Protein- Protein Interaction network analysis for Discovering Potent Candidate Drugs in Female Infertility

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Abstract - This article is based on studying the effects of some potent candidate drugs for the treatment of female infertility. Statistically, it is one of the most important issues experienced by many female groups with the subdivision consisting of primary and secondary infertility. In this article first, genes of female infertility were taken from the Gene Expression Omnibus database, and used to identify the protein-protein interaction network (PPI). Then, three protein modules from the PPI network that is under consideration, were recognized, and followed by drawing the mRNA-miRNA interaction sub-network for each protein module. According to this study, by considering the three main modules, ten miRNAs genes had the most important effect in female infertility, with the genes, MRPS22, BCLAF1, and LYAR having strong positive impact on female infertility. From this study, eventually a drug-gene interaction network for female infertility was obtained. Based on our findings, it is recommended that young couples, use low-risk therapies instead of the prescribed drugs. Finally, to make a scientific conclusion, further studies of the effects of these genes, miRNAs, and drugs in the infertility treatment is needed.

KEYWORDS: Female infertility, microRNA, PPI, mRNA-miRNA, Drug-gene network, POF, PCOS.

I. INTRODUCTION

Infertility is clinically defined as a disease that does not occur within 12 months of regular sex [1], affecting both men and women or even one of them [2]. Today, infertility is one of the hot issues in public health and has affected millions of couples around the world. It has attracted the attention of various researchers to prevent and treat infertility [3, 4] in addition to its significant psychological, medical, and economic consequences [3]. Based on the classification of the World Health Organization (WHO), infertility is divided into two primary and secondary groups. Primary infertility is related to women whom have never being pregnant. On the other hand, secondary infertility is attributed to women whom had a successful pregnancy but later stopped being pregnant [5]. Interestingly, primary infertility in advanced countries is a common problem of couples getting pregnant , while secondary infertility is considered a major problem in developing countries [1]. One of the most important factors associated with infertility is age [6]. The highest level of fertility in women and men is observed in the range of 20-30 and until 40 years old, respectively [7]. According to the WHO, from all cases of infertility, about 30-40%, 35-40%, and 20% of the cases of malfunctionings are related only to men, women, and both of the male and female respectively. Woman-related infertility can come from physical problems, non-ovulation, tubular damage, implantation

failure, hormonal disorders, age, infections, and environmental factors [8]. The reproductive system in women consists of different parts, including ovaries, uterine, fallopian tubes, and uterine spans, and disruption of each of these parts will result in the lack of pregnancy and thus infertility [9], which is a major problem in the fertility of women and is related to fallopian tubes [10].

Premature ovarian failure is the loss of the ovaries over the age of 40, which affects 1% of all women worldwide. Polycystic ovary syndrome (PCOS) is a complication in which the ovary is larger than normal. The cystic means a large number of cysts and follicles in ovaries that can affect women's infertility. It is worth noting that 5-10% of women aged 18-44 years have PCOS, indicating that this disease is the most common endocrine disorder among women of childbearing age. Endometriosis is a chronic disease in women characterized by endometrial growth outside the uterine lining endometriosis disorder leading to pelvic pain and infertility [4]. This disease is found in 50% of infertile women, and patients with endometriosis suffer from infertility in 30-50% of cases, suggesting a profound relationship between endometriosis and infertility [11]. Asherman's syndrome is an acquired disease that is associated with intrauterine adhesion, which is created inside the uterus. It interferes with blastocyst planting and causes frequent abortions or infertility [12]. Preeclampsia is a complication that occurs after the twentieth week of pregnancy, which is associated with high blood pressure and is one of the leading causes of death in pregnant women [13]. According to previous research [14], nearly 5% of all couples are not affected by infertility and an unwilling desire for children. The current treatment usually consists of several hormones or other peptides and laboratory methods, including in vitro fertilization (IVF). Therefore, several types of treatments are available for infertile women [14].

Fertility drugs are an important part of treatment, such as natural hormones for infertility treatment [15]. In recent years, the development of fertility technologies and other treatments has increased to overcome infertility; thus, the number of women using fertility drugs are now more than the past [16]. The most commonly used fertility drugs regulating or stimulating ovulation may include clomiphene citrate, letrozole, gonadotropins, bromocriptine, metformin, and cabergoline [17]. Clomiphene citrate is widely applied alone and with intrauterine inoculation (IUI) to treat unexplained infertility without the labeled method. Clomiphene is often used to treat non-explanatory infertility of women by inducing multiple responses and amazing ovarian disturbances [18]. However, the use of fertility drugs that may cause changes in endogenous hormones and multiple ovulation has created concerns about the long-term safety of such drugs [19]. In recent decades, several studies have shown that vitamin D, due to the expression of vitamin D receptor (VDR) and 1-alpha hydroxylase in reproductive tissues such as ovaries, uterus, placenta, pituitary, and hypothalamus, is involved in modulating the reproductive process in women. The expression of VDRs and enzymes involved in vitamin D metabolism in female genital tissues indicates that it is involved in the pathogenesis of endometriosis due to its regulatory properties of the immune and anti-inflammatory systems. In particular, Evidence has been obtained from epidemiological studies regarding the association between vitamin D and female fertility, indicating seasonal variations in pregnancy rates with higher summer peaks in northern countries [20]. Treatment by new medical techniques for treating infertility is now highly common, and different methods are available for treating this complication depending on the type of

the disease and the diagnosis of a specialist [6]. Assisted reproductive technology (ART) has helped many couples overcome infertility by prescribing fertility drugs, intrauterine insemination (IUI), and IVF [7, 21]. Infertile women with endometriosis often need ART such as IVF and fetal transplantation (IVFET) for fertility. However, the achievement (IVFET) in these patients is about half in comparison with women without endometriosis [11]. Herbal treatment in a small number of herbs is employed for treating female infertility and related problems. The aphrodisiac plant is extensively considered for treating infertility in women using traditional indications and phytochemical studies, and evidence of medicinal fungi [22]. Although some kinds of treatments (e.g., hormone therapy and IVF) increase the rate of treatment of infertility, they are insufficient for elevating this rate [23].

In this paper, first, a number of infertility-related genes were collected using the Gene Expression Omnibus (GEO) database with accession number GSE165004. Then, the protein-protein interaction (PPI) network was plotted for these genes by applying the STRING database [24, 25], and three protein modules were extracted from the network by Cytoscape software and ClusterViz plugin. Furthermore, three mRNA-miRNA networks were separately plotted for each module. Then, gene ontology (GO) [26, 27] and pathway enrichment analysis were performed for the genes of the modules, and finally, the gene-drug network was plotted, followed by introducing candidate drugs for the treatment of female infertility. Figure 1 shows the workflow diagram of the current project.

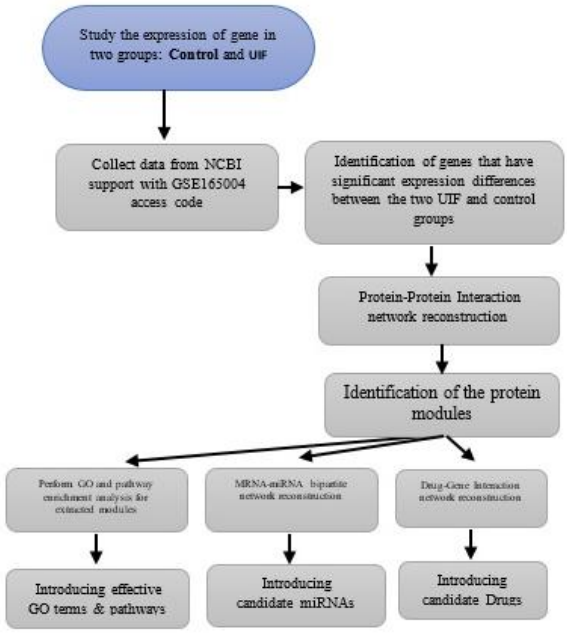


Fig. 1 Workflow diagram of the proposed method *Note*.

II. Materials and Methods

A. *Dataset and preprocessing*

The gene expression data was downloaded from the GEO (Gene Expression Omnibus) using the GSE13507 accession number [28].

First, the genes related to female infertility were extracted from the GEO database, and then 1529 genes related to female infertility with the access code GSE165004 were selected from this site. In order to normalize and calculate DEGs, the GEO2R package has been applied.

B. *PPI network reconstruction*

The PPI network of the obtained genes from the STRING database was plotted based on the study purpose [24]. The STRING database collects and integrates the protein-protein relationship for a large number of organisms by collecting known and predicted data [29, 30]. The parameters used in STRING database are: Network type: physical subnetwork, Active

interaction sources: experiments, Minimum required interaction score:0.4.

Then, Cytoscape software [31] was used to analyze this large network, and a large network of connections between genes and target proteins was obtained accordingly. Cytoscape is applied for visualizing molecular cross-networks and biological pathways and integrating these networks with annotations, gene expression indexes, and other states [32]. For a more detailed analysis of the obtained network, three clusters (each containing different genes) were obtained using the ClusterViz plugin and FAG-EC algorithms.

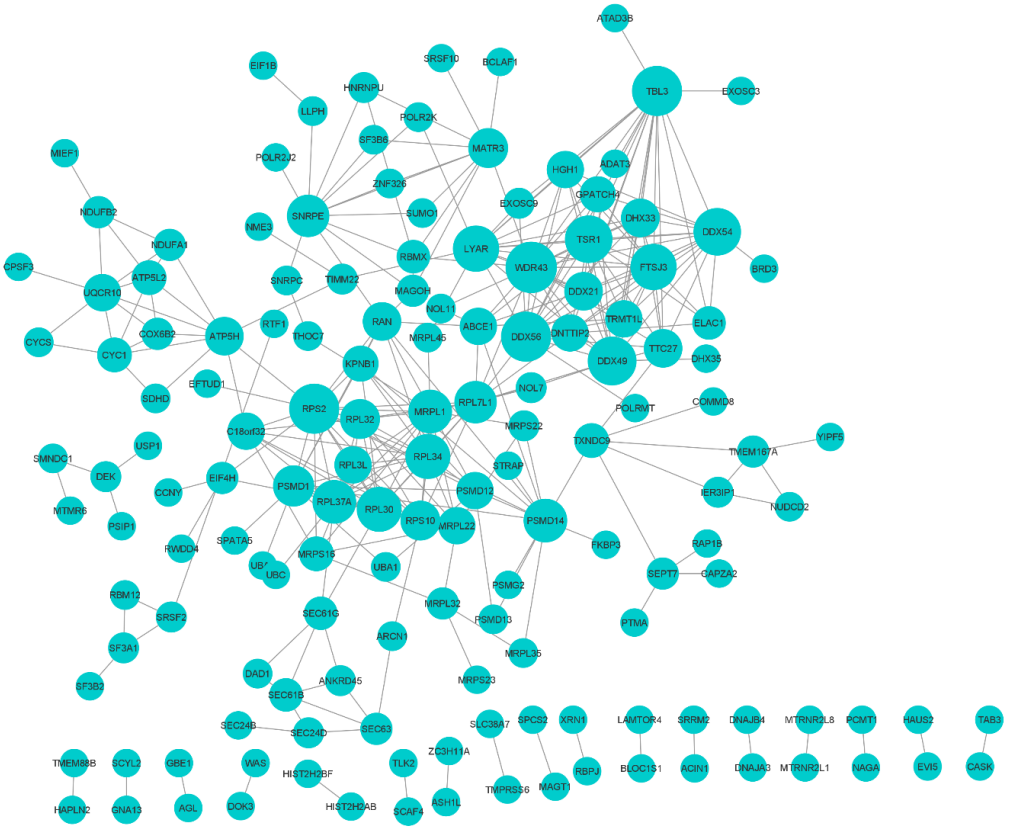


Fig. 2 Protein-protein interaction network.

C. *PPI module extraction*

In order to more accurately analyze the PPI network (Figure 2), and evaluate the performance of proteins, it was necessary to cluster the network with the ClusterViz plugin in Cytoscape software. Biological network clustering is one of the most important approaches for identifying functional clusters and predicting protein performance. In addition, clustering results are highly important for understanding the structure of biological networks. The ClusterViz plugin includes FAG-EC, EAGLE, and MCODE algorithms. The FAG-EC algorithm was employed for clustering the large protein network, with clusters having at least 10 connections between the proteins in that cluster were selected, thus eliminating the weaker clusters.

D. *GO and pathway enrichment analysis*

GO is the world's largest source of information on gene function and the basis for large-scale computational analysis of molecular biology performed by the DAVID online enrichment tool [33]. The pathway enrichment analysis was separately performed for each module to determine in which biological pathways each module is

affected in this study. Then, GO operation was conducted for each obtained module and information about the biological process (BP), cellular component (CC), and molecular function (MF) for the genes of each module based on P<0.05.

E. *miRNA-mRNA network*

After obtaining three modules from the PPI network, the genes of these three modules are separately transferred to the miRWalk 2.0 database, which is an online resource for the prediction of microRNA binding sites [34]. An mRNA-miRNA network [35] was transferred and obtained to determine the link between the gene and the target miRNA. In this section, a large network of genes targeting specific miRNAs for each module was plotted, looking for miRNAs that had the highest impact on female infertility. In Cytoscape software, by selecting the toolbar and the analysis network option, the most effective miRNA having the most role in female infertility was considered for each module.

Table1. The two studied groups

Group	Sample Number of Each Group	Subject Status/Group	Source Name	Tissue
1	24 sample	control	Endometrial _Tissue _Fertile _Control	Endometrial tissue
2	24 sample	patient with UIF	Endometrial_ Tissue _Fertile_ UIF	Endometrial tissue

F. *miRNA set analysis*

The TAM2 [36] database was used to analyze miRNAs and find similar family roots, and finally, the miRNA-family set was obtained for each module. Next, the importance of the obtained families with P-value and false discovery rate (FDR), as previously explained, was determined, followed by selecting five high-grade miRNs for each module and separately entering them into the Tam2 database for analysis to obtain the results.

G. *Drug-gene interaction network*

In this step, to introduce the candidate drugs in female infertility, first, the genes of all three modules were placed in the DGIDB database [37] to determine which drug targets which gene. Then, the gene-drug list was implemented in Cytoscape software to draw a gene-drug interaction network, and finally, high-degree drugs were obtained by selecting the toolbar and analyzing the network option.

III. RESULTS

A. *Dataset*

A total of 1529 genes associated with P<0.001 and the GSE165004 access code were collected as an example of the GEO database. All these genes are involved in female infertility, but looking for genes that play a key role in this regard is challenging. The 1529 obtained genes are presented in Supplementary File S1.

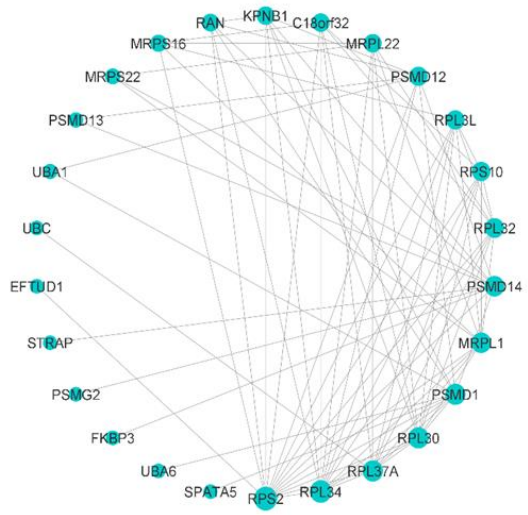
B. *PPI network reconstruction*

The PPI network is plotted using the STRING database and visualized by Cytoscape software. Figure 2 shows the PPI network. ClusterViz plugin was applied for extracting modules, and then three modules were extracted from the PPI network.

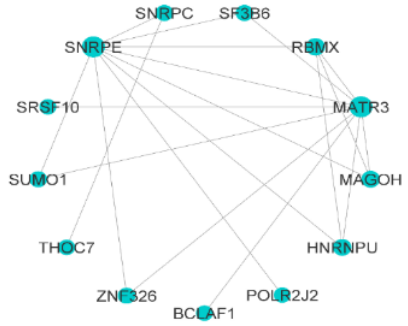
Table 2. List of obtained genes for each module

Module	1	2	3
Gene list	UBA6 UBC RPS10 SPATA5 FKBP3 RPL30 RPL37A RPL32 RPL34 MRPS16 PSMD12 EFTUD1 PSMD1 RAN KPNB1 PSMD14 MRPL22 STRAP MRPS22 PSMG2 PSMD13 RPL3L MRPL1 RPS2 C1Brof32 UBA1	SUMO1 SRSF10 MATR3 BCLAF1 SNRPC ZNF326 SF386 RBMX HNRNPU MAGOH SNRPE ENSG00000267645 THOC7	EXOSC9 POLRMT EXOSC3 ELAC1 TBL3 ADAT3 TSR1 WDR43 RPL7L1 DHX35 DDX54 DDX21 BRD3 HGH1 DNTTIP2 TTC27 TRMT1L GPATCH4 NOL11 ABCE1 NOL7 DHX33 FTSJ3 LYAR DDX56 DDX49 ATAD38

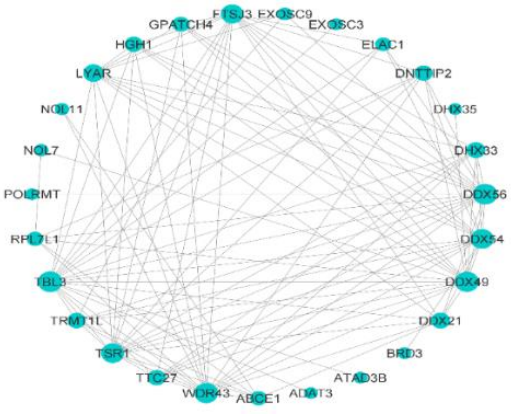
The first, second, and third clusters contain 26 (Figure 3-A), 13 (Figure 3-B), and 27 (Figure 3-C) genes, respectively. The complete list of each cluster is available in Supplementary File S2.



A. Cluster 1



B. Cluster 2



C. Cluster 3

Fig. 3 Initial PPIN clustering

Note. PPIN: Protein-protein interaction networks. Cluster 3 has 27 proteins (blue nodes) and 108 interactions (gray edges). The size of a node represents gene degree.

C. *GO and pathway enrichment analysis*

The DAVID database was employed for the GO of the applied genes in this project, and pathways with a P<0.05 were selected for each module. The ontology indices in all three paths (i.e., BP, CC, and MF) and the KEGG pathway are separately examined for each of the three sub-networks. The obtained results for each module (Table 3) provide an example, and a full file of the results is presented in Supplementary File S3.

Table 3. Results of Gene ontology(GO) for all of the obtained clusters.

Modules	Biological Process		Cellular Component		Molecular Function		KEGG Pathway	
	Term	P-value	Term	P-value	Term	P-value	Term	P-value
1	Cellular macromolecule catabolic process	3/90E-12	Ribosomal subunit	5/70E-14	Poly(A) RNA binding	3/30E-09	Ribosome	4/90E-11
2	mRNA metabolic process	1/30E-09	Spliceosomal complex	9/60E-10	Poly(A) RNA binding	8/90E-10	Spliceosome	1/30E-09
3	RNA processing	1/40E-16	Nucleolus	1/70E-12	Poly(A) RNA binding	1/60E-14	RNA degradation	4/40E-02

Note. GO: Gene ontology.

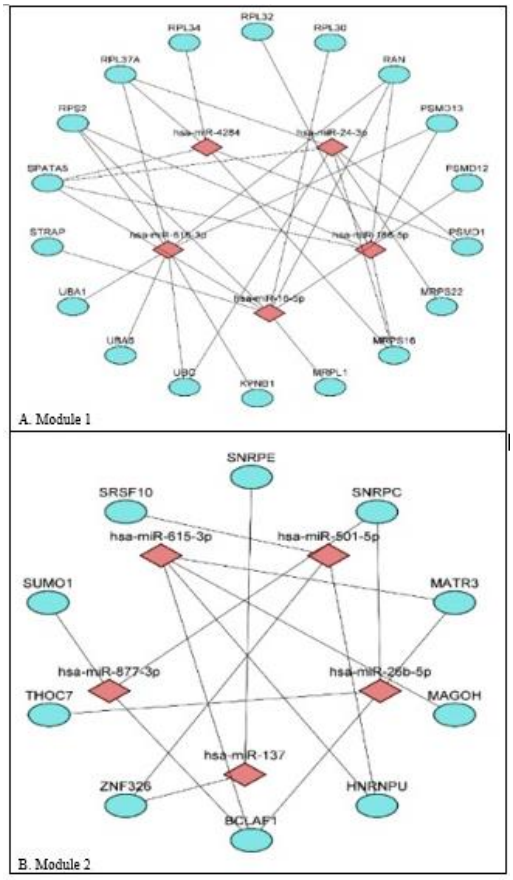
D. *mRNA-miRNA network*

The next step was to separately enter the genes of these three modules into the miRWalk 2.0 database and obtain the target miRNAs. Then, five miRNAs were selected from each module as candidates for the mRNA-miRNA network, which had the greatest effect on female infertility. After analyzing the mRNA-miRNA network (Supplementary File S4) by Cytoscape for each module, five obtained high-degree miRNAs and a bipartite network between these miRNAs and mRNAs for determining this gene were drawn from five high-degree miRNA targets. Eventually, three mRNA-miRNA bipartite networks were obtained for each module.

The first module contained five high-degree miRNAs, including hsa-miR-615-3p, hsa-miR-16-5p, hsa-miR-186-5p, hsa-miR-24-3p, and hsa-miR-4284 (Figure 4-A).

The second module encompassed five high-degree miRNAs, including hsa-miR-615-3p, hsa-miR-26b-5p, hsa-miR-877-3p, hsa-miR-501-5p, and hsa-miR-137 (Figure 4-B).

The third module consisted of five high-degree miRNAs, inclduing hsa-miR-16-5p, hsa-miR-23b-3p , hsa-let-7b-5p, hsa-miR-802, and hsa-miR-484 (Figure 4-C).



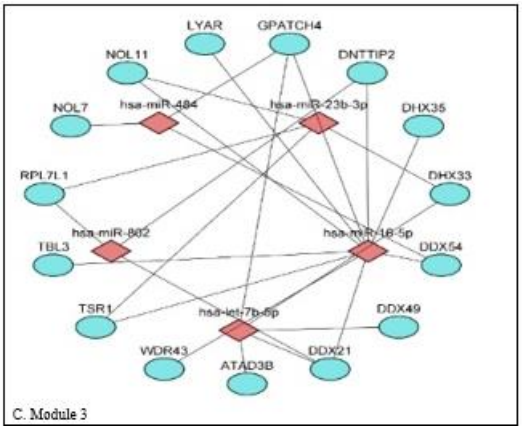


Fig. 4. A high-degree mRNA-miRNA network.

E. *Enrichment of hub miRNAs*

In this article, we focus on the role of miRNAs due to various factors involved in female infertility. Our miRNAs should be tested for biological pathways involved in infertility, for this purpose, we use the TAM2 database for the ontology of miRNAs involved in female infertility.

Similar to GO, P< 0.05 and an FDR index were used to validate miRNAs, which point to different roles of this family of miRNAs in various diseases, including female infertility. An example of miRNA family information for all three modules is presented in Table 4, and full information can be found in Supplementary File S5.

Table 4. Result of enrichment analysis for the obtained miRNAs. These information obtained from the TAM2 online database.

Modules	P-value	FDR	miRNA
Family module 1	1/32E-04	7/35E-03	hsa-mir-16-1 and hsa-mir-16-2
Family module 2	8/42E-03	1	hsa-mir-26b
Family module 3	9/46E-05	9/89E-03	hsa-mir-16-1 and hsa-mir-16-2
Family module 4	9/46E-05	4/67E-03	hsa-mir-16-1 and hsa-mir-16-2

Note. FDR: False discovery rate.

F. *Drug-gene interaction network*

Finally, it was necessary to introduce drugs that can play the most important role in female infertility. Therefore, as in previous steps, a network had to be established between drugs and their target genes. The genes of all three modules were transferred to the DGIdb database in order to construct the drug-gene interaction network (Figure 5).

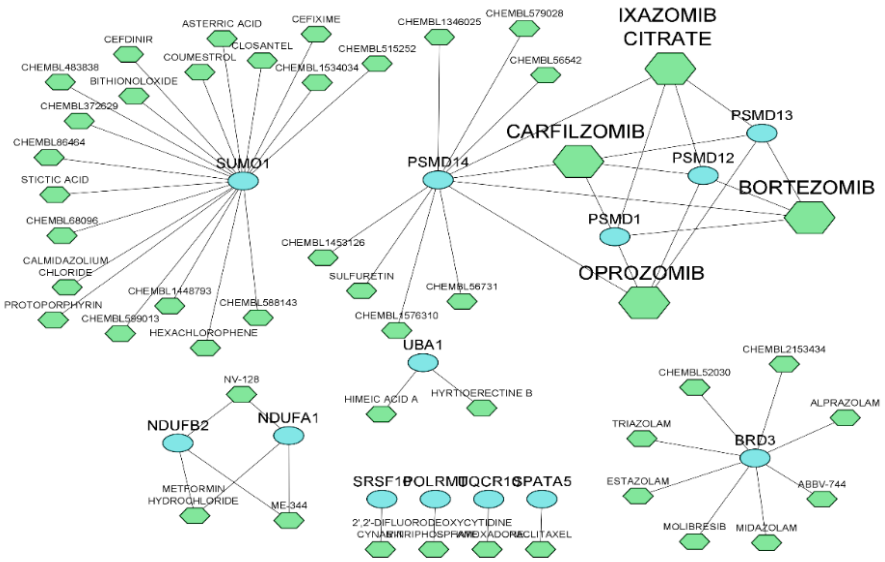


Fig. 5 The drug-gene interaction network consisting of 60 drugs and a gene (node) with 62 interferences (edges). The green hexagon nodes represent drugs and the blue circle nodes represent genes.

As shown in Figure 5, a highly large network of different drugs and genes was obtained via this database. With the release of minor drugs using Cytoscape software, a number of drugs with the greatest possible impact on female infertility were considered to be candidates (Table 5).

Table 5. Candidate drugs in women's infertility

Recommended Drugs	Degree
OPROZOMIB	4
BORTEZOMIB	4
IXAZOMIB CITRATE	4
CARFILZOMIB	4

IV. DISCUSSION

A summary of the project steps is explained in this part. Eventually, the discussion is divided into three parts focusing on miRNAs, genes, and drugs.

As mentioned earlier, the current study addressed female infertility. To this end, first, the gene expression dataset was collected from the NCBI repository, and then the PPI network was reconstructed thanks to the STRING database Next, Cytoscape software was used to construct the network, followed by studying protein modules each containing several genes to identify important genes involved in the disease.

Subsequently, GO was performed for each module, separately. Finally, high-degree candidate drugs were obtained, targeting many genes.

Five high-degree microRNAs were selected for each module, and their role in female infertility. Among the microRNAs, all three modules were separately examined from the first module, and hsa-miR-615-3p was the most important miRNA based on a degree of 8 in the biperiate network. Previous research identified the role of this module in PCOS [38]. Another study reported the role of hsa-miR-16-5p (with a degree of 7) in ovarian stimulation through the expression of exogenous progesterone with an effect on VEGF protein and angiogenesis [39]. Likewise, hsa-miR-186-5p (with a degree of 6) overexpression has been observed in a variety of infertility-induced abortions in women with endometriosis [40]. Although hsa-miR-24-3p (with a degree of 6) is one of the biomarkers of breast and prostate cancer [41, 42], the role of this miRNA has been confirmed in infertility, especially in infertility related to sperm motility in men [42]. Similarly, hsa-miR-4284 (with a degree of 5) is involved in PCOS by reducing endothelin-2 and hypoxic signaling pathways in female granulosa-lutein cells [43]. The findings of a study demonstrated the role of hsa-miR-26b-5p in the infertility of both normospermic men [44] and women with endometriosis [45]. Although a limited body of research is available regarding the role of hsa-miR-877-3p in all types of female infertility, there is some information about the role of this miRNA in uterine aging processes related to non-welding IVF [46]. Moreover, although the role of hsa-miR-501-5p in primary ovarian cancer has been identified [47], there is no precise reference to the contribution of this miRNA to female infertility, and this issue necessitates further research potential to investigate its role in female infertility. The role of hsa-miR-137 and hsa-miR-802 has been further elucidated in testicular germ cell carcinoma [48]. Increased post-transcriptional expression of hsa-miR-23b-3p in the endometrium of women with abnormal uterine bleeding has been reported in a pilot study [49]. In a study on female mice with uterine endometriosis, a decrease was found in hsa-miR-7b-5p expression [50]. hsa-miR-484 overexpression has been observed in signaling processes within oocytes, leading to PCOS [51].

In the next step, the important genes obtained in this article were examined. MRPS22 with a degree of 1 contrary to expectations, an important gene was from Module 1, which in research has pointed to mutations in the mitochondrial ribosomal protein MRPS22 lead to primary ovarian failure [52].

The role of the 3rd degree RPL37A gene from the first module is more related to male infertility [53].

The most important gene from the second module with degree 4 was the BCLAF1. Recent evidence suggests that this gene may play a regulatory role in pre-mRNA (mRNA transplantation or processing) and thus it contributes to molecular level activities in female infertility [54].

Another gene from the second module was the SNRPC, and research has shown that the overexpression of this gene is a potential biomarker candidate for osteosarcoma [55]. DDX21 (DExD-Box Helicase 21) with a degree of 2 from the second module, which is an RNA helicase, can play a role in male fertility and sperm production [56]. From the third module, GPATCH4 and DDX21 with a degree of 3 were the most important genes, and the role of the DDX21 was mentioned in the previous paragraph. GPATCH4 also plays a key role in neurilemmomatosis diseases [57].

To pick out the drugs, four drugs with the highest degree were selected from the drug-gene network (Figure 5). The highest degree (with degree 4) among the drugs was related to oprozomib, bortezomib, ixazomib citrate, and carfilzomib from the drug-gene network (Table 3).

Oprozomib is a small molecule that inhibits a large protein complex called a ‘proteasome’, which contributes to breaking down abnormally folded or damaged proteins. The proteasome also adjusts several proteins that have a role in cell growth, division, and survival. Although this anti-inflammatory drug exerts the main role in the treatment of certain cancers (e.g., leukemia) due to its degree in the drug-gene network, more clinical research is required in this regard [58, 59].

The next drug in this drug network is bortezomib, which is one of the anti-neoplastic drugs that is used in the treatment of cancers, especially myeloma [60]. The link between this drug and female infertility can be explained by the fact that this drug has caused infertility after cancer treatment or other specific side effects in many cases due to its toxicity and side effects [61, 62]. Ixazomib citrate and carfilzomib are also applied for treating leukemias, especially myeloma, but no clear effects have been reported on the role of these drugs in infertility [61, 63-65]. Metformin, which had a degree of 2 in the drug-gene network, has been suggested as a low-risk alternative drug in the treatment of PCOS-associated infertility in women [66]. Another obtained drug was paclitaxel, which is one of the chemotherapy drugs used in endometriosis cancer of the uterus. According to the finding of this study, this drug also confirms the correctness of the previous bioinformatics work [67].

V. CONCLUSION

In general, the present study mainly sought to evaluate female infertility using bioinformatics methods to introduce several microRNAs and genes, and finally, candidate several drugs for this disease. For this reason, the PPI network was drawn, followed by drawing effective genes in female infertility, mRNA-miRNA networks, and the drug-gene network, and examining the important members of these networks. According to the findings, among the three main modules, hsa-miR-615-3p, hsa-miR-16-5p, hsa-miR-186-5p, hsa-miR-4284, hsa-miR-26b-5p, hsa-miR-877-3p, hsa-miR-23b-3p, hsa-miR-7b-5p, hsa-miR-501-5p, and hsa-miR-484 had the most important effect in female infertility. hsa-miR-24-3p was a miRNA that played no direct role in this disease. However, due to the role of this miRNA as a marker of breast and prostate cancer, it can be studied as novel miRNA in infertility. Two microRNAs (hsa-miR-137 and hsa-miR-802) are more common in testicular diseases in men and cancers and require clinical research to link them to female infertility. Among the genes, MRPS22, BCLAF1, and LYAR are involved in female infertility, while the remaining mentioned genes in the Discussion Section are involved in other diseases and need further research as new

candidate genes among the four drugs reviewed in this article (Oprozomib, bortezomib, ixazomib citrate, and carfilzomib). Contrary to expectations, these drugs have no role in the treatment of female infertility, except for oprozomib, which may be involved in female infertility through molecular pathways. The rest of these drugs mainly contribute to the treatment of leukemia myeloma type.

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