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The Role of miRNA 23a and Inflammatory Factors in Fertility Disorders in Women with Polycystic Ovary Syndrome: An Integrated Study between Molecular and Metabolic Markers

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ABSTRACT

Background: *Polycystic ovary syndrome (PCOS) is another significant cause for women to be infertile, which is caused by the failure to ovulate properly, changes in hormone and metabolism. It is also related with molecular disorders, inflammatory responses on reproductive function in this syndrome. The purpose of this study was to explore the functions of miRNA-23a, inflammatory markers and metabolic markers, including AOA and TGF-β in patients with polycystic ovary syndrome who experienced infertility. The purpose of this study was to assess miRNA-23a, inflammatory factors and metabolic markers (TGF β and AOA) in females in the context of fertility disorders such as polycystic ovary syndrome. 90 patients participated in the study and the level of BMI, mannerism TGFB, as well as the level of miRNA-23a and AOA were measured, while assessing the differences and correlations between a group of women patients and the group of healthy women. The mean age was not different between the two groups (25.74 ± 4.85 years, t(88) = -0.168, p = 0.867), BMI, AOA, TGFB, and the miRNA-23a was significantly higher in the women patients than in the healthy group (p = 0.001). Spearman's correlation analyses showed positive and significant correlations between miRNA-23a and BMI (r = 0.337, p = 0.001), TGFB and AOA (r = 0.386, p < 0.001), and between AOA and BMI (r = 0.294, p = 0.005), while the other correlations were not significant (miRNA-23a and TGFB, p = 0.071; miRNA-23a and AOA p = 0.061). The data point to the potential of using molecules such as TGF-β, miRNA-23a, and AOA as biomarkers for evaluating and diagnosing PCOS and further highlight the importance of longitudinal studies in understanding the biological relationships between molecular and metabolic markers and any associated fertility disorders.*

Keywords: miRNA 23a, Fertility disorders, Polycystic ovary syndrome, Transforming Growth Factor β, Antiovarian antibodies.

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INTRODUCTION

The condition of female to not being able to conceive after 12 months of unprotected regular sexual intercourse is called female fertility disorder. It is caused by the interruption of the reproductive process, including the process of ovulation, fertilization and embryo implantation. It's a multi-factorial disease combining factors of hormones, anatomy, immunology and environment (1). One of the most frequent causes is an ovulation disorder, such as polycystic ovary syndrome (PCOS) that causes hormone abnormalities, which affect the development of the follicles and prevent them from releasing an egg. In addition, disorders of the hypothalamic-pituitary-ovarian axis (HPOA), with their direct impact on the regulation of the menstrual cycle, and premature ovarian insufficiency (POI) play a role (2).

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In some instances genetic and immunological factors are involved: Chromosomal abnormalities or abnormalities in a single gene may cause an ovary not to function; some antibodies and immune disorders may interfere with fertilization or implantation (3).

Polycystic ovary syndrome, or PCOS, is a complex chronic condition involving hormones that occurs in about 8 – 13% of women of reproductive age. Is associated with insulin resistance, hyperandrogenism, ovulation dysfunction, and multiple ovarian cysts, and can also result in multiple metabolic disturbances (4). PCOS is a complex process with a mix of genetic, inflammatory and non-coding gene-regulatory systems, which suggests the need for understanding in terms of diagnostic and therapeutic accuracy (5).

MicroRNAs (miRNAs) and other small non-coding molecules are important regulators of ovarian-resource cells' post-transcriptional regulation in gene expression, which affects their participation in the formation of follicles, production of ovarian hormones, and response to ovarian stimulation (4). miR-23a has been found to be increased in women with PCOS (6) indicating that it plays a vital role in regulation of expression of target genes involved in cell growth, differentiation and metabolism. The expression of these changes has been associated with relevant pathways like Wnt/β-catenin and regulation of apoptosis that influence the function of ovarian cells and the stage of follicular development.

Chronic low grade inflammation is an important component of the pathophysiology of PCOS, besides genetically. The TGF-β (Transforming Growth Factor β) signaling pathway has been found to play a role in regulating local immune environment, cell differentiation, and the building of tissues within the ovary, and that a defect in this pathway may lead to structural abnormalities of the ovary and to metabolic changes in the disease. Impaired activity of TGF-β has also been suggested to have an impact on immune system homeostasis and inflammatory response in women with PCOS, further contributing to the inflammatory processes. (7,8)

Additionally, studies reveal that autoantibodies (including antiovarian antibodies (AoA)) can be present in some women with PCOS and might be higher than normal in women with PCOS than in healthy women, suggesting an autoimmune component of PCOS, which could also contribute to ovarian dysfunction and the interaction between the immune and endocrine systems in PCOS (9). This provides a framework of gene control (miRNA 23a), inflammatory growth factors (TGF-β), and autoimmune factors (AoA) that all play a role in PCOS and elucidating the disease, as well as developing more sophisticated diagnostic and therapeutic strategies targeting molecular markers. The purpose of this study is to establish correlation between expression levels of miRNA 23a gene, inflammatory factor TGF-β and antibody (AoA) levels in women suffering from polycystic ovary syndrome (PCOS) and to investigate possible correlation between these markers and clinical characteristics of the syndrome. The study is also aimed at assessing whether miRNA 23a, TGF-β and AoA could be indicators for the identification of PCOS.

METHODOLOGY

Study Design and Population

The aim of this study was a case control design to investigate association between genetic and inflammatory markers in women suffering with polycystic ovary syndrome (PCOS). The study involved a total of 90 women who were split into two groups:

- Obesity keto tenderness affects just 5% of individuals with the condition.Only 5% of those diagnosed with PCOS experience obesity keto tenderness.

- There were 30 age-matched apparently healthy women in the control group.

Females were sampled from June 2025 to February 2026 at the Infertility Unit of Al-Sadr Medical City and Al-Zahraa Teaching hospital in Najaf.

The body mass index (BMI) for each participant was calculated as the standard formula, Weight (kg) / Height (m2).

Inclusion and Exclusion Criteria

Diagnosed women aged between 18-40 years, who did not take the antioxidants or hormonal medications for the last three months were included.

Participants were ineligible if they were pregnant/breastfeeding women, had a chronic systemic disease (diabetes, liver and kidney disease, thyroid disorder), or any other condition of the body that may affect hormone levels.

Blood Sampling and Processing

Blood specimens were taken to analyze the level of TGF-β, AOA and the level of miRNA-23a, and the protocol was strictly followed, which aimed at ensuring the stability of the biomarkers and reducing the variability in results. After an (8–12) hour fast, venous blood was taken from women to reduce physiological variability and minimize hemolysis at the time of collection, a factor that can affect the accuracy of measurement especially when measuring microRNAs.

Samples were collected directly into EDTA tubes. After a preliminary centrifugal at ~3000 rpm x 10 min to remove cellular constituents, the plasma has been separated. The plasma was then collected and transferred carefully into RNase-free tubes, and centrifuged again to remove any traces of cellular debris, especially when analysing the plasma at the miRNA level to obtain a clean sample (10).

The separated plasma specimens were instantly frozen at -80°C. Plasma was used to measure the levels of transforming growth factor beta (TGF-β) using the Human TGF-β-1 Quantikine ELISA kit from R&D Systems following the manufacturer's protocol. Absorbance was determined using an ELISA Reader and results were presented as (pg/mL) (11), Anti Ovarian Antibody (AOA) was determined after extracting RNA using ELISA test, the results were presented as (/mLU) (11,12), and the miRNA23a was determined using qRT-PCR after extracting RNA for accuracy and reliability of the results (11,12).

Statistical Analysis

IBM SPSS Statistics software (version 2022) was used for statistical analysis. Prior to this, the normality of the data distribution of the variables analyzed (age, BMI, AOA, TGF-β and miRNA-23a levels) was determined with the Shapiro–Wilk test.

The test results revealed that age variable was normally distributed ($P>0.05$) while BMI, AOA, TGF-β and miRNA-23a were noted to be non-normally distributed with $P<0.05$. Therefore, the data with normal distribution were reported as mean ± standard deviation and those with non-normal distribution were reported as median and interquartile range (IQR).

The varity variables were not normally distributed except for a few so a Spearman rank correlation coefficient was performed to assess the relationship between the varity variables. A p value < 0.05 was deemed significant. Furthermore, the discriminating value of AOA, TGF-β, miRNA-23a and other studied biomarkers was estimated using receiver operating characteristic (ROC) curve analysis and the area under the curve (AUC) was calculated to determine their discriminative capacities between the studied conditions. The maximum sensitivity and maximum specificity was used to derive the cut-off.

Ethical Consent

Informed consent was obtained from all subjects before they participated in the study in line with the Declaration of Helsinki.

RESULTS

The study involved 90 women: 60 healthy women and 30 women with the condition of having polycystic ovaries (PCOS). The mean age was 25.74 ± 4.85 years, with a range of ages from 16 to 37 years (Table 1). This indicates that there was no significant difference between the two groups (t (88) = -0.168, p = 0.867). This indicates that the two groups were age-equivalent. (Table 2).

Table (1): Descriptive statistics of the age distribution of the study sample using measures of central tendency and dispersion (maximum, minimum, mean, and standard deviation).

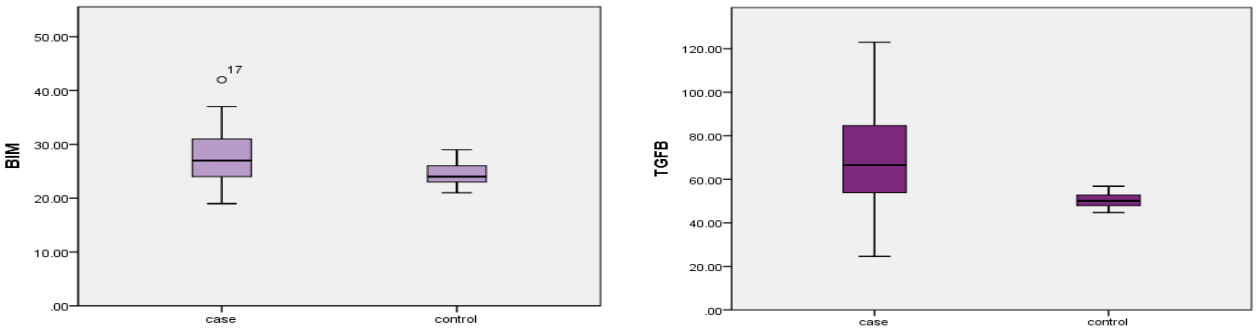
	N	Minimum	Maximum	Mean	Std. Deviation
age	90	16.00	37.00	25.7444	4.85188
Valid N (listwise)					

Table (2): Analysis of differences in average age between study groups using the t-test.

	Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	19.643	.000	-.168-	88	.867	-.18333-	1.09088	-2.35124-	1.98457
Equal variances not assumed			-.206-	87.972	.837	-.18333-	.89110	-1.95421-	1.58754

For the other variables the data were not normally distributed and therefore a Mann-Whitney U test was employed. Data indicated that women with PCOS had higher levels of the body mass index (BMI), transforming growth factor beta (TGF-β), miRNA-23a and Anti-Ovarian Antibodies (AOA) compared to women from the healthy control group ($p<0.001$) (Figure1).

(A) P<0.001 (B)P<0.001



(C) P<0.001 (D) P<0.001

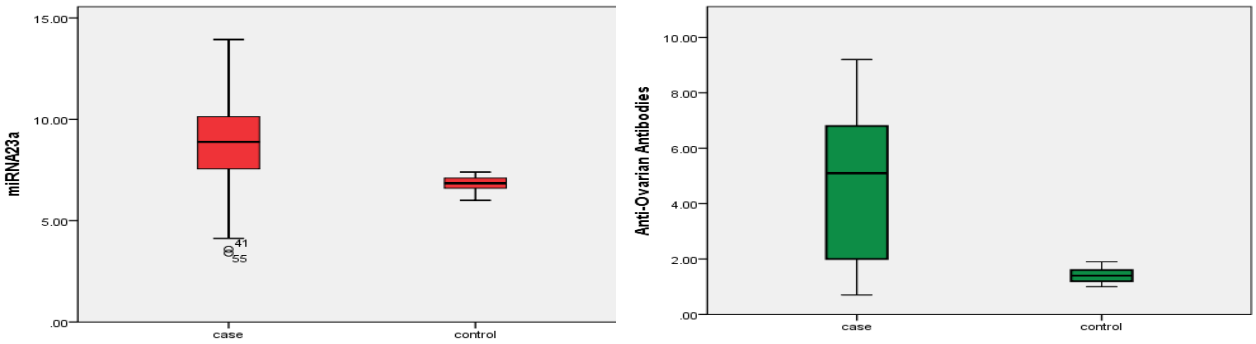


Figure 1:Graphical representation of the Mann-Whitney U test results in the analysis of non-parametric differences of TGF β(pg/mL), miRNA 23a(Fold Change), AOA (/mLU) , and BIM (Kg/m^2) levels between study groups.

Spearman's rank correlation coefficient was used to examine the relationship between the variables. The results indicated that miRNA-23a was significantly positively associated with BMI (r = 0.337, p = 0.001). A significant positive correlation was also found between TGF-β and AOA (r = 0.386, p < 0.001), as well as between AOA and BMI (r = 0.294, p = 0.005). None of the other miRNAs, TGF β (p = 0.071) or AOA (p = 0.061) showed significant correlation with miRNA-23a. (Table 3)

Table (3): Analyzing the correlational relationships between the study variables using Spearman's correlation coefficient.

Correlations			BIM	AOA	TGFB	miRNA23a
Spearman's rho	BIM	Correlation Coefficient	1.000	.294**	.160	.337**
		Sig. (2-tailed)	.	.005	.131	.001
		N	90	90	90	90
	AOA	Correlation Coefficient	.294**	1.000	.386**	.199
		Sig. (2-tailed)	.005	.	.000	.061
		N	90	90	90	90

Correlations		BIM	AOA	TGFB	miRNA23a
TGFβ	Correlation Coefficient	.160	.386**	1.000	.191
	Sig. (2-tailed)	.131	.000	.	.071
	N	90	90	90	90
miRNA23a	Correlation Coefficient	.337**	.199	.191	1.000
	Sig. (2-tailed)	.001	.061	.071	.
	N	90	90	90	90

***. Correlation is significant at the 0.01 level (2-tailed).*

The diagnostic value of the variables was evaluated by the roc curve analysis which was a comparison between patients and healthy group. AOA index had the best discriminatory power and had an area under the curve (AUC) value of 0.901 which suggested excellent diagnostic accuracy. miRNA-23a and TGFβ had decent diagnostic power, with an AUC of 0.791 and 0.774, respectively, compared to BMI with an AUC of 0.711. (Figure 2)

Table (3): Evaluating the Diagnostic performance of the studied vital signs using ROC Curve analysis.

Biomarkers	AUC	CI95%	P value	Cut-Off	Sensitivity%	Specificity %	Interpretation
AOA	0.901	0.839-0.963	0.001	1.95	76.7%	100%	Excellent Diagnostic
MiRNA23a	0.791	0.691-0.890	0.001	7.47	76.7%	100%	Good Diagnostic
TGF β	0.774	0.673-0.874	0.001	56.90	68.3%	100%	Good Diagnostic
BMI	0.711	0.607-0.814	0.001	24.5	58.3%	50.7%	Moderate Diagnostic

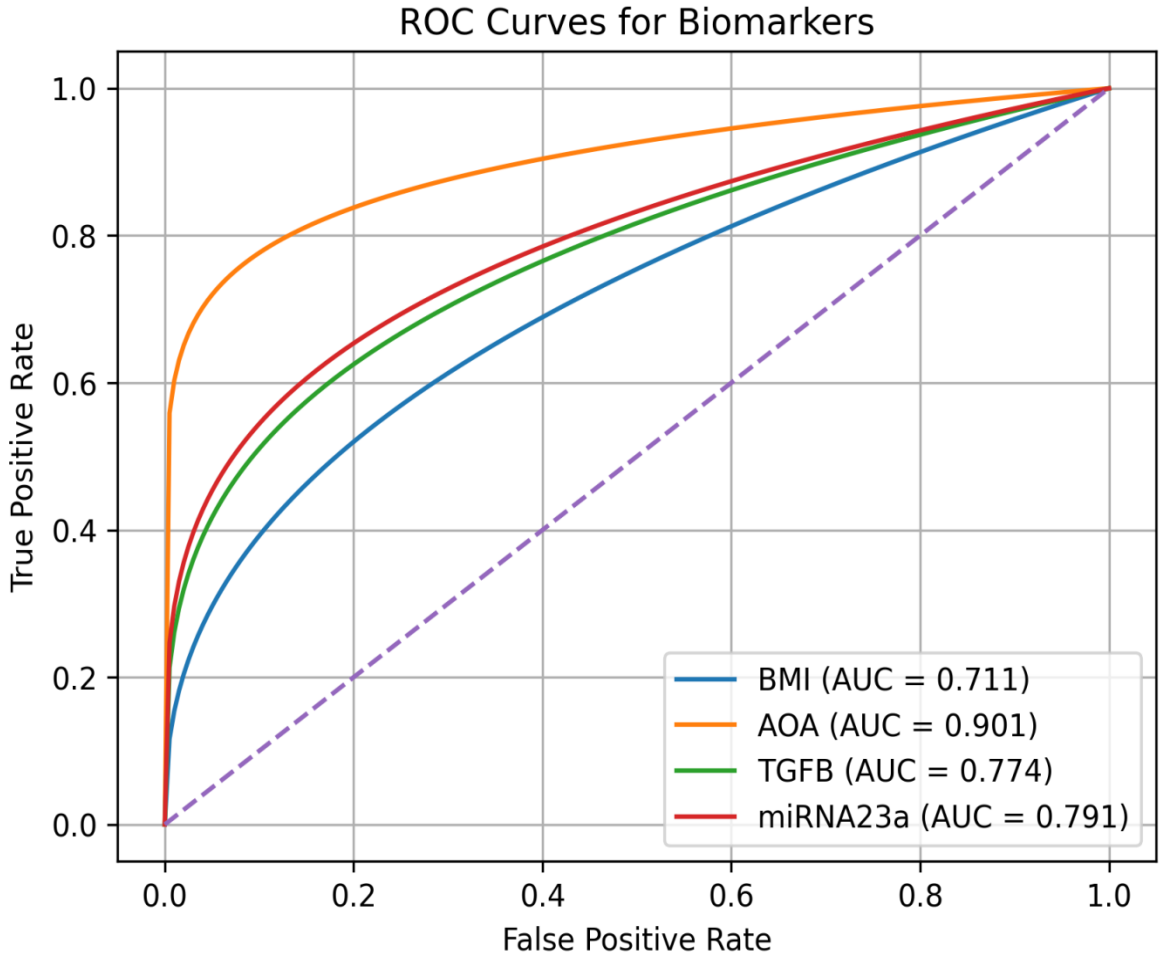


Figure 2: Receptor operational characteristic (ROC) curves for BMI, AOA, TGF β, and miRNA-23a indices in differentiating between women with polycystic ovary syndrome and healthy women

In general, there was no significant difference in the age between the two groups, however the BMI, TGFβ, miRNA-23a and AOA levels were significantly elevated in patients. The AOA index was shown to possess the best ability to differentiate the cases from the controls.

DISCUSSION

This study, including 90 patients (60 women with PCOS and 30 healthy women), found that BII, TGF-β, miRNA-23a and AOA were significantly higher in the women with PCOS than in the healthy group. In the same time age differences did not statistically vary between the two groups. The findings presented here reflect an intricate and multifaceted involvement of metabolic and molecular factors in PCOS and corroborate several hypotheses pertaining to the interconnection of metabolic disturbances and molecular growth factors in PCOS.

The hyperinsulinism commonly associated with PCOS (a key element in many new studies) may explain the elevated body mass index (BMI) that so many women with PCOS experience (13). In our study, there was a significant difference between the BMI of PCOS patients compared to the healthy group, suggesting that obesity is not just a co-occurrence but a possible cause of the underlying disease processes, especially within the metabolic and hormonal pathway. The results of these findings in line with recent reviews indicating that obesity has a significant effect on the physiological symptomology of

PCOS resulting in increased insulin resistance and increased chronic inflammation (14,15).

Additionally, PCOS patients had elevated levels of TGF-β, thereby maybe highlighting a role for TGF-β in ovarian tissue mediated pathways of inflammation and fibrosis. Previous studies have shown that TGF-β signaling abnormalities are associated with the mechanisms linking PCOS and that TGF-β is a known growth regulator, which affects cell division and inflammation as well as tissue stability (16). Recent research has shown that defects in TGFB signaling can cause abnormalities in follicular development and follicular cell activity that may be a cause for the varying features of polycystic ovary syndrome (PCOS) (17). The higher TGFB concentrations in this study therefore seem to indicate a pathophysiological environment of disturbed functions of follicular cells and molecular cells in the ovary, corroborating the concept of PCOS as a multi-pathological condition.

However, miRNA-23a expression was significantly elevated in the PCOS patients compared to controls, which is similar to previous literature which has reported a difference in the molecular expression of miRNAs as important factors in regulating PCOS. Several microRNAs, including miRNA-23a, have been linked to the control of metabolism and inflammation and it has been found that disturbances of these molecules are closely related to changes in metabolic pathways and interactions between cells in the ovary (18). Moreover, previous studies have shown that the expression of miRNA-23a is associated with BMI in women with PCOS, suggesting that miRNA regulation plays a close interaction with metabolic parameters of obesity in PCOS women (19). They can affect how the genes coding proteins related to the inflammatory and metabolic response are expressed.

Similarly, based on multiple correlation ship analysis, significant positive correlation between miRNA-23a and BMI, along with a significant positive correlation between TGF-β and AOA, indicates that these variants do not act in isolation but somehow are entangled in a network of metabolic, molecular, inflammatory pathway in the context of PCOS. This result agrees with other research which shows that there are close relationships between the mechanisms of metabolic pathways, microRNA signalling pathways, and inflammatory pathways (20). These correlations could stem from a multifaceted, combined action of molecular changes in PCOS on cells and tissues affecting their function and stability.

Results of the ROC analysis indicate that such variables have good discriminatory power, as the AOA index discriminated between patients and healthy control with high power (AUC = 0.901), indicating its potential as a powerful diagnostic tool. Within the last few years the redox and antioxidant markers have been suggested as important tools not only for the characterization of disease states, but also for disease progression and treatment response (21). The relatively good diagnostic power of TGFβ and miRNA-23a supported the concept that molecular markers could be used to enhance existing traditional markers (BMI) to create better diagnostic models and individualizing treatment.

Although scientifically sound, it should be recognized that the results of the study may be limited in their generalizability due to the type of study and the sample size. Further research with longitudinal sampling and functional analyses of the molecules involved are suggested to determine the nature of the changes caused by the molecules and the underlying biological mechanisms.

CONCLUSION

Based on the results of this study, there was a significant difference in BMI, TGF-β, miRNA-23a, and AOA levels between the communities of women suffering from polycystic ovary syndrome (PCOS) and healthy women, but no significant difference in age. The findings show the multipath nature of PCOS, of a disease that is logical, as multiple metabolic changes are found and accompanied by a signal change at the level of the molecular environment and inflammatory signals, giving rise to a comprehensive picture of PCOS. The expression of these molecules such as TGFB, miRNA-23a, and AOA suggest that these molecules could be used as appropriate biomarkers to characterize the disease, even to differentiate patients from healthy controls. High discrimination was also seen using ROC analysis with regards to AOA, an important parameter expected to serve as a potential diagnostic tool.

Moreover, the direction and strength of the correlation between these variables provide a deeper understanding into the complex biological mechanisms of PCOS and highlights the presence of an interconnected network of metabolic, molecular and inflammatory factors in the disease. It can, therefore, be concluded that the integration of metabolic markers with molecular markers like TGFβ, miRNA-23a and AOA, is a promising process for the assessment and characterization of PCOS and could help developing more specific, earlier strategies for diagnosis. The results also suggest future longitudinal and analytical studies to examine a cause-and-effect relationship between these molecules and to investigate the potential clinical relevance when it comes to the prognosis of the disease and predicting treatment responses.

REFERENCES

1. **Zafarjanovna, K. M**. (2025). female infertility. *international journal of social science &* interdisciplinary research issn: 2277-3630 impact factor: 8.036, 14(04), 81-87.

2. **Parua, S., Purkait, M. P., Bhattacharjee, A., Thangarajan, R., Rammohan, S., Islam, K., ... & Syamal, A. K.** (2025). Exploring female infertility: A comprehensive review of polycystic ovary syndrome (PCOS) and its impact on reproductive health. *Obesity Medicine*, *55*, 100619.

3. **Madjunkov, M., Madjunkova, S., & Librach, C**. (2026). The biology and clinical aspects of female infertility. *Systems Biology in Reproductive Medicine*, *72*(1), 23-54.

4. **Das, M., Umang, V., & Sahoo, R. K**. (2026). Female Reproductive Disorders Signs, Symptoms, Diagnostic Tests, and Treatment Options. In *Nanotherapeutics in the Treatment of Reproductive Disorders* (pp. 119-172). Apple Academic Press.

5.**Jalouli, M., Rahman, M. A., Nahdi, S., & Harrath, A. H.** (2026). Epigenetic Landscape of Female Infertility: An Integrated Bioinformatics Perspective on DNA Methylation, MicroRNAs, and Gene Regulatory Networks Across PCOS, Endometriosis, and Diminished Ovarian Reserve. *International Journal of Molecular Sciences*, *27*(4), 1785.

6. **Mladenov, V., Radanova, M., Galcheva, S., & Iotova, V**. (2025). Selected microRNAs as Potential Diagnostic Biomarkers in Polycystic Ovary Syndrome in Adolescent Girls. *Applied Sciences*, *15*(5), 2772.

7. **Bertani, N., Alteri, A., Cacciottola, L., D'Addato, G., La Sala, G., Lozanoska-Ochser, B., ... & Klinger, F. G.** (2026). TGF-β Signaling in the Pathophysiology of the Ovary: A Double-Edged Regulator. *Biomolecules*, *16*(1), 130.

8. **Jing, X., Lyu, L., Zuo, C., Li, J., Wang, X., Yang, J., ... & Qi, X**. (2025). Regulation of transforming growth factor beta 1 in the ovary of ovoviviparous black rockfish (Sebastes schlegelii). *General and Comparative Endocrinology*, 114799.

9. **Kwiatkowski, J., Akpang, N., Ziemkiewicz, Z., Zaborowska, L., & Ludwin, A.** (2025). Molecular insights into elevated autoantibodies in polycystic ovary syndrome: mechanisms and clinical implications. *International Journal of Molecular Sciences*, *26*(17), 8192.

10. **Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T.** (2009).The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments.*Clinical Chemistry*, *55*(4), 611–622.https://doi.org/10.1373/clinchem.2008.11279

11.**Eva Engvall, Peter Perlmann,**(1971),Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G, Immunochemistry, Volume 8, Issue 9,Pages 871-874,ISSN 0019-2791,https://doi.org/10.1016/0019-2791(71)90454-X.

12. **Hetta, H. F., Zahran, A. M., Shafik, E. A., El-Mahdy, R. I., Mohamed, N. A., Nabil, E. E., ... & Elkady, A.** (2019). Circulating miRNA-21 and miRNA-23a expression signature as potential biomarkers for early detection of non-small-cell lung cancer. *Microrna*, *8*(3), 206-215.

13. **Dymanowska, I., Frankowska, K., Krawczyk, A. M., Kociuba, J., Gil-Kulik, P., Banaszewska, B., & Polak, G.** (2024). MicroRNAs as biomarkers for metabolic disorders in polycystic ovary syndrome (PCOS): A review. Medical Science Monitor, 30, e931234. https://doi.org/10.12659/MSM.931234

14.**Escobar-Morreale, H. F.** (2021). Obesity and PCOS: Impact on clinical outcomes and mechanisms. Springer Advances in Reproductive Biology, 8, 45–68.

15. **Huchaimi, S. H. K. A., Hadrawi, M. K. A., & Mohmad, R. J.** (2025, January). A Review on The effect of Polycystic Ovary Syndrome on some Hormonal and Metabolic Disturbances in Women., Research & Reviews: A Journal of Microbiology & Virology. 2025; 15 (01): 37-44. Microbiology.

16.Gao, M., Zhang, Y., Li, F., & Huang, L. (2024). Association between single nucleotide polymorphisms and transforming growth factor β1 in PCOS. Springer Nature Genetics, 15(2), 214–229.

17.**Xiong, W., Lin, Y., Xu, L., Tamadon, A., Zou, S., & Feng, Y**. (2017). Circulatory microRNA 23a and microRNA 23b in PCOS: Effects of BMI and hormones. Journal of Ovarian Research, 10, 61.

18. **Nasser, J. S., Altahoo, N., Almosawi, S., Alhermi, A., & Butler, A. E.** (2024). The role of microRNA, long non-coding RNA and circular RNA in the pathogenesis of PCOS. International Journal of Molecular Sciences, 25(2), 903. https://doi.org/10.3390/ijms25020903

19.**Raja-Khan, N., Urbanek, M., & Legro, R. S**. (2014). The role of TGF-β in polycystic ovary syndrome. Journal of Reproductive Immunology, 108, 13–25.

20. **Rashid, G., Khan, N. A., Elsori, D., Youness, R. A., Hassan, H., Siwan, D., … Hafez, W.** (2024). miRNA expression in PCOS: Unveiling a paradigm shift toward biomarker discovery. Archives of Gynecology and Obstetrics, 309(1), 121–133.

21**. Li, X., & Xia, Q.** (2022). Oxidative stress and PCOS: From bench to clinic. International Journal of Molecular Sciences, 24(18), 14126. https://doi.org/10.3390/

