendixes.pdf

Ethics Committee Approval: The study was approved by The Al-Nahrain University Biotechnology Research Center Ethics Committee (No: M. B 52, Date: 02/01/2022).

Authorship Contributions: Concept – F.T.S.; Design – G.J.A.; Supervision – R.S.R.; Materials – M.K.A.S.; Data collection &/or processing – R.S.R.; Analysis and/or interpretation – R.N.A.; Literature search – S.A.J.; Writing – R.N.A.; Critical review – M.A.A.

Conflict of Interest: The authors declare that there is no conflict of interest.

Use of AI for Writing Assistance: No AI technologies utilized.

Financial Disclosure: The authors declared that this study has received no financial support.

Peer-review: Externally peer-reviewed.

References

1. Greil A, McQuillan J, Slauson-Blevins K. The social construction of infertility. Sociol Compass 2011;5(8):736–46. [CrossRef]
2. Öztekin Ü, Caniklioğlu M, Sarı S, Selmi V, Gürel A, Işıkay L. Evaluation of male infertility prevalence with clinical outcomes in Middle Anatolian Region. Cureus 2019;11(7):e5122. [CrossRef]
3. Marzec-Wróblewska U, Kamiński P, Lakota P, Szymański M, Wasilow K, Ludwikowski G, et al. Zinc and iron concentration and SOD activity in human semen and seminal plasma. Biol Trace Elem Res 2011;143(1):167–77. [CrossRef]
4. Chyra-Jach D, Kaletka Z, Dobrakowski M, Machoń-Grecka A, Kasperczyk S, Birkner E, Kasperczyk A. The associations between infertility and antioxidants, proinflammatory cytokines, and chemokines. Oxid Med Cell Longev 2018;2018:8354747. [CrossRef]
5. Sarkar S, Gupta P. Socio-demographic correlates of women's infertility and treatment seeking behavior in India. J Reprod Infertil 2016;17:123.
6. Krausz C, Riera-Escamilla A. Genetics of male infertility. Nat Rev Urol 2018;15:369–384. [CrossRef]
7. Hashem R, Al-Obaidi ZF, Samawi FT, Baher H. Analysis of anti-apoptotic protein (Bcl-xl) levels and mRNA expression in infertile patients. Afr J Reprod Health 2022;26(10):63–71.
8. Ferlin A, Arredi B, Speltra E, Cazzadore C, Selice R, Garolla A, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: A 10-year experience in Italy. J Clin Endocrinol Metab 2007;92:762–70. [CrossRef]
9. Krausz C, Giachini C. Genetic risk factors in male infertility. Arch Androl 2007;53(3):125–33. [CrossRef]
10. Gianotten J, Lombardi MP, Zwinderman AH, Lilford RJ, van der Veen F. Idiopathic impaired spermatogenesis: Genetic epidemiology is unlikely to provide a short-cut to better understanding. Hum Reprod Update 2004;10(6):533–9. [CrossRef]
11. Boyd SD. Everything you wanted to know about small RNA but were afraid to ask. Lab Investig 2008;88:569–78. [CrossRef]
12. Salas-Huetos A, James ER, Aston KI, Carrell DT, Jenkins TG, Yeste M. The role of miRNAs in male human reproduction: A systematic review. Andrology 2020;8:7–26. [CrossRef]
13. Saito A, Horie M, Ejiri K, Akira A, Katagiri S, Maekawa S, et al. MicroRNA profiling in gingival crevicular fluid of periodontitis-a pilot study. FEBS Open Bio 2017;7:981–94. [CrossRef]
14. Arunkumar G, Deva Magendhra Rao AK, Manikandan M, Prasanna Srinivasa Rao H, Subbiah S, Ilangovan R, et al. Dysregulation of miR-200 family microRNAs and epithelial-mesenchymal transition markers in oral squamous cell carcinoma. Oncol Lett 2018;15:649–57. [CrossRef]
15. Wang P, Cao J, Liu S, Pan H, Liu X, Sui A, et al. Upregulated microRNA-429 inhibits the migration of HCC cells by targeting TRAF6 through the NF-κB pathway. Oncol Rep 2017;37:2883–90. [CrossRef]
16. Kawasaki H, Amano H. Anti-inflammatory role of microRNA-429 in human gingival epithelial cells-inhibition of IL-8 production through direct binding to IKKβ mRNA. Mol Med Rep 2021;24(2):581. [CrossRef]
17. Gao Y, Yin Y, Xing X, Zhao Z, Lu Y, Sun Y, et al. Arsenic-induced anti-angiogenesis via miR-425-5p-regulated CCM3. Toxicol Lett 2016;254:22–31. [CrossRef]
18. Liu L, Zhao Z, Zhou W, Fan X, Zhan Q, Song Y. Enhanced expression of miR-425 promotes esophageal squamous cell carcinoma tumorigenesis by targeting SMAD2. J Genet Genomics 2015;42:601–11. [CrossRef]
19. Nakagawa R, Muroyama R, Koike K, Saeki C, Ito S, Morimoto S, et al. Decreased mir-425 induced inflammatory cytokine production via N-RAS upregulation in CD4+ T cells of primary biliar cholangitis. J Hepatol 2016;64(Suppl):S643–4. [CrossRef]
20. He B, Li T, Guan L, Liu FE, Chen XM, Zhao J, et al. CTNNA3 is a tumor suppressor in hepatocellular carcinomas and is inhibited by miR-425. Oncotarget 2016;7:8078–89. [CrossRef]
21. Demir M, Ozdem S. Evaluation of total antioxidant capacity in human seminal plasma. Int J Med Biochem 2022;5(1):54–9. [CrossRef]
22. Parry DC; Work, Leisure, and Support Groups. An examination of the ways women with infertility respond to pronatalist ideology. Sex Roles 2005;53(5–6):337–46. [CrossRef]
23. White L, McQuillan J, Greil AL. Explaining disparities in treatment seeking: The case of infertility. Fertil Steril 2006;85(4):853–7. [CrossRef]
24. Huleihel M, Lunenfeld E. Involvement of intratesticular IL-1 system in the regulation of Sertoli cell functions. Mol Cell Endocrinol 2002;187:125–32. [CrossRef]
25. Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, et al. Cytokine-associated emotional and cognitive disturbances in humans. Arch Gen Psychiatry 2001;58:445–52. [CrossRef]