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Differential Expression of miR-99a and miR-143 in Sperm and Follicular Fluid Samples from Couples Undergoing ART: Implications for Embryo Quality and Human Infertility

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Abstract

Follicular fluid (FF) and semen contain microRNAs (miRNAs), important regulators of reproductive physiology and potential non-invasive biomarkers of infertility. This study examined miR-99a and miR-143 expression in infertile couples undergoing assisted reproductive technology (ART) and their correlations with reproductive characteristics in sperm and FF. Paired FF and semen samples were collected from 36 couples. Quantitative real-time PCR was used to quantify normalized miRNA expression, with G6PD as the internal reference gene. Non-parametric statistical tests were applied to assess correlations between clinical, hormonal, and embryological data, as well as to perform comparative analyses between sexes and tissue types. Significant differences in miR-99a and miR-143 expression were observed between sperm and follicular fluid samples, with higher expression in male than female samples ($p < 0.001$ and $p = 0.004$, respectively). MiRNA expression was significantly associated with selected female reproductive parameters, particularly AMH and FSH levels, whereas no correlations were identified with LH, sperm motility or total sperm count. Moreover, a significant correlation was observed between miR-143 expression and embryo quality, with

higher levels in embryos of superior morphology ($p = 0.046$). These findings indicate differential expression of miR-99a and miR-143 between sperm and follicular fluid and suggest their involvement in early embryonic development and gametogenesis. Specifically, in ART, miR-143 may represent a potential marker of embryo quality, although this finding should be interpreted cautiously due to the limited sample size. Further studies are needed to validate these findings and clarify the underlying mechanisms.

Keywords: miR-99a, miR-143, follicular fluid, sperm, microRNAs, embryo quality, assisted reproduction.

Introduction

A major global health problem, infertility affects 8–12% of couples who are of reproductive age globally. Male factors contribute to approximately 50% of infertility cases overall and account for 20–30% as the primary cause, underscoring the need to assess both partners in clinical practice [1,2]. A significant percentage of infertility cases remain unexplained despite advancements in assisted reproductive technologies (ART), underscoring the need for better genetic indicators and a deeper comprehension of reproductive biology [3].

Small non-coding RNAs, microRNAs (miRNAs) control post-transcriptional gene expression and are essential for various biological functions, including reproduction [4,5]. In the ovary, follicular fluid miRNAs contribute to folliculogenesis, steroidogenesis, oocyte competence and intercellular communication [6,7].

Notably, miR-99a and miR-143 are important regulators of ovarian function. MiR-99a targets the insulin-like growth factor 1 receptor (IGF-1R) to influence granulosa cell proliferation and death, leading to aberrant follicular formation [8]. Similar to this, miR-143 is crucial for granulosa cell physiology because it inhibits the generation of estradiol generated by follicle-stimulating hormone (FSH) and targets KRAS, a key element of the MAPK signaling pathway [9,10]. These findings demonstrate the role of miRNAs in several signaling pathways crucial for ovarian homeostasis, including PI3K/AKT and MAPK.

In the male reproductive system, miRNAs are critical regulators of spermatogenesis and sperm function. Altered miRNA expression can disrupt key molecular pathways, affecting meiosis, chromatin remodeling, and germ cell differentiation [11,12]. Furthermore, members of the miR-99 family exhibit altered expression in the testes of men with non-obstructive azoospermia, suggesting their involvement in male infertility [14,15]. In addition, miR-143 has been associated with sperm maturation and function, further supporting the role of miRNAs as regulators of male reproductive capacity [16].

This study evaluated miR-99a and miR-143 expression in paired sperm and follicular fluid samples from infertile couples and examined their associations with semen quality, hormonal profiles and embryo development, while accounting for tissue-specific and sex related variation.

Materials and Methods

Study Population

All samples were obtained from the Assisted Reproduction Unit of “Attikon” University Hospital (Athens, Greece). The study protocol was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

A total of 36 infertile couples (36 women and 36 men) undergoing ART (IVF/ICSI) between September 2024 and January 2025 were included. Female inclusion criteria were: age 25–45 years, regular menstrual cycles (21–35 days), normal hormonal profile accord-

Table 1. Demographic and clinical characteristics of the included women.

Variables	N	min	max	Mean	SD
AGE	36	26	46	39.6	4.82
AMH	36	0.200	14.000	1.95	2.14
BMI	36	20.4	39.1	27.76	3.74
Duration of Infertility(years)	36	2.0	5.0	2.79	0.86
FSH	36	0.490	18.300	7.630	3.551
LH	36	0.100	11.000	5.068	2.212
Estradiol(E2)	36	8.800	55.100	37.346	8.083
PRL	36	5.900	84.800	27.395	20.43
Retrieved Oocytes	36	1.00	16.00	5.44	4.28
Smoking	N (%)				
No	20/36 (55.6)				
Yes	16/36 (44.4)				

Continuous variables are presented as mean $\pm$ SD, while smoking status is presented as number (%).

Table 2. Male demographic and semen characteristics.

Variables	N	min	max	Mean	SD
Age	36	26	53	41.70	5.19
Total Sperm Motility	36	0.240	0.890	0.425	0.118
Total Sperm Count	36	1.200	252.00	53.60	56.00
Smoking	N (%)				
No	13/36 (36.1)				
Yes	23/36 (63.9)				

Data are presented as mean $\pm$ SD for continuous variables and as n (%) for categorical variables.

ing to WHO guidelines, and the presence of both ovaries. All participants had a history of at least two years of infertility and at least one previous unsuccessful IVF attempt.

Women underwent a short GnRH agonist protocol for controlled ovarian stimulation (COS). No participant had received hormonal treatment for at least three months prior to study inclusion. Male participants provided semen samples following 3–5 days of sexual abstinence. All participants were screened for infectious diseases prior to sample collection. Baseline demographic and clinical characteristics, including age, body mass index (BMI), and duration of infertility, were recorded before initiation of ovarian stimulation. Female and male characteristics are presented in Tables 1 and 2, respectively.

Ovarian stimulation was performed using individualized gonadotropin (Gn) protocols based on ultrasound monitoring and hormonal assessment. Dosage and duration were adjusted until the day of human chorionic gonadotropin (hCG) administration.

On the day of oocyte retrieval, FF samples were collected following transvaginal aspiration. Purified

Table 3. Distribution of embryo quality categories.

Embryos Quality	N (%)
Poor	3/36 (8.3)
Average	15/36 (41.7)
Good	13/36 (36.1)
Excellent	5/36 (13.9)

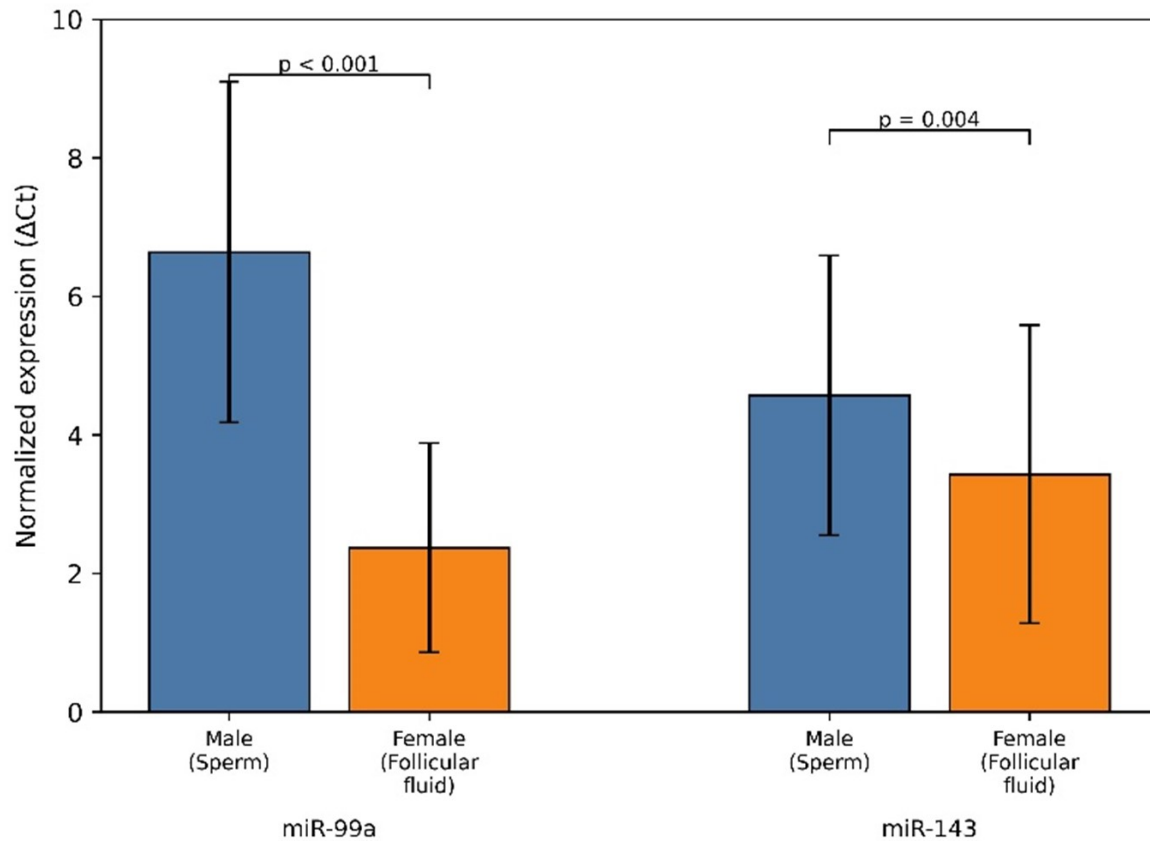


Figure 1. Comparison of miR-99a and miR-143 expression levels. Bar charts represent normalized miRNA expression values in male (sperm) and female (FF) samples. Significant differences were observed between sperm and follicular fluid samples for both miR-99a and miR-143.

samples were either used immediately or stored at -80°C until further analysis.

Semen samples were collected by masturbation into sterile containers and allowed to liquefy for 30 minutes at 37°C . Analysis was performed according to WHO guidelines. Samples were washed with PBS and stored

at -80°C until RNA extraction (within one week).

RNA Isolation, Reverse Transcription, and Quantitative Real-Time PCR

Total RNA was extracted using the Nucleic Acid Extraction Rapid Kit (magnetic bead-based method;

Table 4. Primer sequences used for quantitative real-time PCR analysis.

Gene / miRNA	Primer Type	Sequence (5'-3')
G6PD	Forward	TGGACCTGACCTACGGCAACAGATA
	Reverse	GCCCTCATACTGGAACCC
miR-143	Forward	CTTTCTTCTGCCACTCCTCCT
	Reverse	GAAGGGCTTCAGAATTCCG
miR-99a	Forward	TATTAATAGGGGGCCCATGC
	Reverse	ATTGTTGAACGGCACTGTGT

Table 5. Comparison of miR-99a and miR-143 expression between male and female samples.

miRNA	Group	N	Mean expression	SD	p-value
miR-99a	Male	36	6.64	2.46	<0.001
	Female	36	2.37	1.51	
miR-143	Male	36	4.57	2.02	0.004
	Female	36	3.43	2.15	

Table 6. Association between miR-99a and miR-143 expression and semen parameters in male participants.

(A) Correlation analysis						
Parameter	miR-99a (Spearman)	p-value	N	miR-143 (Spearman)	p-value	N
Motility	-0.146	0.395	36	-0.124	0.470	36
Total count	0.166	0.334	36	0.224	0.190	36
(B) Group comparison (based on motility)						
miRNA	Motility Group	N	Mean normalized expression	SD	p-value	
miR-99a	≤42%	20	7.18	2.23	0.203	
	>42%	16	5.97	2.64		
miR-143	≤42%	20	5.05	1.77	0.214	
	>42%	16	3.96	2.20		

Jiangsu Biopertectus Technologies Co., Ltd.) according to the manufacturer's instructions. RNA samples were stored at -80°C until further processing.

Complementary DNA (cDNA) synthesis was performed using the LunaScript RT SuperMix Kit (New England BioLabs, #E3010L). The resulting cDNA samples were stored at -20°C .

Quantitative real-time PCR (qPCR) was conducted using Luna Universal qPCR Master Mix (New England BioLabs, #M3003) on a LightCycler 480 Real-Time PCR System (Roche Applied Science). The G6PD gene was selected as an internal control based on its stable expression across the analyzed sample types. Primer sequences are presented in Table 4. Normalized expression values were used for comparative analyses. All RNA extraction and amplification procedures were performed under standardized experimental conditions according to the manufacturers' instructions. G6PD was selected as the reference gene based on its stable amplifica-

tion profile and consistent Ct values across the analyzed sample types.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 20.0; IBM Corp., Armonk, NY, USA). Comparisons between two independent groups were conducted using the non-parametric Mann-Whitney U test. Associations between semen parameters and miRNA expression were evaluated using Spearman's rank correlation coefficient, whereas correlations involving female reproductive parameters were assessed using Spearman's rank correlation coefficient due to the non-parametric distribution of the data and the limited sample size. Adjusted correlation analyses were performed to control for the effects of age, body mass index (BMI), smoking status, and number of retrieved oocytes. Comparisons among more than two groups were performed using the non-parametric Kruskal-Wallis

Table 7. Correlation analysis between miR-99a and miR-143 expression levels and female demographic, clinical, and hormonal parameters using Spearman's rank correlation coefficient.

Parameter	mir-99a expression	
	Spearman (rho)	p-value
<i>Demographic & Clinical Parameters</i>		
Age	-0.174	0.310
BMI	0.055	0.751
Infertility (years)	-0.071	0.683
No oocytes	-0.271	0.110
<i>Hormonal Profile</i>		
AMH	0.447	0.006
FSH	0.215	0.208
LH	0.046	0.790
E2	0.081	0.637
PRL	-0.161	0.348

Table 8. Associations between miR-143 expression levels and female reproductive parameters assessed using Spearman's rank correlation analysis.

Parameter	mir-143 expression	
	Spearman (rho)	p-value
<i>Demographic & Clinical Parameters</i>		
Age	-0.058	0.735
BMI	0.237	0.164
Infertility (years)	0.076	0.660
No oocytes	-0.392	0.018
<i>Hormonal Profile</i>		
AMH	0.449	0.006
FSH	0.356	0.033
LH	-0.048	0.783
E2	-0.177	0.303
PRL	-0.286	0.091

Statistically significant correlations are highlighted in bold ($p < 0.05$).

test, followed by multiple pairwise comparisons using Dunn's procedure with Bonferroni correction. A p-value < 0.05 was considered statistically significant.

Study Population and Sample Collection

A total of 36 infertile couples were included in the study. FF samples were collected from women during oocyte retrieval, and semen samples were obtained from their male partners. Expression levels

of miR-99a and miR-143 were analyzed in all samples. The distribution of embryo quality categories is presented in Table 3.

Differential Expression of miR-99a and miR-143 in Sperm and Follicular Fluid Samples

Significant differences in the expression levels of miR-99a and miR-143 were observed between sperm and follicular fluid samples (Figure 1, Table 5). Specifically, miR-99a expression differed signifi-

Table 9. Adjusted correlation analysis between miRNA expression and female hormonal parameters.

Parameter	miR-99a (Mean normalized expression)	
	Model 1: r_s (p-value)	Model 2: r_s (p-value)
AMH	0.445 (0.008)	0.451 (0.007)
FSH	0.248 (0.157)	0.206 (0.243)
LH	0.055 (0.757)	0.160 (0.367)
E2	0.049 (0.784)	0.188 (0.288)
PRL	-0.179 (0.310)	-0.214 (0.225)

Model 1: Spearman's correlation (r_s) adjusted for Age and BMI. Model 2: Spearman's correlation (r_s) adjusted for No oocytes and Smoking status.

Table 10. Adjusted Spearman correlation analysis between miR-143 expression levels and female hormonal parameters after controlling for potential confounding factors.

Parameter	miR-143 (Mean normalized expression)	
	Model 1: r_s (p-value)	Model 2: r_s (p-value)
AMH	0.445 (0.008)	0.451 (0.007)
FSH	0.248 (0.157)	0.206 (0.243)
LH	0.055 (0.757)	0.160 (0.367)
E2	0.049 (0.784)	0.188 (0.288)
PRL	-0.179 (0.310)	-0.214 (0.225)

Model 1: Spearman's correlation (r_s) adjusted for Age and BMI. Model 2: Spearman's correlation (r_s) adjusted for No oocytes and Smoking status.

cantly between sperm (6.64 ± 2.46) and FF samples (2.37 ± 1.51 ; $p < 0.001$), while miR-143 also showed significantly different expression levels between sperm and FF samples (4.57 ± 2.02 vs 3.43 ± 2.15 ; $p = 0.004$).

Association between miRNA Expression and Semen Parameters

No statistically significant correlations were identified between miR-99a expression and sperm motility ($r_s = -0.146$, $p = 0.395$) or total sperm count ($r_s = 0.166$, $p = 0.334$). Similarly, miR-143 expression showed no significant associations with semen parameters ($p > 0.05$) (Table 6).

Subgroup analysis revealed that men with lower sperm motility ($\leq 42\%$) exhibited higher mean miR-99a and miR-143 levels compared to those with higher motility ($> 42\%$); however, these differences were not statistically significant ($p = 0.203$ and $p =$

0.214 , respectively). Likewise, men with higher total sperm counts (> 39 million) showed slightly increased miRNA expression levels, without statistical significance ($p = 0.222$ and $p = 0.168$). The motility group comparisons are presented in Table 6B.

Association between miRNA Expression and Female Clinical Parameters

In the female group, significant positive correlations were observed between miR-99a or miR-143 expression levels and AMH concentrations. In addition, miR-143 expression was positively associated with FSH levels and negatively correlated with the number of retrieved oocytes. No significant associations were found with age, body mass index (BMI), duration of infertility, LH, estradiol or prolactin ($p > 0.05$). Results for miR-99a and miR-143 are presented in Tables 7 and 8, respectively. Adjusted correlations between hormonal parameters and

Table 11. Evaluation of miR-99a and miR-143 expression in relation to embryo quality parameters.

miRNA	Embryo Quality	N	Mean normalized expression	SD	p-value
miR-99a	Poor	3	3.18	2.65	0.436
	Average	15	1.88	1.51	
	Good	13	2.53	1.35	
	Excellent	5	2.93	1.07	
miR-143	Poor	3	2.06	3.31	0.046
	Average	15	2.73	2.11	
	Good	13	3.69	1.73	
	Excellent	5	5.65	0.88	

miR-99a and miR-143 are presented in Table 9 and Table 10.

Association between miRNA Expression and Embryo Quality

No statistically significant differences were observed in miR-99a expression across embryo quality groups (Poor, Average, Good, Excellent; $p = 0.436$). Embryo quality was assessed on Day 3 according to standard morphological criteria based on blastomere symmetry, fragmentation rate, and cell number. In contrast, miR-143 expression differed significantly among these groups ($p = 0.046$).

Post hoc Bonferroni analysis demonstrated that embryos of excellent quality exhibited significantly higher miR-143 expression levels compared with those of poor quality ($p = 0.042$) (Table 11).

Summary of Statistical Findings

No statistically significant differences were observed in miR-99a expression across embryo quality groups (Poor, Average, Good, Excellent; $p = 0.436$). In contrast, miR-143 expression differed significantly among these groups ($p = 0.046$). A summary of statistical associations is presented in Table 12.

Discussion

This study demonstrated differential expression of

miR-99a and miR-143 between sperm and follicular fluid samples from ART patients, likely reflecting tissue-specific and sex-related differences. Limited associations were observed with female hormonal parameters (AMH and FSH), while higher miR-143 expression was positively associated with embryo quality.

The observed differences in miR-99a and miR-143 expression between sperm and follicular fluid samples are consistent with previous studies highlighting the role of miRNAs in spermatogenesis [5,12]. Dysregulated miRNAs, which regulate key processes such as meiosis, germ cell development, and chromatin remodeling, may contribute to male infertility [17,18]. The genes targeted by these microRNAs, including FGFR3, SMAD3, AKT3, and TGFBR1, are associated with signaling pathways that regulate germ cell survival and differentiation [19]. These pathways are essential for maintaining spermatogonial stem cell self-renewal and preventing apoptosis during early germ cell development, thereby ensuring proper progression through spermatogenesis. Moreover, miR-143 is enriched in epididymis tissues, while miR-99a is present in seminal plasma exosomes, supporting their role in intercellular communication [16].

Despite these molecular links, no correlation was found between the expression of these miRNAs and standard semen analysis parameters, including sperm motility and total number of spermatozoa.

Table 12. Summary of statistical associations between miR-99a and miR-143 expression and reproductive parameters.

Parameter Category	Variable/ Comparison	Statistical Test	Result	p-value	Significance
Sex difference	miR-99a (Male vs Female)	Mann-Whitney U	Higher in males (6.64 vs 2.37)	<0.001	Significant
	miR-143 (Male vs Female)	Mann-Whitney U	Higher in males (4.57 vs 3.43)	0.004	Significant
Semen Parameters	miR-99a vs motility	Spearman	$r = -0,146$	0,395	NS
	miR-99a vs total sperm count	Spearman	$r = 0,166$	0,334	NS
	miR-143 vs semen parameters	Spearman	No correlation	>0.05	NS
Motility groups	≤42% vs >42%	Mann-Whitney U	Higher miRNA levels in low motility	0.203/0.214	NS
Sperm count groups	≤39M vs >39M	Mann-Whitney U	Slightly higher miRNA levels in higher counts	0.222/0.168	NS
Female parameters	miR-99a & miR-143 vs AMH/miR-143 vs	Spearman	Positive correlation	0.006/0.033/0.018	Significant
	FSH/ miR-143 vs		No correlation	>0.05	NS
	retrieved oocytes miRNAs vs age, BMI, LH, E2, PRL, infertility duration				
Embryo quality	miR-99a across groups	Kruskal-Wallis	No difference	0.436	NS
	miR-143 across groups	Kruskal-Wallis	Significant difference	0.046	Significant
	miR-143 (Excellent vs Poor)	Bonferroni post hoc	Higher in excellent embryos	0.042	Significant

This apparent contradiction suggests that these miRNAs may exert more pronounced effects at the molecular level or through processes not detected by conventional semen analysis. In fact, previous studies have shown that sperm function can be impaired through signaling pathways such as calcineurin signaling without necessarily altering classical semen parameters [20,21].

Both miR-99a and miR-143 are also involved in female reproductive processes, including steroidogenesis, granulosa cell function, and folliculogenesis [4,6]. While miR-143 regulates granulosa cell proliferation and estrogen secretion through inhibition of components in the MAPK pathway, miR-99a regulates folliculogenesis and ovarian signaling pathways. In particular, miR-99a has been shown to target IGF-1R, a key regulator of granulosa cell survival and proliferation, thereby influencing follicular development and oocyte maturation. Additionally, studies indicate that miR-143-3p induces granulosa cell apoptosis

and contributes to follicular dysfunction in PCOS [22,23].

The functional relevance of miR-143 in male reproduction may also be supported by its evolutionary conservation across species, suggesting a fundamental regulatory role. Previous studies have demonstrated that miR-143-3p interacts with key regulators of spermatogenesis, including CREBBP, a transcriptional coactivator involved in chromatin remodeling and energy metabolism during early germ cell development [24]. In this context, circular RNA circ-CREBBP has been shown to act as a molecular sponge for miR-143-3p, thereby modulating the expression of genes associated with cell survival and sperm function, such as MCL1 and CREB1. Given that MCL1 is an anti-apoptotic member of the BCL-2 family, these interactions suggest a role for miR-143 in regulating sperm survival and motility. Indeed, dysregulation of circ-CREBBP and its associated pathways has been linked to impaired sperm motility and increased

apoptosis, further supporting the involvement of miR-143 in male reproductive function [25].

In the present study, significant correlations were observed between miRNA expression and selected female reproductive parameters, particularly AMH levels. In contrast, no significant associations were identified with age, BMI, LH, estradiol, prolactin, or duration of infertility. Despite this, a correlation was observed between miR-143 expression and embryo morphology, with higher levels in embryos displaying better morphology. Our results are consistent with previous reports indicating that FF miRNAs influence oocyte developmental potential through intracellular pathways and granulosa cell activity [7,26–29]. These miRNAs are capable of modulating gene expression within granulosa cells after uptake, thereby altering the follicular microenvironment and influencing oocyte competence. Thus, miR-143 appears to be associated with early embryonic development through modulation of the follicular microenvironment. However, these findings should be interpreted cautiously, as the small subgroup sizes, particularly in the poor and excellent embryo quality groups, limit the robustness of subgroup comparisons.

Interestingly, significant positive correlations were observed between both miR-99a and miR-143 expression levels and AMH concentrations. These associations remained statistically significant even after adjustment for potential confounding factors, including age, BMI, smoking status, and number of retrieved oocytes. Since AMH reflects ovarian reserve and granulosa cell function, these findings may support the involvement of both miRNAs in follicular physiology and ovarian microenvironment regulation. MiR-99a and miR-143 regulate granulosa cell function via IGF-1R, MAPK, and TGF- β -related pathways.

In addition, miR-143 expression demonstrated a positive association with FSH levels and a negative correlation with the number of retrieved oocytes. Although the underlying mechanisms remain unclear,

these findings may indicate a relationship between miR-143 expression and ovarian response during ART cycles. Higher miR-143 levels in women with lower oocyte yield could reflect altered follicular microenvironment dynamics or compensatory regulatory mechanisms associated with diminished ovarian responsiveness.

No statistically significant correlations were observed between miRNA expression and age, BMI, LH, estradiol, prolactin, or duration of infertility. This may suggest that the observed miRNA alterations are closely associated with specific ovarian functional pathways rather than with general demographic or endocrine characteristics.

All results support the view that miRNAs act as active regulators in the reproductive system rather than merely as diagnostic markers of physiological conditions [30]. Through major cellular pathways such as PI3K/AKT, TGF- β /SMAD, and MAPK/ERK, miRNAs may influence various functions in reproductive physiology. Such physiological processes may include follicular development, spermatogenesis, and even embryo implantation. The process of fertilization and early embryogenesis might be negatively affected if the complex interactions are somehow impaired.

Although miR-143 showed an association with embryo quality, further validation in larger cohorts is required before any potential clinical application can be considered. The complexity of miRNA regulation is evident from its lack of association with clinical indicators. This indicates that the usefulness of miRNAs as biomarkers is contingent upon their combined use with other biomarkers [3].

This study is limited by its small size, lack of causal inference, evaluation of only two miRNAs, absence of lifestyle confounder assessment and independent validation, and limited adjustment for clinical confounders.

Further research should focus on large-scale, multicenter studies integrating clinical, transcriptomic,

and proteomic data. Advanced approaches such as next-generation sequencing (NGS) may provide deeper insights into reproductive miRNA networks. Further studies should investigate the mechanisms governing miRNA transport, stability, and intercellular communication in the reproductive system. Advances in miRNA biology may facilitate the development of targeted diagnostic and therapeutic approaches for infertility.

Conclusions

In conclusion, miR-99a and miR-143 expression differed significantly between sperm and follicular fluid samples, with miR-143 showing positive associations with embryo quality and morphology, and selected female hormonal and ovarian response parameters, but not with conventional semen parameters.

These findings suggest that miR-99a and miR-143 may regulate gametogenesis and early embryonic development, with miR-143 representing a potential biomarker of embryo quality pending further validation.

Furthermore, RT-PCR-based methods are used in the majority of recent investigations, including this one. NGS may make it possible to find more pertinent miRNAs.

These results require more confirmation. Larger, more diverse populations, combined with molecular and clinical research, will be necessary to clarify the mechanisms by which miRNAs influence human fertility and reproductive outcomes.

Author Contributions

Conceptualization, E.N. and S.S.; methodology, D.M., E.M. and E.D.; validation, D.M., P.C. and A.P.; investigation, E.M., and E.N.; writing—original draft preparation, E.N. and S.S.; writing—review and ed-

iting, D.M., A.P., E.M., P.P., E.D., G.D. and P.D.; visualization, S.S.; supervision, P.D.; project administration, P.D. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee.

Informed Consent Statement

Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data presented in this study are available on reasonable request from the corresponding author. Due to ethical and privacy considerations related to patient data, the datasets are not publicly available.

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Conflicts of Interest

The authors declare no conflicts of interest.

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