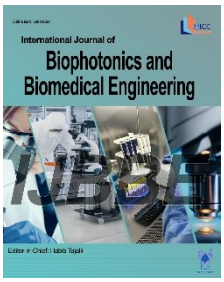

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Restoration of Exosomal miR-125b, lncRNA TUG1 and lncRNA NEAT1 Homeostasis by Vit-D in Polycystic Ovary Syndrome: Insights from an Experimental Rat Study

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Abstract

Polycystic ovary syndrome (PCOS) is a disease of ovarian dysfunction, hyperandrogenism and metabolic disorders in which exosomal noncoding RNAs have been reported to play a key role in its development. Vit-D (Vit-D) is known to modulate ovarian and systemic metabolism, but the impact of Vit-D on exosomal RNA networks has not been well studied. Thus, this work evaluated the regulatory role of Vit-D supplementation on exosomal microRNA (miR-125b) and long non-coding RNAs (Lnc-TUG1, Lnc-NEAT1) in PCOS rat model, offering potential mechanisms by which dietary intervention influences molecular regulation.

Keywords: Vit-D; PCOS; Exosomes; miRNA; LncRNAs; Precision Therapy

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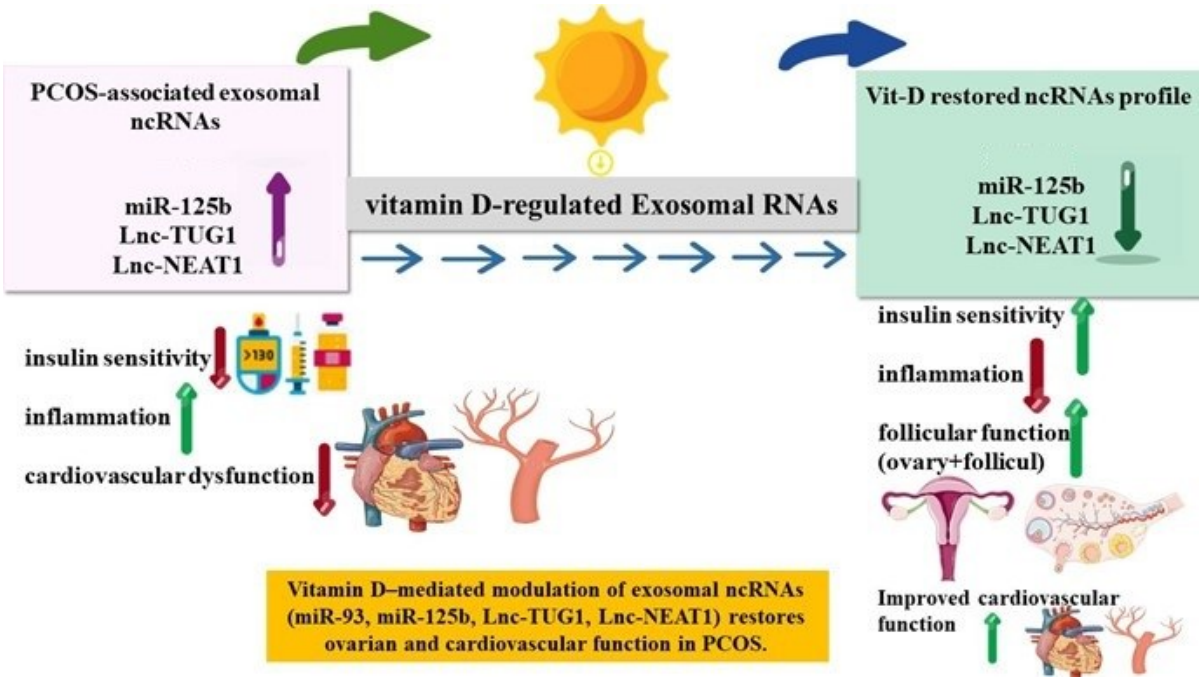
Graphical Abstract



1. Introduction

Polycystic Ovary Syndrome (PCOS) is a common and complex endocrine-metabolic disorder in women of reproductive age, affecting 8–11% worldwide, with regional prevalence ranging between 7.1% – 14.6%. In the clinical setting, PCOS is presented as ovulatory disturbance, androgen excess, and polycystic ovaries. Rather, it predisposes women to serious reproductive and metabolic long-term sequels such as insulin resistance (IR), dyslipidemia type 2 diabetes (T2D) hypertension and CVD [1-3].

Recent evidence confirms that Vitamin-D_3 (Vit-D), besides its classical bioactivities of regulating calcium and bone metabolism, has a pleiotropic effect on the reproductive system by affecting such processes as folliculogenesis, adaptation to AMH signalling or steroid hormone biosynthesis. Vit-D insufficiency or deficiency is widespread in PCOS women, and it could be linked to increased androgen levels, disturbed ovulatory function, and metabolic impairment. Ovarian adipocyte local and systemic levels of 1,25-dihydroxyVit-D (1,25(OH)2D3), a biologically active form of VD hormone, is significantly higher in women with PCOS compared to matched controls [2, 4, 5].

Concurrently, ncRNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have been recognized as important gene expression/gene function regulators in the pathogenesis of PCOS. The expression of miRNAs and lncRNAs has also been found to be dysregulated in granulosa cells (GCs), follicular fluids, serum, and adipose tissue (AT) from PCOS patients. These changes are associated with the disruption of folliculogenesis, steroidogenesis, cell growth, apoptosis and insulin signaling pathways. Systematic reviews and meta-analyses have reported an upregulation of miR-125b as well as other circulating miRNAs in PCOS individuals, highlighting the potential applicability of ncRNAs as disease biomarkers and therapeutic targets [6, 7].

Extracellular vesicles, especially exosomes, have emerged as a new means of intercellular communication by employing the horizontal transfer of functional RNA species including miRNAs and lncRNAs. Exosomal ncRNAs from follicular fluid or serum have been demonstrated to regulate the fate of granulosa cells, insulin resistance, and ovary dysfunction in PCOS. For instance, differentially expressed exosomal miRNAs and lncRNAs have been found in PCOS, and co-expression network analyses indicate that the exosomal lncRNA–miRNA–mRNA regulatory axes possibly participate in disease-related signal pathways such as MAPK/PI3K/AKT/infectaion [8, 9].

Although accumulating evidence has indicated the association of Vit-D status with deregulation of ncRNA in PCOS pathophysiology, few experimental studies have explored the mechanistic role of Vit-D supplementation on exosomal miRNAs and lncRNA expression profile in PCOS models. More importantly, few studies have investigated miR-125b, miR-93, lncRNA TUG1 and lncRNA NEAT1 within the

exosomal compartment at the same time, although considerable evidence has been shown to link these molecules with metabolic and reproductive regulation [10, 11].

Our previous work [15] focused on the effects of Vit-D on ovarian histology and a selected panel of exosomal microRNAs and lncRNAs, including miR-21, miR-29, miR-186, Lnc-ROR, Lnc-H19, and Lnc-MALAT1. In contrast, the present study expands this framework by investigating a distinct and previously unexamined exosomal ncRNA axis—namely miR-125b, Lnc-TUG1, and Lnc-NEAT1—which are mechanistically linked to insulin signaling, granulosa cell fate, and androgen regulation in PCOS. Importantly, although the same well-established PCOS animal model and exosome isolation methodology were employed to ensure phenotypic consistency, all ncRNA expression data generated in this study represent newly acquired datasets and have not been reported previously.

2. Research Aim

The present study was to investigate the effect of Vit-D supplementation on the expression levels of exosomal miR-125b, lncRNA TUG1 and lncRNA NEAT1 in PCOS rats. Our results may lead to new explanations about the molecular mechanisms of PCOS through detection of some epigenetic regulatory pathways in which Vit-D could contribute to the recovery of ncRNA homeostasis and provide a novel therapeutic strategy for the treatment of it.

homeostasis and provide a novel therapeutic strategy for the treatment of it.

3. Method and Materials

3.1. Animal Model and Experimental Design

Twenty-four prepubertal female Wistar rats (21 days old, 250 ± 20 g) were obtained and acclimatized under controlled environmental conditions (22 ± 2 °C; 12-h light/dark cycle; ad libitum access to food and water) at the Animal Research Facility of the School of Basic Sciences, Islamic Azad University, Mashhad. Animals were randomly allocated into four groups (n = 6 per group). Estrous cyclicity was verified by vaginal cytology for 12–14 days, and only rats exhibiting two to three consecutive regular cycles were included for further experimentation. All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the university and were performed in accordance with international guidelines for the ethical treatment of laboratory animals [12-15].

3.2. Assessment of Estrous Cycle Regularity

The estrous cycle phase of each rat was determined using daily vaginal smears. Samples were obtained with a sterile saline-moistened cotton swab and examined under a light microscope to identify the predominant cell types linked to the proestrus, estrus, metestrus, and diestrus phases. Only animals exhibiting reliable cyclic patterns were incorporated into the experimental methodology [15, 16].

3.3. Induction of the PCOS Phenotype

PCOS was induced experimentally by administration of testosterone enanthate (Iran Hormone Co., Tehran, Iran) for induction of the syndrome according to previous published articles which has been established and applied as a well-known and classic animal model that mimics human PCOS in terms of its endocrine and metabolic features. The testosterone enanthate dose (6 mg/100 g body weight; equivalent to 60 mg/kg) was selected based on well-established prepubertal androgenization models of PCOS, which consistently demonstrate persistent anovulation, cystic follicle formation, and hyperandrogenism resembling the human PCOS phenotype. This dosing regimen has been widely validated in previous studies and is distinct from adult-onset PCOS models that typically employ lower androgen doses [14, 15].

3.4. Preparation and Administration of Vit-D

Vit-D_3 (cholecalciferol; Iran Caspian Supply Co., Tehran, Iran) was freshly prepared at a working concentration suitable for intraperitoneal administration. The stock solution (100 µL Vit-D_3 + 900 µL sesame oil) was serially diluted as described in prior studies to yield a final concentration of 120 ng/g body weight per injection. The Vit-D treatment was initiated following PCOS induction and administered once weekly for five consecutive weeks using an insulin syringe to minimize injection-related stress [14, 15].

3.5. Experimental Groups

Animals were allocated to four experimental groups (n = 6 per group):

1. Negative Control (Control): Received no testosterone or Vit-D administration.
2. Positive Control (Pos.): Received the vehicle of testosterone enanthate (0.2 mL sesame oil + 0.01 mL 95% ethanol) subcutaneously for 35 days.

3. PCOS Group: Induced by daily subcutaneous testosterone injections (6 mg/kg body weight) for 35 days.

4. PCOS + Vit-D Group (Vit D): Induced with testosterone as previously described and subsequently administered Vit-D_3 (120 ng/g body weight, intraperitoneally, once weekly for 5 weeks). The incorporation of both vehicle and non-PCOS controls facilitated precise distinction between disease-specific and treatment-related effects [13, 15].

3.6. Collection of Sample and Tissue Processing

At the end of experiment period, animals were anesthetized with chloroform inhalation and then sacrificed. The blood samples were obtained by cardiac puncture and spun at 3,000 rpm for 5 min to obtain serum. Serum samples were aliquoted and stored at −70 °C for subsequent exosomal RNA analysis. The ovaries were removed, washed with PBS and fixed in 10% neutral buffered formalin for histologic examination by hematoxylin-eosin staining [15, 16].

3.7. Isolation and Characterization of Serum Exosomes

Exosomes were isolated from serum samples Second, to isolate exosomes from serum samples, we utilized the exoRibo™ Exosome Isolation Kit (Anasal, Tehran, Iran) according to manufacturer's instructions. After purification, the exosomes were subjected to ultracentrifugation and morphological analysis by TEM of fixed (1% glutaraldehyde) samples. The particle size distribution and concentration were determined by dynamic light scattering (DLS) with a Zetasizer Nano system. This in situ, non-destructive method assured exosome purity and a size distribution in the nanometer scale [15, 17].

3.8. RNA Extraction and Quantitative Real-Time PCR

Total RNA, including small RNA species, was extracted from serum-derived exosomes using the miRNeasy Mini Kit (Qiagen, Germany). The purity and concentration of RNA was analysed by spectrophotometry. First-strand cDNA was prepared according to the manufacturer's instructions (Applied Biosystems, USA). Quantitative real-time PCR (qRT-PCR) was performed on a LightCycler® system (Roche Diagnostics, Mannheim, Germany) using gene-specific primer sets for miR-125b, lncRNA-TUG1 and lncRNA-NEAT1 (Macrogene; Seoul of Korea) [15, 18].

Quantitative real-time PCR was performed using gene-specific primers for miR-125b, Lnc-TUG1, and Lnc-NEAT1. Primer sequences are provided in Supplementary Table S1. Amplification was carried out using an initial denaturation step at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 30 s and annealing/extension at 58 °C for 60 s. All reactions were performed in triplicate."

"Amplification efficiency for all primer sets was confirmed to be within the acceptable range of 90–110% using standard curve analysis. Melt curve analysis was performed at the end of each run to verify the specificity of amplification and the absence of primer-dimer formation.

The PCR amplification procedure was organized as follows: initial denaturation at 95 °C for 15 min, followed by 35–40 cycles of 95 °C for 30 s and 58 °C for 60 s. Relative expression levels were normalized against GAPDH and quantified using the 2^−ΔΔCT method.

3.9. Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical differences between groups were determined by one-way ANOVA followed by Bonferroni post hoc test. A p-value < 0.05 was considered statistically significant. All analyses were performed using GraphPad Prism® 8.0 (GraphPad Software, San Diego, CA, USA).

The sample size (n = 6 per group) was determined based on prior experimental studies using similar PCOS animal models and exosomal RNA analyses, which demonstrated sufficient statistical sensitivity to detect biologically relevant differences. Post hoc power analysis revealed that the study achieved a statistical power >80% for the primary outcome measures. Additionally, sample size was minimized in accordance with ethical guidelines for animal research to reduce unnecessary animal use.

3.10. Isolation and Characterization of Serum Exosomes

Exosome isolation procedures and physicochemical characterization (TEM and DLS) were performed using the same standardized protocol as described in our previous publication (Attarian et al., 2025) to ensure methodological consistency across studies. These characterization data are reproduced here solely to confirm cohort equivalence and exosome quality. Notably, all downstream RNA extraction, quantitative PCR analyses, and expression profiling of miR-125b,

Lnc-TUG1, and Lnc-NEAT1 were conducted independently for the present study and constitute novel experimental data [15].

4. Results

The TEM micrographs, DLS measurements and representative ovarian histology images presented in Figures 1–3 was generated in our previously published study and are reproduced here to demonstrate cohort equivalence and to contextualize the current molecular data [15].

4.1. Transmission electron microscopy (TEM)

Transmission electron microscopy confirmed the expected ultrastructural features of serum-derived exosomes. Representative micrographs (Figure 1) display cup-shaped vesicles with a homogeneous electron-dense rim, morphology consistent with bona fide exosomes and previously reported characterization standards for extracellular vesicles. The observed particle sizes fall within the canonical exosomal range (~30–150 nm). These morphological images were previously generated and published in our earlier study; they are reproduced here to ensure methodological continuity and permit direct comparison with the current molecular analyses [15].

4.2. Dynamic light scattering (DLS)

Analysis of dynamic light scattering complemented the hydrodynamic size measurements, for the purified vesicle population (Figure 4). The intensity-weighted mean diameter was around 139.6 nm, with a particle hydrodynamic average diameter of 224.2 nm and a polydispersity index (PDI) of 0.17, indicating a narrowly distributed, monodisperse population. The TEM data supported the DLS findings and substantiate the necessary high sample homogeneity for following mRNA extraction from exosomes. As with TEM, the same DLS characterization was done as described in our previous study and it is presented herein to provide context for the current exosomal ncRNA data [15].

4.3. Ovarian histology

Histological examination of ovarian tissue from each experimental group is shown in Figure 5. The negative control and vehicle (Pos. groups) showed an increased richness of antral follicles and corpora lutea compared with the testosterone-induced PCOS animals, suggestive of maintained ovulatory function.

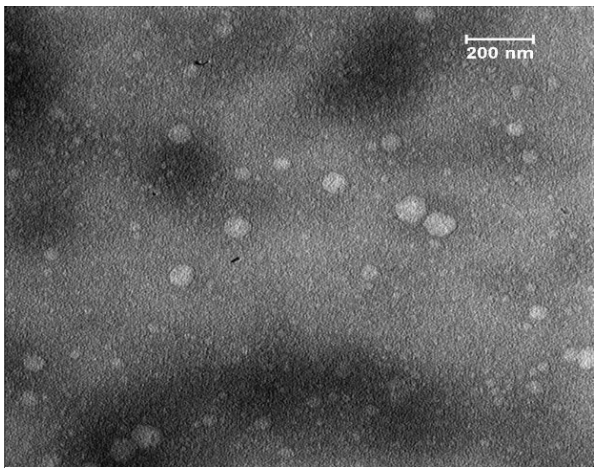


Figure 1. Transmission electron microscopy (TEM) of serum-derived exosomes. Representative micrograph showing characteristic cup-shaped extracellular vesicles within the canonical size range for exosomes. Note: Panel is reproduced from our prior publication [15] with permission for methodological reference

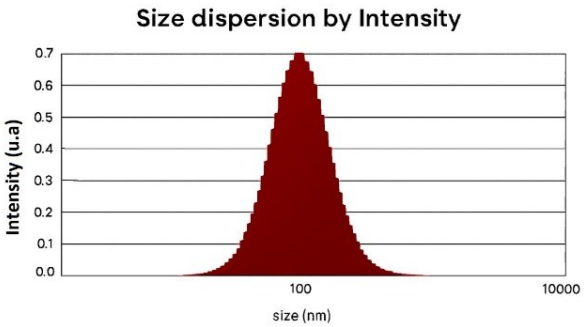


Figure 2. Dynamic light scattering (DLS) profile of isolated exosomes. Intensity distribution indicates a mean particle diameter of ~139.6 nm; hydrodynamic diameter = 224.2 nm; PDI = 0.17. Note: Panel is reproduced from our prior publication [15] with permission for methodological reference

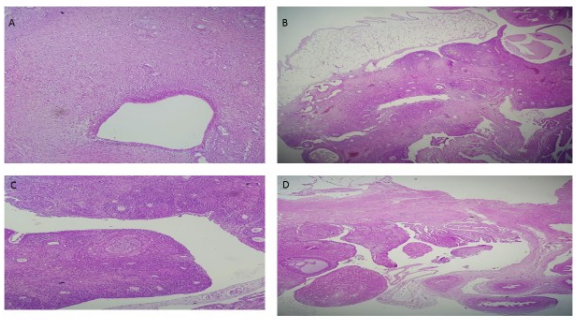


Figure 3. Representative hematoxylin & eosin–stained ovarian sections from (A) Negative control, (B) Vehicle (Pos.), (C) PCOS, and (D) PCOS + Vit-D groups. Images illustrate follicular development and corpus luteum frequency across groups. Note: All Panel are reproduced from our prior publication [15] with permission for methodological reference

FG counts and antral and luteal phenotypes were increased in PCOS compared with C group, showing follicular arrest and anovulation. Vit-D treatment partially restored such dynamics to control values, as

indicated by the lower proportion of secondary follicles and a higher number of antral follicles.

The representative histological images presented herein are reproduced from our previous paper and show the phenotypic homogeneity of the cohort used for ncRNA analyses here [15].

4.4. Vit-D influenced the expression of miR-125b in serum exosomes in PCOS

Figure 4 Comparison of relative expression level of miR-125b in serum exosome after Vit-D (Vit-D) treatment. miR-125b was significantly different in the experimental groups compared with Control.

The expression of miR-125b was significantly downregulated in Pos Control ($P<0.05$) and Vit D groups ($P<0.001$) when compared with Control group. The difference was very thin between Control and PCOS, but significant down-expression difference presented when comparing to Positive Control group ($P<0.05$). Interestingly, miR-125b in Vit-D group was markedly lower as compared with PCOS ($P<0.01$), suggesting that the regulation of this microRNA in different targets might be differently affected by treatment of Vit-D against PCOS. These findings enhance our previous data on exosomal miRNA modulation (miR-21, miR-29, miR-186) recorded in (15), demonstrates that Vit-D has a opposite effect on known and novel microRNAs related to PCOS pathophysiology.

Relative expression of miR-125b in serum-derived exosomes across the same experimental groups. miR-125b levels were decreased in Pos. and Vit-D groups relative to Control, with Vit-D treatment further reducing expression compared to the PCOS group. Data are presented as mean ± standard deviation (SD). Statistical significance was assessed using ANOVA followed by Bonferroni's post hoc test. (*) indicates a significant difference versus Control, and (#) indicates a significant difference between specified experimental groups.

4.5. Effects of Vit-D on Serum Exosome Lnc-TUG1 and Lnc-NEAT1 Expression in PCOS

The levels of Lnc-TUG1 and Lnc-NEAT1 were examined to detect the role of Vit-D on exosomal lncRNAs (Figure 5). Lnc-TUG1 was significantly downregulated in Positive Control ($P<0.01$), PCOS ($P<0.05$) and Vitamin D groups compared with the Control group, respectively. And Vit-D treatment induced the most remarkable decline which was extremely lower than the Positive Control ($P<0.01$), suggesting that VitaminD could rectify abnormal Lnc-TUG1 expression in PCOS.

Lnc-NEAT1 exhibited a similar pattern. Expression levels were significantly lower in the PCOS ($P<0.05$) and Vit-D groups ($P<0.001$) than those in the Control group. Furthermore, Vit-D treatment significantly decreased ($P<0.05$) the expression of Lnc-NEAT1 than PCOS only, which was indicative to the potential role of exosomal lncRNA neia in this process.

These results extend our previous study on exosomal lncRNAs (Lnc-ROR, Lnc-H19, Lnc-MALAT1) [15], and indicate that Vit-D regulates selective as well as broad effects on exosomal non- coding RNA networks in PCOS.

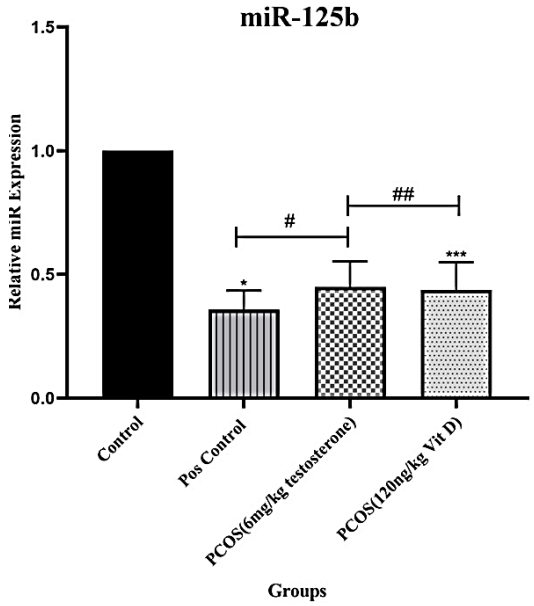


Figure 4. Effects of Vit-D on exosomal miRNA expression in a rat model of PCOS

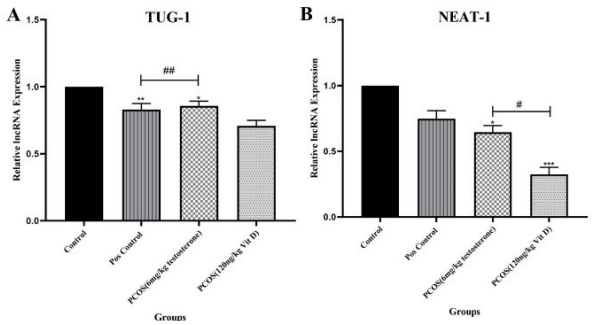


Figure 5. Effects of Vit-D on exosomal long non-coding RNA expression in a rat model of PCOS. (A) Relative expression of Lnc-TUG1 in serum-derived exosomes across experimental groups: Negative Control (Control), Positive Control (Pos.), PCOS, and PCOS treated with Vit-D (Vit-D). Lnc-TUG1 expression was significantly downregulated in Pos., PCOS, and Vit-D groups compared to Control, with Vit-D treatment inducing the most pronounced suppression relative to Pos., indicating its potential regulatory effect. (B)

Table S1. Primer sequences used for qRT-PCR analysis, including forward (F) and reverse ® primers and the corresponding product sizes

Target	Primer Sequence (5′–3′)	Product Size
miR-125b	F: TCCCTGAGACCCTAACTTGT R: GAACATGTCTGCGTATCTC	68 bp
LncTUG1	F: TTAAGGGCCAAACGCCATCA R: GGGCCAGTTGGGTATAGCAG	120 bp
LncNEAT1	F: ACTGCTTGACACCCCATGCC R: CGGTGATGACCACGGCTACC	119 bp
GAPDH	F: ATGGGGAAGGTGAAGGTCG R:GGGGTCATTGATGGCAACAATA	107 bp

Relative expression of Lnc-NEAT1 in serum-derived exosomes across the same groups. Lnc-NEAT1 levels were significantly reduced in PCOS and Vit-D groups compared to Control, with further significant downregulation following Vit-D treatment relative to PCOS alone.

Data are expressed as mean ± standard deviation (SD). Statistical significance was determined by ANOVA followed by Bonferroni's post hoc test. (*) indicates a significant difference versus Control, and (#) indicates a significant difference between specified experimental groups difference between specified experimental groups.

5. Discussion

Polycystic ovarian syndrome (PCOS) is a multifaceted endocrine disorder characterized by hormonal dysregulation, insulin resistance, and reproductive complications. Recent research has emphasized the important role of non-coding RNAs, particularly microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), in the pathophysiology of PCOS. Vit-D (Vit-D) which is known for its immune-modulation, anti-inflammatory effects are proposed as regulator of these ncRNAs. This study expands upon our previous research, which investigated the effects of Vit-D on exosomal miRNAs and lncRNAs within a rat model of PCOS. We particularly evaluated the expression levels of miR-125b, Lnc-TUG1, and Lnc-NEAT1 in exosomes derived from serum after Vit-D treatment.

5.1. miR-125b: Regulator of PCOS Pathogenesis

Our results demonstrate that Vit-D treatment markedly reduced the expression of miR-125b in the serum exosomes of PCOS rats. This corresponds with prior studies demonstrating that miR-93 is overexpressed in the granulosa cells of individuals with PCOS, associated with insulin resistance and reduced GLUT4 expression in adipose tissue [19]. Likewise, miR-125b has been associated with the modulation of ovarian granulosa cell proliferation and death, exhibiting elevated levels in instances of PCOS [20].

The downregulation of these miRNAs by Vit-D proposes use as a therapeutic agent for normalizing the abnormal gene expression-profiles in PCOS. T This is in line with the results of our previous study, which reported that Vit-D treatment influenced the expression of other miRNAs, including miR-21 and miR-186, in PCOS rats [15]. It should be noted that the present study evaluated ncRNA expression changes at the exosomal level and did not directly assess downstream functional endpoints such as insulin signaling proteins or granulosa cell viability. Therefore, the proposed mechanistic links should be interpreted as hypothesis-generating rather than definitive causal evidence. Taken together, these results highlight the importance of Vit-D in regulating miRNA levels and possible use as a therapeutic in treating PCOS.

5.2. Lnc-TUG1 and Lnc-NEAT1: Emerging Biomarkers in PCOS

In our present investigation, Vit-D therapy resulted in a notable downregulation of Lnc-TUG1 and Lnc-NEAT1 expression in PCOS rats. Lnc-TUG1 has been demonstrated to facilitate follicular activation and development in PCOS through the modulation of estrogen production [10]. Lnc-NEAT1 is associated with the regulation of insulin receptor substrate-2 and androgen receptor pathways, which are essential to the etiology of PCOS [8].

The downregulation of these lncRNAs by Vit-D indicates that Vit-D may partially exert its effects by modulating lncRNA expression. This corresponds with our previous findings that Vit-D treatment influenced the expression of additional lncRNAs, such as Lnc-ROR and Lnc-H19, in PCOS rats [15]. These findings underscore the potential of lncRNAs as indicators and therapeutic targets in PCOS.

5.3. Mechanistic Insights and Therapeutic Implications

The exact mechanisms through which Vit-D regulates miRNA and lncRNA expression in PCOS have yet to be completely clarified. Numerous studies have decisively identified the underlying causes. Vit-D has been demonstrated to mitigate oxidative stress and ferroptosis in several cell types, potentially influencing its regulatory effects on non-coding RNAs [21]. Moreover,

Vit-D insufficiency has been linked to elevated testosterone synthesis and insulin resistance, both of which are pivotal in the pathophysiology of PCOS [22]. Our present research, together our prior findings, indicates that Vit-D administration may provide a comprehensive strategy for controlling PCOS by influencing both miRNA and lncRNA expression. This may result in the normalization of atypical gene expression patterns, enhancement of insulin sensitivity, and reinstatement of reproductive function.

5.4. Comparative Analysis with Existing Literature

Our conclusions are in accordance with other recent work on the impact of Vitamin-D on PCOS. A study by [4] reported high frequency of Vit-D insufficiency in patients with PCOS, and their suggestion was that Vit-D supplementation could cure menstrual irregularities and insulin resistance [4]. A review investigated mechanisms by which Vit-D deficiency may lead to the development of PCOS such as increased testosterone production and insulin resistance [22].

Furthermore, our study validates the results of another study which reported miR-125b regulate Vit-D metabolism by targeting CYP24A1, which contributed to the development of gestational diabetes mellitus [23]. It suggests that Vit-D and miR-125b have a mutual effect, such as the Vit-D may influence miR-125b expression in tumorigenesis and vice versa.

While several studies have documented the dysregulation of ncRNAs in PCOS [8–11], most reports have examined isolated miRNAs or lncRNAs within ovarian tissue or circulation, rather than within the exosomal compartment. The present study uniquely integrates miR-125b, Lnc-TUG1, and Lnc-NEAT1 into a unified exosomal framework and demonstrates their coordinated modulation by Vit-D supplementation. This approach provides novel insight into how dietary and hormonal factors may reshape intercellular RNA-mediated communication in PCOS.

5.5. Integrative ncRNA network perspective

Looking back on the studies we have conducted presently and before, the above mentioned exosomal ncRNAs are conveyed by Certified Email towards those sharded molecular trails which should be involved in the pathophysiology of PCOS. Such as miR-21,,miR-29, miR-186, miR-125b have been reported to have the ability to aim a punch at some components in insulin signaling, blaze up inflammatory cascade, and regulate inducing apoptosis;

Table S2. Raw Ct values, ΔCt calculations, and relative expression (fold change, 2^-ΔΔCt) for Target genes across experimental groups

Target	Group	Raw Ct (miRNA/lncRNA)	Raw Ct (GAPDH)	ΔCt (Target – GAPDH)	Fold Change (2^-ΔΔCt vs Control)
miR-125b	Neg Control	22/13	13/93	8/19	1
miR-125b	Neg Control	21/87	13/69	9/927	0/299992846
miR-125b	Pos Control	25/484	14/83	9/51	0/400534939
miR-125b	Pos Control	23/98	14/78	9/68	0/356012549
miR-125b	PCOS	24/21	14/29	7/935	1
miR-125b	PCOS	23/41	14/31	9/255	0/400534939
miR-125b	PCOS + Vit-D	25/53	15/44	8/93	0/501735874
miR-125b	PCOS + Vit-D	24/65	15/38	8/97	0/48801588
TUG1	Neg Control	18/37	13/76	4/765	1
TUG1	Neg Control	18/78	13/73	5/105	0/790041312
TUG1	Pos Control	19/98	13/42	5/06	0/815072332
TUG1	Pos Control	19/84	13/37	5/33	0/675955417
TUG1	PCOS	18/87	14/59	6/15	1
TUG1	PCOS	19/85	14/59	6/375	0/855595026
TUG1	PCOS + Vit-D	20/99	13/21	6/32	0/888842681
TUG1	PCOS + Vit-D	20/49	13/33	6/61	0/726986259
NEAT1	Neg Control	21/77	14/66	7/085	1
NEAT1	Neg Control	21/78	14/72	7/56	0/71946679
NEAT1	Pos Control	22/32	13/89	7/785	0/615572207
NEAT1	Pos Control	21/38	14/69	8/71	0/324209889
NEAT1	PCOS	21/65	13/98	7/48	1
NEAT1	PCOS	22/63	14/73	7/82	0/790041312
NEAT1	PCOS + Vit-D	24/99	14/65	8/025	0/685391412
NEAT1	PCOS + Vit-D	21/27	14/19	8/895	0/375009747

While lncRNAs such as Lnc-ROR, Lnc-H19, Lnc-MALAT1, Lnc-TUG1 and Lnc-NEAT1 are concerned with transcription control, chromatin changes and steroidogenic regulation in ovarian and metabolic tissues [8, 11] .

Although no formal bioinformatic network analysis was implemented in this study, the previous unification by literature suggests that Vit-D might be upstream to interconnected exosomal ncRNA networks as a whole, rather than scattered transcripts. System-level bioinformatic modelling and functional tests in the future is necessary to further show these regulatory axes in action.

5.6. Limitations and Future Directions

Although our work substantially elucidates the effect of Vit-D on Noncoding RNA expression in PCOS, it also has its own limitations. The experiment was conducted in a rat model which is good for determining the effects of Vit-D, though not necessarily transferable to humans. Furthermore, the study only concentrated on changes in the expression of individual miRNAs and lncRNAs, without investigating putative functional consequences pertaining to shifts in targeted gene expression and cellular pathways.

Further studies should focus upon these strains using clinical trials that will definitively assess the impact of Vit-D supplementation on non-coding RNA expression in women with PCOS. Finally, functional analyses are needed to unravel the downstream effects of Vit-D-mediated changes in miRNA and lncRNA expression. Further longitudinal studies are needed to demonstrate the long-lasting efficacy as well as safety of Vit-D therapy in successful PCOS treatment.

6. Conclusion

In conclusion, our study provides evidence that Vit-D (Vit-D) supplementation changes the profile of rat model of polycystic ovarian syndrome (PCOS) exosomal non-coding RNAs including microRNA (miR-125b) and long non-coding RNAs (Lnc-TUG1, Lnc-NEAT1). These discoveries extend previous knowledge on the modulation of Vit-D of exosomal miRNAs and lncRNAs (miR-21, miR-29, miR-186, Lnc-ROR, Lnc-H19, Lnc-MALAT1), thus furthering our visceralization by filing additional RNA networks in ovarian dysfunction and metabolic dysregulation.

Vit-D functions as a primary upstream regulator capable of altering the exosomal RNA landscape. The noted downregulation of miR-125b may be associated with molecular pathways related to insulin sensitivity and granulosa cell homeostasis, warranting further functional validation, while the inhibition of Lnc-TUG1 and Lnc-NEAT1 indicates a more extensive epigenetic regulation of follicular growth, steroidogenesis, and inflammatory mechanisms. Overall, these consequences further emphasize the pleiotropic and integrative aspects of Vit-D signaling across complex ovarian cellular systems.

The data we observed may support Vit-D as a safe, natural and physiologically rational supplemental therapy for PCOS and from the translation point of view. Modification of exosomal non-coding RNAs could have an effect on both local ovarian physiology, and systemic metabolic pathways associated with insulin resistance, dyslipidaemia and cardiovascular risk. This study strengthens the concept of exosomes as essential players in intercellular communication and delivery, and that they can transport molecular messages indicative for disease status and response to treatment, with exosomal RNAs emerging as biomarkers for precision diagnostics and monitoring treatment.

Future studies should clarify downstream molecular pathways, determine dose-dependent effects, and identify combinatory regimens with other treatments. Clinical trials are essential to confirm these preclinical findings in humans and to assess long-term reproductive and metabolic consequences.

From a biomedical engineering perspective, exosomes represent naturally occurring nanoscale delivery systems capable of transporting regulatory RNA cargo between cells. The characterization of exosomal ncRNA signatures in response to Vit-D intervention aligns with the journal's scope by contributing to the development of precision diagnostics, biomarker discovery, and RNA-based therapeutic strategies.

In conclusion, Vit-D supplementation is a mechanistically informed and therapeutically pertinent intervention that can modulate essential exosomal non-coding RNA networks. This study establishes a conceptual foundation for next-generation, precision-based therapeutics targeting the restoration of reproductive and metabolic equilibrium in PCOS by connecting dietary and hormonal interventions to epigenetic and post-transcriptional control.

Deceleration

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Ethical statement

The research ethics committee has approved the project (Approval ID: IR.IAU.MSHD.REC.1401.042). https://ethics.research.ac.ir/IR.IAU.MSHD.REC.1401.042

Declaration on the Use of AI Tools

No generative AI or AI-assisted tools were used to create, modify, or process any figures, images, or artwork in this manuscript. AI tools were used only for minor grammar and language editing, fully in accordance with Elsevier's policy. The scientific content and conclusions are solely the responsibility of the authors.

Authors Contribution
Fatemeh Attarian**:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft. Mohammad Mahdi Forghanifard**:** Conceptualization, Methodolgy, Writing – original draft, Writing – review & editing. Saeedeh Zafar Balanezhad**:** Conceptualization, Funding acquisition, Investigation, Writing – review & editing. Jina khayatzadeh**:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Availability of data and materials
Data will be made available on request.

Conflict of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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