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Original Article

Clinical significance of microRNA-30d-5p and TGF-β/Smad2 pathway expression levels in polycystic ovary syndrome patients

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Abstract: Objective: To investigate the clinical significance of miR-30d-5p and transforming growth factor-β (TGF-β)/Smad2 pathway expression levels in polycystic ovary syndrome (PCOS) patients. Methods: A total of 82 PCOS patients undergoing their first *in vitro* fertilization-embryo transfer (IVF-ET) (PCOS group) and 82 non-PCOS patients undergoing their first IVF-ET due to fallopian tube factors (control group) were included in this retrospective study. The levels of miR-30d-5p, TGF-β1, Smad2, sex hormones, and insulin in follicular fluid exosomes on the day of oocyte retrieval were compared between the two groups. Correlation analysis was used to analyze the correlation between these indicators in the PCOS group. ROC curve analysis was performed to predict the clinical value of miR-30d-5p, TGF-β1, and Smad2 levels in the PCOS group for low 2PN numbers. Results: There were statistically significant differences between the groups in miR-30d-5p, estrogen, progesterone, TGF-β1, Smad2, testosterone, and insulin levels (all P<0.05). In the PCOS group, miR-30d-5p was negatively correlated with testosterone and insulin levels, and positively correlated with estrogen and progesterone levels; TGF-β1 and Smad2 were positively correlated with testosterone and insulin levels, and negatively correlated with estrogen and progesterone levels (all P<0.05). miR-30d-5p, TGF-β1, and Smad2, alone or in combination, had an AUC >0.7 in predicting low 2PN numbers in PCOS patients. Conclusion: The miR-30d-5p and TGF-β/Smad2 pathways are closely related to ovarian sex hormone and insulin levels in patients with PCOS and may participate in the development and progression of PCOS.

Keywords: Polycystic ovary syndrome, miR-30d-5p, TGF-β/Smad2, follicular fluid

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders in women of reproductive age, with a global prevalence of approximately 5%-20%. Its clinical manifestations are highly heterogeneous, including ovulation disorders, hyperandrogenemia, and polycystic ovarian changes [1-3]. PCOS not only leads to female infertility but also poses a serious threat to the long-term physical and mental health and quality of life of patients [4]. Although the etiology and pathogenesis of PCOS are not fully understood, current evidence suggests that PCOS is a complex disease involving multiple systemic disorders, including the hypothalamic-pituitary-ovarian axis, insulin signaling pathway, adrenal function, and adipokines network, under the combined influence of genetic susceptibility and environmental factors [5-7].

In the local microenvironment of the ovary, follicular fluid, as the direct intrinsic environment for follicular growth and development, directly reflects the metabolic and endocrine status of follicles and affects oocyte quality, fertilization potential, and early embryonic development [8]. Studies have shown that there are significant hormonal imbalances and metabolic abnormalities in the follicular fluid of PCOS patients, including elevated androgen levels, increased insulin concentrations, and an imbalance in the estrogen/progesterone ratio [9]. These changes are closely related to follicular development arrest, ovulation disorders, and adverse preg-

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nancy outcomes. Therefore, in-depth exploration of the molecular regulatory mechanisms of the local ovarian microenvironment in PCOS is of great clinical significance for revealing the nature of the disease and improving assisted reproductive outcomes. In recent years, microRNAs (miRNAs), as a class of non-coding single-stranded RNA molecules with a length of about 18-25 nucleotides, regulate gene expression at the posttranscriptional level and are widely involved in physiologic and pathologic processes such as cell proliferation, differentiation, apoptosis, and hormone secretion [10]. Various miRNAs are abnormally expressed in the serum, ovarian tissue, and follicular fluid of PCOS patients, suggesting that they may participate in the occurrence and development of PCOS by regulating key signaling pathways [11]. Among them, microRNA-30d-5p (miR-30d-5p) plays an important role in various metabolic diseases and tumor processes and is related to insulin sensitivity, adipocyte differentiation, and inflammatory response regulation [12]. Previous studies have confirmed that the level of miR-30d-5p in the peripheral blood of PCOS patients is significantly higher than that in normal individuals, and it can serve as a biomarker for the early diagnosis of PCOS [13]. However, the expression characteristics of miR-30d-5p in the local microenvironment of the ovary in PCOS and its relationship with hormone secretion disorders remain to be clarified. Transforming growth factor-β (TGF-β) superfamily signaling pathways play multiple regulatory roles in ovarian physiologic processes. The TGF-β/Smad signaling pathway is its classic transduction pathway, in which TGF-β1 binds to cell surface receptors, phosphorylates and activates downstream Smad2/3 proteins, and then enters the cell nucleus to regulate the transcription of target genes. Studies have found that abnormalities in the TGF-β/Smad pathway may be related to abnormal follicular development, fibrosis tendency, and hormone synthesis disorders in PCOS patients [14]. Previous in vitro studies have confirmed that miR-30d-5p can regulate the TGF-β/Smad2 pathway and thus participate in the development of ovarian granulosa cells [15]. Based on this, this study analyzed the expression characteristics of miR-30d-5p and TGF-β/Smad2 pathways in the follicular microenvironment of PCOS and evaluated the potential clinical value of the above indicators for the diagnosis of PCOS and the prediction of 2 pronucleus (2PN) fertilization outcome, aiming to provide experimental evidence for exploring the pathogenesis of PCOS and finding new therapeutic targets.

Materials and methods

Study participants

This retrospective study included PCOS patients who underwent *in vitro* fertilization-embryo transfer (IVF-ET) for the first time at the Reproductive Medicine Center of the First Affiliated Hospital of Yunnan University of Chinese Medicine (PCOS group) between January 2021 and June 2023, and 82 non-PCOS patients who underwent IVF-ET for the first time due to tubal factors (control group). This study was approved by the Ethics Committee of the First Affiliated Hospital of Yunnan University of Chinese Medicine.

Inclusion and exclusion criteria

PCOS group: Inclusion criteria: Diagnosis met the 2003 Rotterdam criteria, i.e., the presence of at least two of the following three criteria: (1) Infrequent ovulation and/or anovulation; (2) Clinical and/or biochemical hyperandrogenism; (3) Ultrasound findings indicating polycystic ovarian changes (≥12 follicles with a diameter of 2-9 mm in one or both ovaries, and/or ovarian volume ≥10 mL). Exclusion criteria included congenital adrenal hyperplasia, Cushing's syndrome, and androgen-secreting tumors.

Control group: Inclusion criteria: (1) Patients undergoing IVF-ET for the first time with infertility due to tubal factors (tubal obstruction or hydrosalpinx); (2) Regular menstrual cycles (25-35 days), normal endocrine hormone levels, and no clinical manifestations or biochemical evidence of hyperandrogenism; (3) No polycystic ovarian changes on ultrasound examination.

Exclusion criteria: (1) Comorbid endometriosis, uterine fibroids (diameter >4 cm or affecting uterine cavity morphology), or adenomyosis; (2) Other endocrine disorders such as thyroid dysfunction or hyperprolactinemia; (3) Comorbid diabetes mellitus, severe hepatic or renal dysfunction, autoimmune diseases, or hematological disorders; (4) History of ovarian surgery; (5) Chromosomal abnormalities in either spouse;

(6) Severe oligoasthenospermia, asthenospermia, or teratospermia due to male factors.

Ovulation induction protocol

All subjects underwent mid-luteal controlled ovarian hyperstimulation. In the mid-luteal phase of the previous menstrual cycle (approximately 7 days after ovulation), daily subcutaneous injections of 0.05-0.1 mg of gonadotropin-releasing hormone agonist (leuprolide acetate sustained-release microspheres for injection, Beijing Bornte Pharmaceutical Co., Ltd., National Drug Approval Number: H20093809) were initiated for pituitary downregulation. After 10-14 days, once downregulation criteria were met (serum estradiol <50 pg/mL, progesterone <1.5 ng/mL, endometrial thickness <5 mm, no follicles with a diameter >10 mm), exogenous gonadotropins (Gn) were administered to initiate ovulation induction. The initial dose of Gn was individualized based on the patient's age, weight, baseline antral follicle count, and anti-Müllerian hormone levels, generally 150-300 IU/day. The Gn dosage was adjusted by monitoring follicular growth and serum hormone levels via transvaginal ultrasound. When at least one dominant follicle has an average diameter ≥18 mm or two follicles have a diameter ≥17 mm, 5,000-10,000 IU of human chorionic gonadotropin is injected subcutaneously that evening, followed by oocyte retrieval under transvaginal ultrasound guidance 34-36 hours later.

Follicular fluid collection

On the day of oocyte retrieval, follicular fluid was aspirated from dominant follicles with a diameter >16 mm using a sterile, heparin-free retrieval needle. Follicular fluid from all retrieved oocytes of the same patient was pooled into a sterile centrifuge tube. Within 30 minutes, the samples were centrifuged at 2,000×g for 15 minutes at 4°C to remove cellular debris and red blood cells. The supernatant was carefully collected, aliquoted into cryogenic tubes, and stored at -80°C for subsequent analyses.

Observation indicators

Clinical data collection: The following data were collected for all study subjects: age, period of infertility, body mass index (BMI), baseline antral follicle count (AFC), daily Gn dose, number of days of Gn use, basal hormones [luteinizing hormone (LH), estradiol (E2), follicle-stimulating hormone (FSH), progesterone (P), testosterone (T), anti-Müllerian hormone (AMH)], number of retrieved oocytes, number of MII oocytes, number of 2PN embryos, number of usable embryos on day 3, and number of high-quality embryos on day 3.

Detection of miR-30d-5p in follicular fluid exosomes: Follicular fluid exosomes were extracted using the ExoQuick Exosome Extraction Kit (SBI, USA, catalog number: EXOQ5A-1). RNA was extracted using an RNA extraction kit (QIAGEN, Germany, catalog number: 217084). The relative expression level of miR-30d-5p was detected using RT-PCR (SYBR RT-PCR kit, QIAGEN, Germany, catalog number: 204245) on an Agilent PCR instrument. The reaction system was: 95°C pre-denaturation for 30 s, 95°C for 5 s, 62°C for 30 s, for 35 cycles. The 2-ΔΔCt method was used to determine the relative expression levels of LncRNAs BLACAT1 and miR-17-5p in each group. miR-30d-5p upstream primer: 5'-GCCGAGTGTAAACATCCCCGAC-3'; downstream primer: 5'-CTCAACTGGTGTCGTGGA-3'. Internal control U6 upstream primer: 5'-GCCAGCTCCTACATCTCAGC-3'; downstream primer: 5'-AGCCTGACTTGCTAGTGGATTAT-3'. Primers were synthesized by Beijing Dingguo Biotechnology Co., Ltd., China.

Detection of TGF-β1 and Smad2 levels: The levels of TGF-β1 and Smad2 in follicular fluid were determined using an MR-96A multifunctional microplate reader via enzyme-linked immunosorbent assay (ELISA). (Shanghai Enzyme-Link Biotechnology Co., Ltd.; TGF-β1 kit catalog number: mlC30466; Smad2 kit catalog number: ml106249).

Detection of sex hormone and insulin levels in follicular fluid: The levels of estradiol (E2), progesterone (P), testosterone (T), and insulin in follicular fluid were measured using a CL-2600i fully automated electrochemiluminescence analyzer (Mindray) and matching reagent kits.

Statistical analysis

Data were processed using SPSS 26.0 software. Normally distributed continuous data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and independent samples t-tests were used for comparisons between two groups.

Table 1. Comparison of general data

Index	PCOS group (n=82)	Control group (n=82)	*t*	*P*
Age (year)	30.78±4.42	31.04±5.17	0.346	0.730
Infertility period (years)	1.71±0.31	1.55±0.42	1.041	0.300
BMI (kg/m^2)	22.84±1.89	23.09±2.21	0.779	0.437
AFC	19.75±3.84	13.71±2.65	11.723	<0.001
Gn	191.70±24.49	187.06±31.32	1.057	0.292
Gn usage days	9.22±1.17	8.96±1.02	1.517	0.131

PCOS, polycystic ovary syndrome; Gn, gonadotropins; BMI, body mass index; AFC, baseline antral follicle count.

Table 2. Comparison of basic hormone levels

Index	PCOS group (n=82)	Control group (n=82)	*t*	*P*
LH (IU/L)	7.95±1.84	4.01±1.13	16.523	<0.001
E2 (pg/mL)	48.71±6.69	47.58±7.91	0.988	0.325
FSH (IU/L)	5.24±1.07	5.53±1.26	1.589	0.114
P (μg/L)	0.51±0.17	0.45±0.22	1.954	0.053
T (μg/L)	1.42±0.34	0.75±0.32	12.994	<0.001
AMH (μg/L)	7.73±1.26	3.76±1.04	22.004	<0.001

PCOS, polycystic ovary syndrome; LH, luteinizing hormone; E2, estradiol; FSH, follicle-stimulating hormone; P, progesterone; T, testosterone; AMH, anti-Müllerian hormone.

Table 3. Comparison of sex hormone and insulin levels in follicular fluid between the two groups

Index	PCOS group (n=82)	Control group (n=82)	*t*	*P*
E2 (pg/mL)	24091.48±339.45	30176.45±445.94	98.391	<0.001
P (μg/L)	20977.21±498.15	26015.79±514.73	63.696	<0.001
T (μg/L)	4.92±1.13	3.86±1.24	5.722	<0.001
Insulin (U/L)	19.47±3.62	12.45±3.91	11.93	<0.001

PCOS, polycystic ovary syndrome; E2, estradiol; P, progesterone; T, testosterone.

Table 4. Comparison of miR-30d-5p, TGF-β1, and Smad2 levels in follicular fluid exosomes between the two groups

Index	PCOS group (n=82)	Control group (n=82)	*t*	*P*
miR-30d-5p	1.06±0.22	1.95±0.27	23.14	<0.001
TGF-β1 (ng/L)	706.25±81.43	544.17±78.28	12.994	<0.001
Smad2 (ng/L)	78.92±10.17	39.08±10.94	24.153	<0.001

PCOS, polycystic ovary syndrome.

Counted data were expressed as percentages, and χ^2 tests were used. Pearson correlation analysis was performed for correlation analysis. In the PCOS group, the mean 2PN (6.12) was used as the cutoff; patients below this value were considered to have low 2PN. ROC curve analysis was used to assess the clinical value of sex hormone and insulin levels in the PCOS group in predicting low 2PN. Decision curve analysis was used to evaluate the clinical applicability of the model at different thresholds. A *p*-value <0.05 was considered significant.

Results

Comparison of baseline characteristics

The results showed no statistically significant differences between the two groups in terms of age, duration of infertility, BMI, AFC, daily Gn dosage, and number of days of Gn use (all P>0.05). See **Table 1**.

Comparison of basal hormone levels

There were no significant differences in basal E2, FSH, and P levels between the two groups (P>0.05); however, the PCOS group had higher LH, T, and AMH levels compared to the control group (all P<0.05). See **Table 2**.

Comparison of follicular fluid sex hormone and insulin levels between the two groups

Compared to the control group, the PCOS group had lower E2 and P levels and higher T and insulin levels in follicular fluid (all P<0.05). See **Table 3**.

Comparison of miR-30d-5p, TGF-β1, and Smad2 levels in follicular fluid exosomals

Compared to the control group, the PCOS group had lower levels of miR-30d-5p in follicular fluid exosomals, while having higher levels of TGF-β1 and Smad2 (all P<0.05). See **Table 4**.

Table 5. Correlation of miR-30d-5p, TGF-β1, Smad2 with sex hormones and insulin levels in the observation group

Indicator	Statistical value	E2 (pg/mL)	P (μg/L)	T (μg/L)	Insulin (U/L)
miR-30d-5p	*r*	0.514	0.494	-0.473	-0.445
	P	<0.001	<0.001	0.027	<0.001
TGF-β1 (ng/L)	*r*	-0.493	-0.476	0.484	0.427
	P	0.013	<0.001	<0.001	0.004
Smad2 (ng/L)	*r*	-0.478	-0.507	0.454	0.463
	P	0.005	<0.001	<0.001	0.017

E2, estradiol; P, progesterone; T, testosterone.

Correlation of miR-30d-5p, TGF-β1, and Smad2 with sex hormone and insulin levels in PCOS group

Correlation analysis revealed that in the PCOS group, miR-30d-5p was negatively correlated with testosterone and insulin levels, but positively correlated with E2 and P levels (P<0.05); TGF-β1 and Smad2 were positively correlated with testosterone and insulin levels, but negatively correlated with E2 and P (all P<0.05). See **Table 5**.

Comparison of ovulation induction cycles

No significant differences were observed between the two groups in the number of oocytes retrieved, the number of MII oocytes, the number of usable embryos on day 3, and the number of high-quality embryos on day 3 (all P>0.05). See **Table 6**.

ROC curve analysis of sex hormone and insulin levels for predicting low 2PN number in PCOS group

The results showed that miR-30d-5p, TGF-β1, and Smad2 alone had an AUC >0.7 for predicting low 2PN numbers in PCOS patients; the combined detection of all three had an AUC >0.8 for predicting low 2PN numbers in PCOS patients. See **Table 7** and **Figure 1**.

Decision curve analysis

The curves showed that the model had the highest clinical net benefit over a wide range of threshold probabilities. See **Figure 2**.

Discussion

Polycistic ovarian syndrome (PCOS) is the most common endocrine and metabolic disorder in women of reproductive age. One of its core pathophysiologic links lies in the abnormality of the local microenvironment of the ovary. Follicular fluid, as the direct microenvironment for oocyte development, has an important window reflecting the functional status of the ovary [16]. In recent years, the interaction between epigenetic regulation and classical signaling pathways has become an important direction for exploring its pathogenesis. This study analyzed the expression of miR-30d-5p and the activity of the TGF-β/Smad2 signaling pathway in the exosomes of follicular fluid of PCOS patients, and explored its association with the imbalance of hormone secretion in follicles and its predictive value for early IVF outcomes, aiming to provide relevant evidence for exploring the pathophysiologic mechanism of PCOS. The TGF-β superfamily signaling pathway is widely expressed in the ovary and exerts complex bidirectional regulatory effects on follicle recruitment, growth, dominant selection, atresia, and steroid hormone production [17]. TGF-β1, as a major member of this family, is closely related to tissue fibrosis due to its overexpression [18]. Smad2 serves as a major intracellular signal transduction molecule downstream of TGF-β1. After phosphorylation and nuclear translocation, it can regulate the transcription of a series of target genes [19]. Previous studies have shown that TGF-β signaling activity is enhanced in ovarian granulosa cells of PCOS patients, which may inhibit the selection and ovulation of dominant follicles by inhibiting granulosa cell proliferation, promoting premature luteinization or extracellular matrix deposition [14, 20]. The results of this study showed that the protein levels of TGF-β1 and its downstream signal transduction molecule Smad2 in the follicular fluid of PCOS patients were significantly increased, and the levels of TGF-β1 and Smad2 were positively correlated with hyperandrogenism and hyperinsulinism, and negatively correlated with hypoestrogenism, suggesting that overactivation of this pathway may be involved in the pathologic remodeling of ovarian structure. The possible mechanisms are as follows: on the one hand, TGF-β1 can promote the proliferation of theca cells and upregulate the activity of androgen synthesis

Table 6. Comparison of ovulation induction cycles

Index	PCOS group (n=82)	Control group (n=82)	*t*	*P*
Number of retrieved oocytes	12.53±4.45	11.49±4.02	1.57	0.118
MII number of oocytes	11.26±4.49	9.91±4.52	1.919	0.057
2PN number	6.12±2.27	7.39±2.08	3.735	<0.001
Number of available embryos on day 3	5.32±2.47	4.86±1.94	1.326	0.187
Number of high-quality embryos on day 3	3.12±1.43	2.77±1.25	1.669	0.097

2PN, 2 pronucleus; PCOS, polycystic ovary syndrome.

Table 7. Results of the ROC curve for predicting low 2PN numbers of sex hormones and insulin levels in the PCOS group

Indicator	Optimal cutoff value	AUC (95% CI)	P	Sensitivity	Specificity	Youden Index
miR-30d-5p	1.065	0.740 (0.627, 0.853)	<0.001	0.615	0.833	0.448
TGF-β1 (ng/L)	686.30	0.792 (0.686, 0.897)	<0.001	0.900	0.654	0.554
Smad2 (ng/L)	81.84	0.788 (0.688, 0.887)	<0.001	0.733	0.808	0.541
Joint factor	-	0.834 (0.749, 0.919)	<0.001	0.900	0.673	0.573

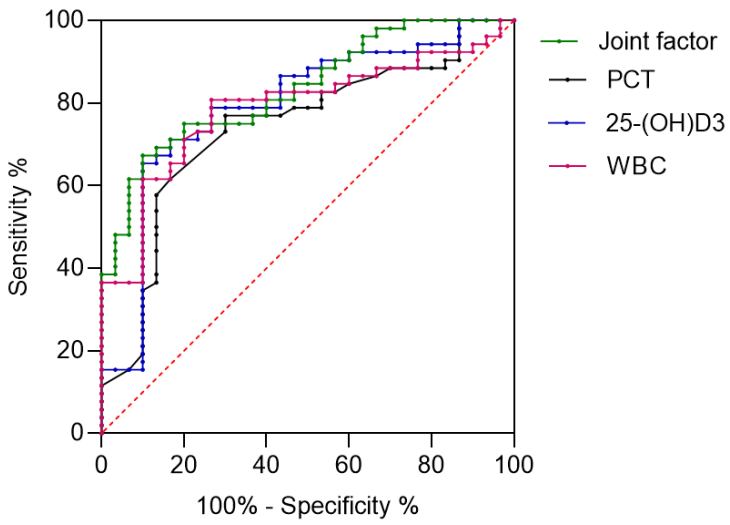

Figure 1. ROC curve graph.

rate-limiting enzymes such as CYP17A1, leading to excessive androgen production; on the other hand, excessively strong TGF-β signaling may inhibit granulosa cell differentiation, downregulate aromatase expression and FSH sensitivity, thereby hindering the conversion of androgens to estrogens; in addition, the TGF-β pathway interacts with the insulin-like growth factor system, which may jointly exacerbate insulin resistance in theca cells, amplify the stimulatory effect of insulin on androgen synthesis, and form a vicious cycle. Based on this, the results of this study showed that the levels of TGF-β1 and its downstream signaling molecule Smad2 protein in the follicular fluid of PCOS patients were significantly increased and closely related to hyperandrogenism, hyperinsulinemia and hypoestremia. Overactivation of this pathway may participate in the pathological remodeling of ovarian tissue through multiple mechanisms such as promoting androgen synthesis, inhibiting granulosa cell differentiation and exacerbating insulin resistance. MicroRNAs can be transmitted between cells through vesicles such as exosomes, thereby regulating gene expression in target cells [21, 22]. The miRNA profile in follicular fluid exosomes is considered an important biomarker reflecting the state of oocytes and surrounding granulosa/thecal cells. miR-30d-5p is a member of the miR-30 family, and previous studies have suggested that it plays an important role in regulating insulin sensitivity, lipid metabolism, and inflammatory response [23, 24]. PCOS is generally associated with insulin resistance and chronic low-grade inflammation. The results of this study found that the relative expression level of miR-30d-5p in follicular fluid exosomes of the PCOS group was significantly lower than that of the control group with fallopian tube factors. Furthermore, miR-30d-5p in the PCOS group was negatively correlated with

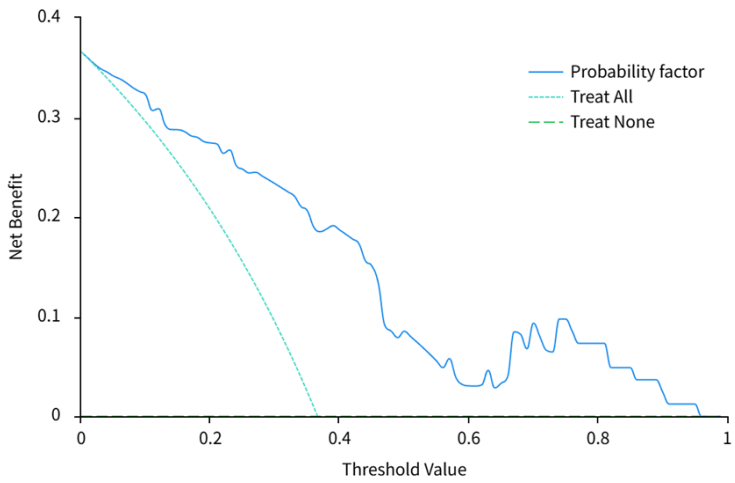

Figure 2. Decision curve analysis chart.

testosterone and insulin levels, but positively correlated with estrogen and progesterone levels. This suggests that the decreased expression of miR-30d-5p may be closely related to the pathologic environment of high androgen, high insulin, and low estrogen in the follicles of PCOS patients. Previous studies have found that miR-30d-5p can regulate TGF-β1 expression, thereby inducing apoptosis in lung cancer cells [15]. Other studies have confirmed that Smad2 is a direct and functional target of miR-30d-5p [25]. Therefore, this study proposes that downregulation of miR-30d-5p may relieve the inhibition of pathways such as TGF-β/Smad2, thereby participating in the pathologic processes of PCOS ovarian stromal hyperplasia and theca cell hyperfunction. However, this study did not further confirm this hypothesis through experiments such as dual-luciferase reporter gene assay, so further research is needed to confirm this hypothesis.

The 2PN number reflects the number of normally fertilized oocytes and is a key indicator for assessing the early outcome of an IVF cycle [26, 27]. In this study, the 2PN number in the PCOS group was significantly lower than that in the control group, indicating that the fertilization capacity of oocytes in PCOS patients may be impaired to some extent. In addition, there were no significant differences in clinical outcome indicators such as the number of oocytes retrieved, the number of MII oocytes, and the number of usable embryos between the groups, which may be related to factors such as sample size and the choice of stimulation protocol. ROC curve analysis revealed that the AUC for miR-30d-5p, TGF-β1, and Smad2 in follicular fluid alone in predicting low 2PN numbers in PCOS patients was >0.70, while the combined AUC was >0.8. This indicates heterogeneity in the molecular pathology of the follicular microenvironment among patients clinically diagnosed with PCOS, directly affecting oocyte fertilization capacity. This further suggests that oocyte fertilization capacity may be impaired in PCOS patients. The levels of miR-30d-5p, TGF-β1, and Smad2 in follicular fluid can all individually predict low fertilization rates in PCOS patients, with combined detection showing even better predictive power. Detecting these factors can aid in accurate risk stratification and clinical decision-making for PCOS patients during IVF treatment.

In summary, the exosomes of follicular fluid in PCOS patients showed downregulated expression of miR-30d-5p and overactivation of the TGF-β1/Smad2 signaling pathway, both closely related to the disordered microenvironment of high androgen, high insulin, and low estrogen/progesterone levels within the follicle. This suggests that the miR-30d-5p/TGF-β/Smad2 axis may be involved in the development and progression of PCOS. Furthermore, these indicators show good predictive potential for normal fertilization outcomes in IVF cycles of PCOS patients, and are expected to become one of the biomarkers for assessing oocyte quality.

However, this study also has several limitations. This is a cross-sectional observational study, and the causal regulatory relationship between miR-30d-5p and TGF-β/Smad2 cannot be clearly defined. Furthermore, follicular fluid is a mixture of various cellular secretions, requiring further separation of granulosa cells, theca cells, and stroma, for cell-specific analysis. Therefore, further research is needed in *in vitro* granulosa cell models and PCOS animal models to verify the targeted regulatory effect of miR-30d-5p on the TGF-β/Smad2 pathway through gain-of-function and loss-of-function experiments, and to further explore the effects of intervention on this regulatory axis on the

improvement of follicular microenvironment and reproductive outcomes, thereby providing new theoretical basis for the etiological research and targeted therapy of PCOS.

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Disclosure of conflict of interest

None.

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References

[1] Wang M, Zhang S, He J, Zhang T, Zhu H, Sun R and Yang N. Biochemical classification diagnosis of polycystic ovary syndrome based on serum steroid hormones. J Steroid Biochem Mol Biol 2025; 245: 106626.

[2] Alesi S, Ee C, Moran LJ, Rao V and Mousa A. Nutritional supplements and complementary therapies in polycystic ovary syndrome. Adv Nutr 2022; 13: 1243-1266.

[3] Helvaci N and Yildiz BO. Polycystic ovary syndrome as a metabolic disease. Nat Rev Endocrinol 2025; 21: 230-244.

[4] Herman R, Sikonja J, Jensterle M, Janez A and Dolzan V. Insulin metabolism in polycystic ovary syndrome: secretion, signaling, and clearance. Int J Mol Sci 2023; 24: 3140.

[5] Su P, Chen C and Sun Y. Physiopathology of polycystic ovary syndrome in endocrinology, metabolism and inflammation. J Ovarian Res 2025; 18: 34.

[6] Wang K and Li Y. Signaling pathways and targeted therapeutic strategies for polycystic ovary syndrome. Front Endocrinol (Lausanne) 2023; 14: 1191759.

[7] Armanini D, Boscaro M, Bordin L and Sabbadin C. Controversies in the pathogenesis, diagnosis and treatment of PCOS: focus on insulin resistance, inflammation, and hyperandrogenism. Int J Mol Sci 2022; 23: 4110.

[8] Sayutti N, Abu MA and Ahmad MF. PCOS and role of cumulus gene expression in assessing oocytes quality. Front Endocrinol (Lausanne) 2022; 13: 843867.

[9] Hood RB, Liang D, Tan Y, Ford J, Souter I, Jones DP, Hauser R and Gaskins AJ. Characterizing the follicular fluid metabolome: quantifying the correlation across follicles and differences with the serum metabolome. Fertil Steril 2022; 118: 970-979.

[10] Zhu M, Gao Y, Zhu K, Yuan Y, Bai H and Meng L. Exosomal miRNA as biomarker in cancer diagnosis and prognosis: a review. Medicine (Baltimore) 2024; 103: e40082.

[11] Insenser M, Quintero A, de Lope S, Álvarez-Blasco F, Martínez-García MÁ, Luque-Ramírez M and Escobar-Morreale HF. Validation of circulating microRNAs miR-142-3p and miR-598-3p in women with polycystic ovary syndrome as potential diagnostic markers. Hum Reprod 2023; 38: 951-960.

[12] Zhou F, Zheng Z, Zha Z, Xiong T and Pan Y. Nuclear paraspeckle assembly transcript 1 enhances hydrogen peroxide-induced human vascular smooth muscle cell injury by regulating miR-30d-5p/A disintegrin and metalloprotease 10. Circ J 2022; 86: 1007-1018.

[13] ElMonier AA, El-Boghdady NA, Fahim SA, Sabry D, Elsetohy KA and Shaheen AA. LncRNA NEAT1 and MALAT1 are involved in polycystic ovary syndrome pathogenesis by functioning as competing endogenous RNAs to control the expression of PCOS-related target genes. Noncoding RNA Res 2023; 8: 263-271.

[14] Gao M, Liu X, Gu H, Xu H, Zhong W, Wei X and Zhong X. Association between single nucleotide polymorphisms, TGF-β1 promoter methylation, and polycystic ovary syndrome. BMC Pregnancy Childbirth 2024; 24: 5.

[15] Zeng Q, Shi W, Xie L, Dai Y and Wei C. TGF-β1/miR-30d-5p axis regulating the expression of EMT key factors to induce apoptosis of lung cancer cells. Heliyon 2024; 10: e37801.

[16] Zhou W, Zhang T, Lian Y, Zhang W, Yang M, Li Y, Wang L and Yan X. Exosomal lncRNA and mRNA profiles in polycystic ovary syndrome: bioinformatic analysis reveals disease-related networks. Reprod Biomed Online 2022; 44: 777-790.

[17] Lee JH, Sánchez-Rivera FJ, He L, Basnet H, Chen FX, Spina E, Li L, Torner C, Chan JE, Yarlagadda DVK, Park JS, Sussman C, Rudin CM, Lowe SW, Tammela T, Macias MJ, Koche RP and Massagué J. TGF-β and RAS jointly unmask primed enhancers to drive metastasis. Cell 2024; 187: 6182-6199, e29.

[18] Xin X, Cheng X, Zeng F, Xu Q and Hou L. The role of TGF-β/SMAD signaling in hepatocellular

carcinoma: from mechanism to therapy and prognosis. Int J Biol Sci 2024; 20: 1436-1451.
[19] Zhang T, He X, Caldwell L, Goru SK, Ulloa Severino L, Tolosa MF, Misra PS, McEvoy CM, Christova T, Liu Y, Atin C, Zhang J, Hu C, Vukosa N, Chen X, Krizova A, Kirpalani A, Gregorieff A, Ni R, Chan K, Gill MK, Attisano L, Wrana JL and Yuen DA. NUAK1 promotes organ fibrosis via YAP and TGF-β/SMAD signaling. Sci Transl Med 2022; 14: eaaz4028.
[20] Nanda D, Venkatraman AC and Kalaivanan K. A cross talk study on sitagliptin mediated reclamation on TGF β signalling, DPP 4, miR-29a and miR-24 expression in PCOS rats fed with high fat-high fructose diet. Tissue Cell 2024; 88: 102375.
[21] Vijayakumar S, Yesudhason BV, Anandharaj JL, Kalaivanan L and Selvan Christyraj JRS. Exosomes and their miRNA as a potential biomarker in the diagnosis of Parkinson's disease. ACS Chem Neurosci 2025; 16: 2756-2766.
[22] Sharma S and Bhonde R. Applicability of mesenchymal stem cell-derived exosomes as a cell-free miRNA therapy and epigenetic modifiers for diabetes. Epigenomics 2023; 15: 1323-1336.
[23] Zheng L, Rao Z, Wu J, Ma X, Jiang Z and Xiao W. Resistance exercise improves glycolipid metabolism and mitochondrial biogenesis in skeletal muscle of T2DM mice via miR-30d-5p/SIRT1/PGC-1α axis. Int J Mol Sci 2024; 25: 12416.
[24] Song M, Wang J, Mu W, Zheng Y, Cai Y, Liu J and Xia Y. MiR-30d-5p modulates glucose and lipid metabolism by targeting CD73 through the AMPK pathway. Cell Biochem Biophys 2025; 83: 4947-4960.
[25] Yu M and Liu J. MicroRNA-30d-5p promotes ovarian granulosa cell apoptosis by targeting Smad2. Exp Ther Med 2020; 19: 53-60.
[26] Huang S, Wang Z, Yang R, Li R and Qiao J. Embryological and pregnancy outcomes of IVF-ET in overweight/obese and normal-weight women with PCOS. Front Endocrinol (Lausanne) 2025; 16: 1552561.
[27] Guo W, Zheng X, Zheng D, Yang Z, Yang S, Yang R, Li R and Qiao J. Effectiveness, flexibility and safety of switching IVF to IVM as a rescue strategy in unexpected poor ovarian response for PCOS infertility patients. J Clin Med 2023; 12: 1978.