Original Article

Association Between Vitamin D Levels, miRNA Expression, and Sperm Parameters in Infertile Men

Impact of Vitamin D and Sperm miRNA on Sperm Parameters

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ABSTRACT

Objective: To examine the impact of vitamin D levels and the miRNA profiles of miR-34b, miR-19a-3p, and miR-122 on sperm parameters of men with male-factor infertility.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: This study was conducted at the Department of Urology, Teaching Hospital of Hilla, Hilla City, Iraq, between June 2024 and August 2025.

Methods: The current case control study was conducted involving 75 infertile and 75 fertile men. Semen samples were collected through masturbation after a time of abstinence and sperm analysis was performed on computer-assisted semen analysis (CASA). Serum vitamin D levels were measured by chemiluminescence, and RT-qPCR approach was used to investigate expression profiles of sperm-born miR-34b, miR-19a-3p, and miR-122.

Results: Infertile men were presented with significantly lower semen quality, including low semen volume, reduced sperm concentration, and lower motility. Infertile men were also presented with lower levels of vitamin D compared to control, ($p < 0.001$). MiRNA expression revealed miR-34b was reduced in infertile men, whereas miR-19a-3p and miR-122 levels were found upregulated. Examining correlation between vitamin D and miRNA expression revealed inverse correlation with miR-19a-3p ($r = –0.267$, $p < 0.001$), while no significant relationships were observed for miR-34b or miR-122. ROC analysis displayed moderate diagnostic potential for miR-19a-3p (AUC = 0.696, $p < 0.001$) and miR-122 (AUC = 0.614, $p = 0.015$).

Conclusion: Vitamin D deficiency and dysregulation of specific sperm miRNAs are closely linked to reduced sperm motility and viability. The inverse relationship between vitamin D and miR-19a-3p indicates a regulatory function through which vitamin D may control expression of miR-19a-3p and preserve normal sperm function. These results support the use of sperm-borne miRNAs as promising biomarkers for the evaluation of male infertility.

Key Words: Vitamin D, microRNA, sperm parameters, male infertility.

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INTRODUCTION

Male fertility mostly depends on the quality of the produced spermatozoa.[1] Sperm with good morphology, motility and viability could predict successful fertilization and pregnancy outcomes, while those with impaired sperm quality usually suffer from infertility and pregnancy loss.[2] Vitamin D was reported to play an important role in sperm cell formation and spermatozoa maturation.[3] Patients with vitamin D deficiency found with reduced semen quality and low pregnancy outcomes.[4] Vitamin D was also found with significant antioxidant properties against oxidative stress–induced sperm damage.[5] Higher reactive oxygen species (ROS) in semen reported to negatively impact the integrity of the sperm membrane, sperm proteins and DNA, as well as resulting in excessive lipid peroxidation, leading to male infertility through impacting sperm motility, viability, and morphology.[6] Recent findings suggest that higher ROS concentrations in semen, along with impaired sperm quality, are attributed to decreased serum levels of vitamin D.[5]

Spermatogenesis and sperm maturation processes are controlled by extensive sets of microRNAs (miRNAs), which regulate gene expression essential for sperm development.[7] Recent clinical studies associated abnormal miRNA expression profiles with reduced sperm functions and lower reproductive outcomes.[8] Several miRNAs were investigated, and many have been suggested as potential biomarkers for male fertility.[9] MiR-34b expression showed a positive association with sperm concentration and improved pregnancy outcomes.[10] MiR-19a/b-3p expression was

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found upregulated in patients with oligoasthenozoospermia, and higher miR-122 expression was associated with improved sperm concentration, which when downregulated resulted in impaired spermatogenesis.[11, 12] Since vitamin D and miRNAs are both recognized for their roles in maintaining male reproductive health and preventing infertility, their potential interaction in the context of male fertility remains mostly unknown. Therefore, this study was designed to examine the impact of vitamin D levels and expression profiles of miR-34b, miR-19a-3p, and miR-122 on sperm parameters of men with male-factor infertility.

METHODS

Patients and healthy volunteers' recruitment was conducted at the Department of Urology, Teaching Hospital of Hilla, Hilla City, Iraq, between June 2024 and August 2025. The protocol of the study was granted by the Ethics Committee of the College of Medicine, University of Al-Qadisiyah (Reference no. 77/315) and conducted in accordance with the Declaration of Helsinki. Each participant was informed about the study's objectives and informed consents were collected for these regards. In brief, 75 patients with male-factor infertility were selected according for being unable to achieve pregnancy for at least 12 months following regular unprotected intercourse, and presenting with at least one abnormality related to semen quality (low sperm count, reduced motility, or poor morphology), as defined by World Health Organization (WHO) reference values. The control group consisted of 75 men with proven fertility, normal semen parameters, and absence of history related to infertility or other diseases, such as sexually transmitted diseases, varicocele, cystic fibrosis, hormonal imbalance, obstructive azoospermia, or chemotherapy. Both groups were also selected based on the absence of significant female factor infertility, as well as the absence of systemic illnesses, endocrine disorders, prior medications or supplements that could impact levels of vitamin D or oxidative stress. To control any confounders, the two groups were paired in accordance with age and body-mass index.

Semen was collected through masturbation in a sterile container after three to five days of abstinence. After incubation for half an hour at 37 °C, sperm motility parameters were assessed using a computer-assisted semen analysis (CASA). In brief, (10 µL) of the semen sample was loaded into the counting chamber of the CASA apparatus (ASCEN technology, China), and percentages of progressive motility, non-progressive motility, as well as immotile sperm were reported for all samples.

For miRNA expression profile analysis, liquefied semen samples were centrifuged at 3000×g for 10 minutes to form a pellet of the sperm. The sperm pellet was then washed twice by resuspending it in phosphate-buffered saline (PBS) and centrifuging before adding 200 µL of QIAzol reagent (Qiagen, Germany). An equal volume of chloroform was then added to the solution and centrifuged to separate phases followed by collection of the upper RNA layer in fresh tubes and mixing with an equal volume of isopropanol, before centrifugation, removing supernatant, and washing the RNA pellet in 70% ethanol. RNase-free water was used to dissolve the RNA pellet, and total RNA yield and purity were assessed on a NanoDrop spectrophotometer (Thermo Scientific, UK).

Reverse transcription and complementary DNA (cDNA) production were performed with the use of Qiagen miScript II RT Kit (Qiagen, Germany), according to the manufacturer instructions. The reaction mix included 50 ng of total RNA, miScript HiSpec Buffer, nucleotides, reverse transcriptase, and a mix of oligo-dT and random primers. Tubes with the reaction mix were then incubated at 37 °C for 60 minutes to generate the cDNAs before incubation at 95 °C for 5 minutes to inactivate the reverse transcription enzyme. cDNA products were used as templates for quantitative PCR, which was carried out in 96-well plates on a real-time PCR system (Applied Biosystems StepOnePlus, USA), following instruction protocols of miScript SYBR Green PCR kit (Qiagen, Germany). The PCR thermal profile for SYBR Green assays was 15 minutes on 95 °C, 10 seconds on 95 °C (45 cycles), 30 seconds on 56 °C, then 30 seconds on 70 °C. miRNA's relative expressions were quantified at the end of the cycle.

For measurement of vitamin D, (5 mL) peripheral blood was collected in serum separation tubes. Serum samples were then aliquoted into labeled tubes followed by measuring 25-hydroxyvitamin D [25(OH)D] using a chemiluminescence immunoassay on an automated analyzer with a commercially available kit on Cobas e411 analyzer (Roche, Germany). All samples were run in duplicate and results were reported in nanograms per milliliter (ng/mL).

Statistical analysis was conducted on SPSS version 28 (IBM, USA). Independent sample t-test was used to compare variables between findings of groups of the study. Pearson correlation (r) was applied to examine relationships between miR-34b, miR-19a-3p, and miR-122 expression profiles, semen parameters, and serum vitamin D levels. Curve analysis (ROC) was used to assess the potential applications of these miRNAs as markers that could predict male infertility. Statistical significance was set at $p < 0.05$.

RESULTS

Infertile patients showed lower volume of semen (2.05 ± 0.92 ml) compared to the control (3.28 ± 1.18 ml; $p < 0.001$), lower sperm concentration (16.03 ± 5.78 million/ml) vs (53.84 ± 20.19 million/ml; $p < 0.001$) of the control, lower total sperm motility p

(25.85 ± 10.38%) compared to controls (62.91 ± 22.89%; $p < 0.001$), and lower progressive motility (15.15 ± 7.64% vs. 47.60 ± 15.55%; $p < 0.001$). Infertile patients were also found with reduced serum vitamin D (15.04 ± 5.75 ng/ml) compared to the control group (23.75 ± 7.92 ng/ml; $p < 0.001$). Table 1.

Table No.1: Comparison of semen parameters, serum vitamin D levels, and miRNA expression (miR-34b, miR-19a-3p, miR-122) between infertile patients and healthy fertile controls.

Characteristics	Healthy controls		Patients		P value
	Mean	S.D	Mean	S.D	
Age (years)	30.83	7.49	31.15	7.82	0.798
Volume (ml)	3.28	1.18	2.05	0.92	<0.001
Concentration (Million/ml)	53.84	20.19	16.03	5.78	<0.001
Total motility (%)	62.91	22.89	25.85	10.38	<0.001
Progressive motility (%)	47.60	15.55	15.15	7.64	<0.001
Vitamin D (ng/ml)	23.75	7.92	15.04	5.75	<0.001
miR-34b	2.70	1.53	1.08	0.65	<0.001
miR-19a-3p	0.80	0.43	1.29	0.71	<0.001
miR-122	1.38	0.70	1.69	0.87	0.017

Table No.2. Pearson correlation coefficients between microRNA expression levels (miR-34b, miR-19a-3p, miR-122) and semen parameters and serum vitamin D levels.

		miR-34b	miR-19a-3p	miR-122
Volume	r	0.219	-0.285	-0.165
	P-value	0.007*	<0.001*	0.043*
Concentration	r	0.480	-0.348	-0.171
	P-value	<0.001*	<0.001*	0.037*
Total motility	r	0.446	-0.310	-0.113
	P-value	<0.001*	<0.001*	0.167
Progressive motility	r	0.433	-0.286	-0.162
	P-value	<0.001*	<0.001*	0.048*
Vitamin D	r	0.128	-0.267	-0.082
	P-value	0.119	<0.001*	0.318

*Significant association

Table No.3: The table shows the Area Under the Curve (AUC) for the Receiver Operating Characteristic (ROC) analysis, p-values, and 95% Confidence Intervals (CI) for each microRNA was investigated.

	AUC	P value	95% CI	
			Lower Bound	Upper Bound
miR-34b	0.208	<0.001	0.134	0.283
miR-19a-3p	0.696	<0.001	0.610	0.781
miR-122	0.614	0.015	0.524	0.705

Results from MicroRNAs expression analysis showed significant differences in expression levels between groups. Infertile men exhibited significantly lower expression of miR-34b (1.08 ± 0.65) in comparison to controls (2.70 ± 1.53; $p < 0.001$). Conversely, miR-19a-3p expression was significantly higher in the infertile group (1.29 ± 0.71) than in controls (0.80 ± 0.43; $p < 0.001$). miR-122 was also significantly higher in infertile patients (1.69 ± 0.87) relative to fertile men (1.38 ± 0.70; $p = 0.017$). Table 1.

The association between miRNA expression and sperm parameters was also examined and showed a significant positive correlation between miR-34b expression and seminal volume ($r = 0.219$), concentration ($r = 0.480$), overall motility ($r = 0.446$), and progressive motility ($r = 0.433$). However, miR-19a-3p was inversely correlated with seminal volume ($r = –0.285$), concentration ($r = –0.348$), motility ($r = –0.310$), and progressive motility ($r = –0.286$). The inverse correlation was also evident in miR-122 expression profile, where miR-122 was negatively associated with volume ($r = –0.165$), concentration ($r = –0.171$), and progressive motility ($r = –0.162$). With respect to the correlation with vitamin D, neither miR-34b nor miR-122 was significantly associated with vitamin D levels. However, miR-19a-3p levels showed a significant inverse association with vitamin D ($r = –0.267$, $p < 0.001$). Table 2.

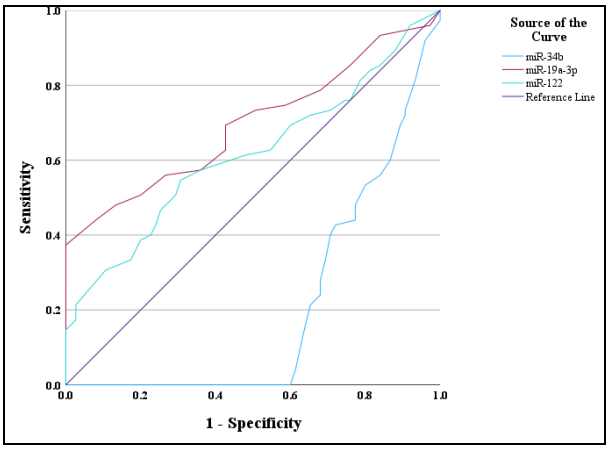

Figure No.1: Receiver Operating Characteristic (ROC) curves for miR-34b, miR-19a-3p, and miR-122 in distinguishing infertile men from fertile controls.

The diagnostic performance of the three microRNAs through the implementation of the ROC curve analysis showed the area under the curve (AUC) of 0.208 ($p < 0.001$) for miR-34b, miR-19a-3p with an AUC of 0.696 ($p < 0.001$), and miR-122 with an AUC of 0.614 ($p = 0.015$). The results suggest that these miRNA expression profiles may serve as molecular indicators of impaired sperm quality in infertile men. Table 3 and Figure 1.

DISCUSSION

In this study which included infertile men, we found that normal levels of vitamin D and semen miRNA can significantly maintain semen quality and male reproductive function. Studies linked vitamin D to better semen quality and normal androgen levels, while lower vitamin D levels were presented in cases with gonadal insufficiency, decreased sperm count, and infertility.[1] Vitamin D insufficiency was also associated with lower live birth rates and lower outcomes from assisted reproductive technologies.[4] Sufficient vitamin D levels are important to maintain the quality of semen parameters, and the observed inverse association, by the current analysis, between vitamin D and sperm parameters aligns with these reports.

Our infertile patients also displayed altered expression profiles of miRNA-19a/b-3p, miR-34b and miR-122. miR-19a-3p levels were shown to be higher than control, combined with reduced vitamin D levels. Those with higher miR-19a/b-3p levels in sperm cells were identified as infertile men with oligoasthenozoospermia, presenting with reduced sperm count, motility, and sperm morphology.[11] Our finding indicate an important role for miR-19a-3p in reproductive health where higher levels of miR-19a-3p could negatively impact male fertility.[7]

Our findings regarding miR-34b and miR-122 are in line with previous studies, showing significant reductions in male patients which were associated with reduced semen quality compared to healthy individuals. Abdolmabood et al. reported downregulation of miR-34b in infertile men, which was found to be indicative of impaired spermatogenesis.[13] Lower miR-34b levels were also linked to reduced sperm motility and sperm concentration.[14] Yeh et al. showed that higher miR-34b expression was positively correlated with implantation, pregnancy, and live-birth rates.[15] Similarly, studies have also associated the elevated miR-122 levels with enhanced male reproductive competence. Joshi et al.[9] provided evidence for a link between miR-122 and male infertility, and Mokánszki et al.[10] confirmed a positive correlation between miR-122 and sperm concentration, while Tomic et al.[12] correlated lower miR-122 levels to teratozoospermia in infertile men. However, serum levels of vitamin D did not show a significant correlation with seminal levels of miR-122, suggesting no impact of vitamin D levels on these miRNAs in sperm.

In contrast, the observed inverse relationship between vitamin D and miR-19a-3p by the current analysis holds considerable significance in the context of male reproduction. It may provide an insight into a novel mechanism where vitamin D directly or indirectly regulates expression of miR-19a-3p, limiting inhibitory actions of the miRNA on key regulatory genes which are controlling spermatogenesis and improving semen quality. Recent studies demonstrated a role for vitamin D in regulating expression of a wide range of miRNA profiles across various physiological and pathological contexts.[16] Although evidence regarding the relationship between vitamin D and miR-19a-3p is very limited in the literature, findings from a recent study point toward a possible regulatory mechanism of vitamin D supplementation on improving the clinical signs of allergic rhinitis and reduce miR-19a levels in B cells, an effect that was linked to lower IgE levels, reduced Th2 cytokines, and increased interleukine-10.[17] The impact of vitamin D on miR-19a expression levels is still not completely understood. However, recent evidence suggests that sufficient levels of vitamin D could restrain aberrant miRNA expression, thereby preserving normal physiological processes.[18] One of these suggested routes is through VDR signaling pathways, where VDR was shown to influence miRNA expression levels in various conditions.19 A study reported that supplementation of vitamin D in animal models with PCOS showed higher expression of specific miRNAs that are required for oogenesis while decreasing other aberrant miRNAs in the ovaries.[20] Those animal models were also presented with significant improvements in number of molecular markers related to ovarian dysfunction.[20] Additional studies described that certain miRNAs can also modulate expression of VDR, and low levels of miR-19a-3p could permit vitamin D to promote expression

of genes that are responsible for maintaining male fertility.[2] It is clear from our findings that sufficient vitamin D can favorably modify genetic expression in reproductive tissues to prevent infertility by creating an environment where normal expression profiles of pro-fertility genes are maintained.

Vitamin D is also known for its effective anti-inflammatory and antioxidative properties, which are widely recognized for regulating extensive cellular activities.[18] Hence, sufficient levels of vitamin D plus maintaining normal miRNA expressions could effectively alleviate excessive inflammatory or oxidation factors that generate apoptotic signaling in the sperm of infertile individuals.[3]

The findings first provide insight into the mechanisms that govern male reproductive health through endogenous levels of vitamin D and sperm miRNA expression profiles. Our findings align with recent reports supporting the roles of vitamin D and sperm miRNAs in maintaining normal sperm quality, in addition to the potential applications of sperm miRNAs and vitamin D levels as valuable biomarkers for male fertility. Larg cohort studies to validate these results are required, which could help in providing a better understanding of the molecular mechanisms that govern sperm pathophysiology.

CONCLUSION

The study showed that altered expression profiles of miR-34b, miR-19a-3p, and miR-122 were significantly associated with reduced normal sperm characteristics in infertile patients. The study also revealed an inverse relationship between vitamin D and miR-19a-3p, suggesting a mechanism that could contribute to enhanced male reproductive health through the regulatory actions of vitamin D on miR-19a-3p expression. The findings also described a potential application of miR-19a-3p, miR-34b, and miR-122 levels as viable biomarkers for assessing male fertility.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Alaa Hachem, Asaad Al-Shouk
Drafting or Revising Critically:	Alaa Hachem, Asaad Al-Shouk
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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