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Upregulation of Oxidative Phosphorylation Genes in Cumulus Cells of The Polycystic Ovary Syndrome Patients with or without Insulin Resistance

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Abstract

Objective: The relationship between oxidative stress (OS), insulin resistance (IR), and polycystic ovary syndrome (PCOS) is an important medical issue in human reproduction. Some of the oxidative phosphorylation (OXPHOS) genes have been previously studied in granulosa and muscle cells of PCOS patients. Cumulus cells (CCs) remain close to the oocyte even after ovulation. This research has been designed to compare the expression of OXPHOS genes in CCs of PCOS, with or without insulin resistance.

Materials and Methods: In this experimental study, patients were included in PCOS insulin-resistant, PCOS insulin-sensitive (IS), and control (fertile women with male infertility history) groups. The expression of *NCF2, TXNIP, UCP2, NDUFB6, ATP5H, COX7C, NDUFA3, SDHA,* and *SDHB* was studied by real-time polymerase chain reaction (PCR), and normalization was performed considering *HPRT1* and *CYC1* as reference genes. One-way ANOVA and Tukey test were used for data analysis.

Results: The results showed that the expression of *NCF2, TXNIP, UCP2,* and *ATP5H* was significantly higher in the IR group than IS and control groups (P<0.01). *NDUFB6* showed the highest expression in the IS group, which was significantly different from other groups (P<0.01). The other genes of interest, except *COX7C,* were observed with the most transcriptional levels in the IS group, although there was no significant difference for those genes.

Conclusion: Altered expression of genes involved in mitochondrial function compared to the control group in CCs of both IR and IS categories of the PCOS patients suggests that alteration in OXPHOS genes can contribute to the pathophysiology of PCOS.

Keywords: Cumulus Cells, Insulin Resistance, Mitochondrial Function, Polycystic Ovary Syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine diseases in females and a complex disorder that affects women who complain of anovulatory infertility (1, 2). Even though women with PCOS are generally characterized by the generation of more oocytes retrieved during *in vitro* fertilization (IVF), the lower oocyte quality and unsuccessful pregnancy outcomes after IVF and embryo transfer remain controversial (3).

Women with this syndrome suffer from symptoms caused by androgen elevations and reproductive disorders as well as other metabolic disorders, including polycystic ovaries, significant prevalence of insulin resistance (IR), chronic inflammation, and oxidative stress (OS) (2, 4). According to Liu et al. (5), alterations in the lipid-related genes' expression in PCOS patients are known to contribute to hyperandrogenism, IR, OS, and infertility in PCOS patients. Adenosine triphosphate (ATP), created by oxidative phosphorylation (OXPHOS) in the mitochondria, accounts for more than 90% of the energy in eukaryotic cells (6). Evidence has shown that inadequate energy generation is linked to impairments in fertilization and embryo development, as the relationship between cumulus cells (CCs), the embryo's cellular ATP levels, and its implantation has been established previously (7). OS and IR both play an essential role in the pathophysiology of PCOS. One of the main reasons for low oocyte quality is OS. The CCs in a healthy female tract work with the physiological environment to protect the oocyte from the antioxidant defenses if required for optimal development. In the mammalian ovary, oocytes

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are housed inside follicles, which, when fully matured, are composed of somatic granulosa cells (GCs) and filled with follicular fluid, and a specific subtype of GCs called CCs surround the gamete in the antral follicle (8).

CCs contribute to the energy supply for oocyte maturation. Therefore, fertilization, early embryonic development, and the quality of the oocytes are highly dependent on CCs. these cells play essential roles in the oocyte's growth and maturation processes. They deal with substrates that the oocyte cannot metabolize and shield the oocyte from OS damage (9). Oocytes derived from follicles with defective CCs development have limited potential for implantation. Mitochondrial dysfunction is the main reason for producing the majority of reactive oxygen species (ROS), which induces OS, and is also linked to IR and as a result, it may be a key player in the pathogenesis of PCOS. The direct relationship between mitochondrial dysfunction and IR is yet unknown, and it is also uncertain whether mitochondrial abnormalities are a primary defect or a subsequent occurrence concurrently with IR (10). There are two theories explaining how mitochondrial malfunction contributes to IR. Firstly, the reduced oxidation of fuels, such as fatty acids, leads to lipid accumulation, which inhibits insulin signaling (11). Therefore, a potential connection between mitochondrial malfunction and IR could be caused by an accumulation of fatty acid mediators. Another pathway linking mitochondrial dysfunction to IR is alterations in electron flow within the electron transport chain (ETC).

PCOS is classified as a weak-oxidative state and has shown a relationship between increased OS and decreased antioxidant concentrations. Mentioned conditions and mitochondrial dysfunction can cause ovarian follicle damage, fractions in nuclear and mitochondrial DNA, OXPHOS defect, impaired ROS production by mitochondria, and, eventually, cell apoptosis (10, 12).

Limited studies have investigated the expression profile in the CCs of PCOS patients. In this study, we examined the differences in the transcriptional level of OXPHOS genes between PCOS and healthy individuals and the differences between patients with or without insulin resistance.

Materials and Methods

Patients

In this experimental study, all the cases were chosen from individuals who were referred for fertility treatment by assisted reproductive technologies. This study was approved by the Institutional Ethics Committee (IR.ACECR.ROYAN.REC.1398.70) in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants. Twenty-three women under the age of 36 participated in this study, who were candidates for intracytoplasmic sperm injection (ICSI). In this case, the age cutoff of 36 years was used as the first inclusion criteria because of the accelerated decline in fecundity and oocyte quality at higher ages (13). Fifteen patients with PCOS were divided into two groups of IR or IS according to fasting insulin levels (FI, cutoff: 12 mU/L) and the homeostasis model assessment of IR (HOMA-IR, cutoff: 2.57). Our criteria for categorizing the patients were like our recent study (14). PCOS patients were diagnosed based on the Rotterdam 2004 criteria and whose partners had normal spermogram results (2). In addition, women with regular menstrual cycles, normal sonographic appearance of ovaries, and no diabetes or clinical signs of PCOS but, due to male factors, were referred to infertility clinics were included in the control group. Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as follows: [(fasting serum insulin [mU/L]×fasting serum glucose [mmol/L])/22.5] (15).

The patients who had been affected by thyroid or hormonal disorders, diabetes, ovarian hyperstimulation syndrome (OHSS), or had poor responses to ovulation stimulation were excluded.

Sample collection

Controlled ovarian stimulation commenced on day three of the cycle. All cases were treated according to the stimulation protocol by recombinant follicle-stimulating hormone (rFSH, Gonal-F, Serono, Switzerland). When the ovarian follicles had grown to a diameter of 12 mm, they were treated with subcutaneous injections of a gonadotropin-releasing hormone (GnRH) antagonist, cetrorelix (Cetrotide®, Merck Serono, Germany). The protocol included daily Cetrotide® subcutaneous injections until the criteria for administering human chorionic gonadotropin were met. When more than three follicles reached diameters of 18 mm at least, and estradiol levels of 1000-4000 pg/mL were detected, intramuscular injection of 10000 IU of human chorionic gonadotropin (hCG, Pregnyl®, Organon, Netherlands) or subcutaneous injection of 250 μg Ovidrel (Merck Serono, Germany) was performed. Follicle collection was done 36 hours after the hCG injection. Afterward, blood and extra cells were removed from the cumulus-oocyte complexes (COCs) by washing 3 to 5 times in G-IVFTM medium (Vitrolife, Sweden); then 80 IU of hyaluronidase (Sigma, USA) was added for Oocyte denudation. CCs were extracted from the oocytes in the metaphase II (MII) stage. Immediately after oocyte denudation, CCs were washed with phosphate-buffered saline (PBS), RNA protection was added, and the cells were snap-frozen in liquid nitrogen and stored at -80˚C until RNA extraction.

RNA extraction and cDNA synthesis

The PicoPure RNA Isolation Kit (Arcturus, USA) was used to extract the total RNA from the CCs of each sample. The RNA was treated by RNase-free DNase I (Qiagen, USA). Then, the purification of RNA was evaluated by a Nanodrop 2000 Spectrophotometer (Thermo, USA). Following the RNA extraction, cDNA was synthesized using QuantiTect whole transcriptome cDNA synthesis kit (Qiagen, USA) according to the manufacturer's instructions.

Real-time polymerase chain reaction

The relative expression of genes of interest was investigated by quantitative real-time polymerase chain reaction (qPCR) on a Step One Plus Real-time PCR system (Applied Biosystems, USA). SYBR Green reagent (Ampliqon, Denmark), specific primers, and 25 ng cDNA, making the total volume of 20 µl, were performed in triplicate on a 96-well plate for amplification. The system was set for a cycle of 15 minutes at 95˚C followed by 40 cycles of 15 seconds at 95˚C and 1 minute at 60˚C.

The selection criteria for the target genes included involvement in OXPHOS, their presence in the OMIM database, and association with either PCOS or diabetes, according to available publications in Pubmed. In this regard, OXPHOS genes were selected based on previous publications (16) and then, shortlisted based on the aforementioned selection criteria. Finally, NCF2, TXNIP, UCP2, ATP5H, NDUFB6, COX7C, NDUFA3, SDHA, and SDHB were selected for the current study. Table 1 represents their association with PCOS and diabetes. In addition, the mRNA sequences of the genes of interest were taken from the NCBI site to design the primers. Then, the primers were designed by Perl Primer, and selected primers were rechecked by Primer Blast, Gene Runner, and UCSC in-Silico PCR tool sites. Primer sequences are available in Table 1.

Table 1: The selected OXPHOS and reference genes primer sequences used for the qPCR

Gene	Primer sequences (5´-3´)	Product size (bp)	Diabetes related	PCOS related
NCF2	F: TGATGCTAATAACCAGACAACAG R: TTCATTCACCTTTGATAACACCA	142	✓	✓
TXNIP	F: CTGTGAAGGTGATGAGATTTCCA R: TATGATTGCCTCTGACTGATGAC	158	✓	✓
UCP2	F: CACAGATGTGCCCCCTACTG R: TCCTTGGATCTGTAACCGGAC	118	✓	✓
SDHB	F: AATGAAGTTGACTCTACTTTGACC R: ATAGAAGTTGCTCAAATCGGG	201	✓	-
NDUFB6	F: TCACTCATGTACTTGTACCTGTCTG R: CTCCAGAATTGTATCACCAGGGA	122	✓	-
COX7C	F: TGTCCTCATTCTCTGCGCCT R: ATGGCAAATTCTTCCCAGGGC	128	✓	-
NDUFA3	F: CCCCCATTGAGCCCCTACTT R: CATCATCACGGACGGGCACT	85	-	✓
SDHA	F: CAACTACAAGGGGCAGGTCC R: ACATGCCCGACCAAAGACAA	157	✓	✓
ATP5H	F: CGTTACTTGCTGCGGAGGAC R: GCAAGTTTTCGCCCAGCCAT	73	✓	✓
HPRT1	F: TAATCCAAAGATGGTCAAGGTC R: CACAAACATGATTCAAATCCCTG	161	Reference gene	
CYC1	F: CTACCATGTCCCAGATAGCC R: CATCATCAACATCTTGAGCCC	103	Reference gene	

Statistical analysis

This study was designed to evaluate the relative expression of specific genes, involved in OXPHOS from the CCs of the PCOS patients compared to the control women. According to our previous study (14), genes with a threshold cycle (CT) variation of less than one among all replicates in the current study, including patients and controls, were selected as reference genes for the accuracy and reliability of qPCR comparative data analyses (17). The normalized ΔCT value of each sample was computed using Cytochrome c1 (*CYC1*) and hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) for these selected reference genes. The relative expression level of the chosen genes was assessed using the fold-change $2^{-\Delta\Delta CT}$ formula. The Kolmogorov-Smirnov test verified the data's normal distribution. The data analysis was performed using SPSS Statistics version 21 (IBM, SPSS Inc., USA) using one-way analysis of variance (ANOVA) followed by Tukey's test for the comparison between IR, IS, and control groups. Independent sample t test was performed to compare PCOS and control groups. The Graph-Pad Prism 7 software (Graph-Pad Software Inc., California) was used in bar graph preparation based on mean values, and the error bars depicted the standard error of the mean (SEM). A P<0.05 was considered a significant alteration.

Results

This study was designed to evaluate the relative expression of specific genes, involved in OXPHOS from the CCs of PCOS patients compared to the control women. None of the studied clinical parameters significantly differ among the three groups (P>0.05, Table S1, See supplementary Online Information at www.celljournal.org). A comparison of cycle characteristics and IVF/ICSI outcomes in controls compared to PCOS patients showed significantly higher numbers of MII oocytes and good-quality embryos in control and insulin-resistant groups (Table S2, See supplementary Online Information at www.celljournal.org).

The relative expression of genes that had significant differences between groups has been shown in Figure 1. The qPCR analysis indicated significant upregulation of *NCF2, TXNIP, UCP2,* and *ATP5H* in the IR than the control with P values of 0.003, <0.001, <0.001, and 0.004, respectively. Also, their expression in the IR was higher than that of the IS, with P values of 0.005, 0.003, <0.001, and 0.015, respectively. *NDUFB6* had higher expression in the IS than the control and the IR with P values of 0.014 and 0.001, respectively. These four genes have known roles in both PCOS and diabetes (Table 1).

The relative expression of *COX7C, NDUFA3, SDHA,* and *SDHB* are indicated in Figure 2. Except for *COX7C* highest expression in the sensitive group, all of these genes had higher expression in the resistant group compared to the other groups. However, none of the differences was statistically significant.

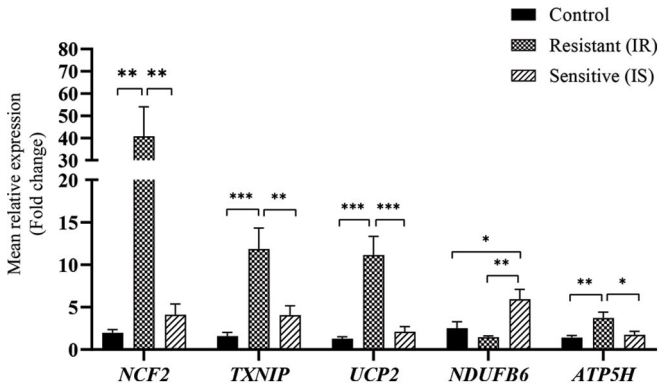

Fig.1: The relative expression of genes with significant differences among groups. Values are presented as mean ± SEM. *; P<0.05, **; P<0.01, and ***; P<0.001.

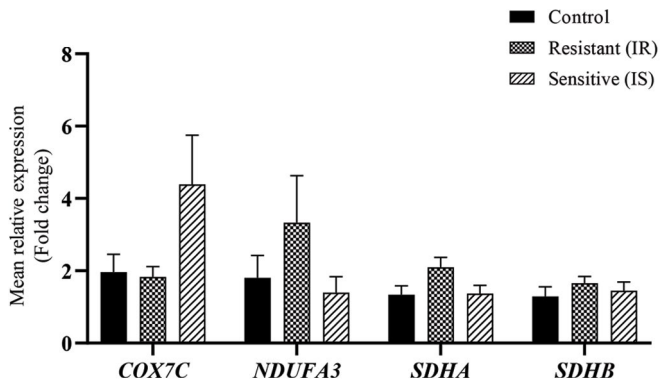

Fig.2: The relative expression of genes without significant differences among groups. Values are presented as mean ± SEM.

Comparing the relative expression of OXPHOS genes in control and PCOS samples, regardless of the insulin response, demonstrated that *UCP2, TXNIP, NCF2,* and *ATP5H* had a higher expression, with P values of <0.001, <0.001, 0.002, and 0.002 in samples from patients diagnosed with PCOS than the control samples (Fig.3).

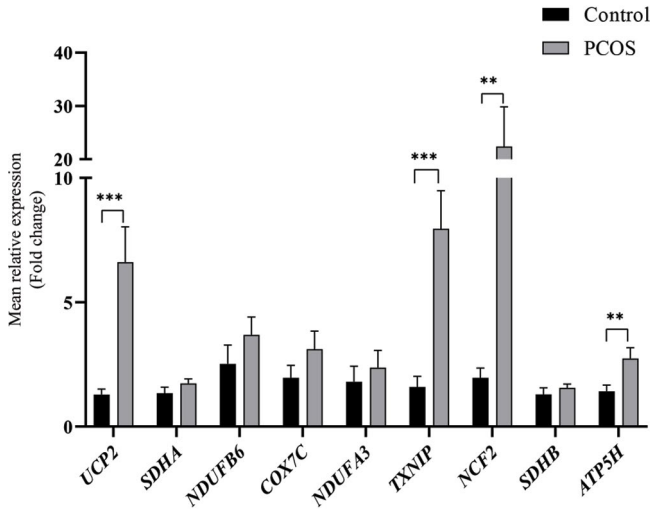

Fig.3: The relative expression of OXPHOS genes in control and cases regardless of the insulin response. Values are presented as mean ± SEM. **; P<0.01 and ***; P<0.001.

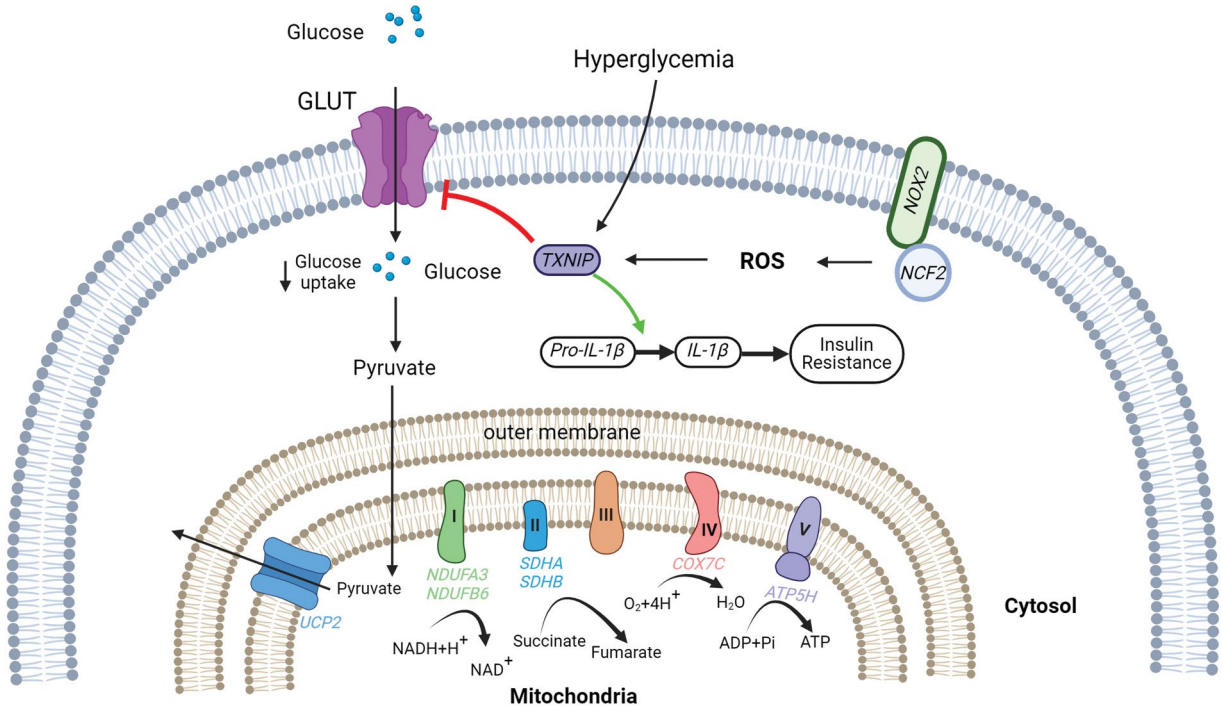

Fig.4: Possible functions of the OXPHOS genes were investigated in this study. Related details have been addressed in the discussion. The graph has been designed in BioRender.

The main interaction between the investigated genes has been highlighted in Figure 4. The genes of interest typically play crucial roles within mitochondria, contributing significantly to the maintenance of cell metabolism and overall homeostasis.

Discussion

To assess the impact of PCOS and IR on the expression of OXPHOS genes in CCs, it was essential to first evaluate their influence on clinical parameters and the outcomes of assisted reproductive technology (ART). According to our variables, non-significant differences in clinical parameters of the patients indicate adjustment of the groups for further evaluations. Regarding cycle characteristics and ART outcome, PCOS was associated with a lower oocyte maturity rate, but this did not affect the fertilization rate. Moreover, PCOS negatively affected the number of good-quality embryos compared to the control, which is in accordance with previous findings (2, 3).

Most metabolites and energy are provided by CCs during oogenesis, suggesting that the mitochondria in CCs are crucial for oocyte development and rejuvenation (18). On the other hand, mitochondrial dysfunction in oocytes has been found in women with PCOS (1). In this study, CCs were considered as available samples to evaluate the physiological functions of the oocytes, and they were separated from the oocytes during ART using a non-invasive method. The primary purpose was to assess the expression levels of some OXPHOS genes from CCs of both insulin-resistant and insulin-sensitive PCOS patients. Insulin is a central regulator of OXPHOS, which can directly affect mitochondrial function, and conversely, mitochondria plays a pivotal role in insulin function (19). On the other hand, ROS acts as a necessary molecule for some biological functions like involvement in signaling; it can also interact with lipids and nucleic acids and cause mutation in DNA, also, ROS can reduce the general quality of the oocytes by inducing OS (20).

A previous investigation has shown that patients with PCOS are exposed to OS, and the level of that was higher in IR patients than in the IS group (12). Considering the necessity of interaction between mitochondrial function and nuclear genome to maintain cellular homeostasis, it has been revealed that women with PCOS have higher DNA damage markers (4). Any damage to the mitochondria will affect the cellular integrity, and vice versa may lead to pathological conditions. In recent years, insights into oocytes, GCs, or CCs in women with PCOS have been provided through microarray or RNA sequencing studies (21).

According to a recent study, GCs in PCOS exhibit impaired glucose metabolism, contributing to IR and altered cellular signaling pathways. Mitochondria plays a crucial role in these cells, contributing to energy production and insulin secretion (22). Transcriptomic studies have revealed altered expression of genes related to OXPHOS and mitochondrial function in PCOS GCs.

On the other hand, mitochondrial dysfunction in PCOS can lead to the production of ROS, causing OS, disrupting the balance between oxidants and antioxidants, and damaging DNA and mitochondrial DNA (mtDNA) (22, 23). Genome mutations, particularly in mtDNA genes, can induce mitochondrial dysfunction, insulin resistance, and hormonal imbalances. In addition, the dysregulation of glycolysis and glyoxylate production in GCs leads to insufficient energy substrates for both GCs and oocytes, resulting in excessive ROS production due to dysfunctional mitochondrial oxidative phosphorylation. Moreover, is revealed that elevated mitochondrial ROS and altered insulin signaling pathways impact the maturation of oocytes in the PCOS GCs. This incapacity of GC mitochondria to maintain ATP synthesis promotes OS, impairs glucose metabolism, and negatively influences oocyte maturation in PCOS (23).

NCF2, as a rate-limiting cofactor of *NADPH* oxidase and TXNIP as a suppressor of thioredoxin disulfide reductase function, had an increased expression, leading to elevated OS (24). Another recent study also suggested that *NCF2* and other *NADPH* genes are highly expressed in PCOS, contributing to OS. IR commonly associated with PCOS may influence the expression of these genes, as indicated by their elevated levels in PCOS patients (25). The results of our study also illustrated an overexpression of NCF2 and TXNIP in the PCOS group compared to the controls, while only in the insulin-resistant group the differences were significant.

UCP2 plays a crucial role in energy metabolism regulation by uncoupling oxygen consumption from *ATP* synthesis, and it is implicated in various physiological processes such as thermogenesis, lipid metabolism, and the prevention of OS accumulation (26). *UCP2* is an exporter of pyruvate from the mitochondria (27). Upregulation of pyruvate in the PCOS group compared to the control group has been previously reported in rat models (28). The expression of the *UCP2* gene in PCOS patients appears to be influenced by a combination of factors, including IS, androgen synthesis, and genetic variations. The increased *UCP2* expression in previous research has been correlated with IR in PCOS patients, suggesting a potential regulatory role of insulin in *UCP2* gene expression (29). We found the highest level of expression of *UCP2* in IR compared to both IS and control groups,

NDUFB6 subunit of mitochondrial respiratory chain complex *I* has been found to be more highly expressed in PCOS ovaries (30). Additionally, the ethnicity-specific expression of *NDUFB6* suggests contributing to the heterogeneity of PCOS manifestations across different populations, emphasizing the importance of genetic variations (31). Ling et al. (32) have indeed demonstrated that IR is associated with the downregulation of specific OXPHOS genes, including *NDUFB6*. Additionally, they have highlighted that the dysregulation of the *NDUFB6* gene expression is influenced by various factors, including genetic, epigenetic, and nongenetic factors in human skeletal muscle. Significant upregulation of NDUFB6 in the sensitive group and lower expression of this gene in the resistant group compared to the control group can be discussed by such factors, which suggests a complex interplay between insulin resistance, gene expression, and epigenetic regulation in the context of OXPHOS that needs to be evaluated in future studies with more samples. Interestingly, in our research, *NDUFB6* was the only gene that downregulated significantly in the IR group.

ATP5H, a subunit of mitochondrial complex V (ATP synthase), plays a crucial role in catalyzing ATP synthesis using an electrochemical gradient of protons within the inner mitochondrial membrane during oxidative phosphorylation. In the context of PCOS, Skov et al. (33) have reported reduced expression of this gene in skeletal muscle of PCOS patients. A recent study in 2019 suggests a decrease in the expression of *ATP5H* in PCOS patients, indicating a potential link between mitochondrial dysfunction and PCOS (1). On the other hand, higher expression of ATP5H in the present study compared to further research might be due to various sources of biopsies. Therefore, the expression of the *ATP5H* gene in PCOS patients may be influenced by mitochondrial dysfunction and the interconnected molecular mechanisms associated with PCOS. The exact role and implications of *ATP5H* in PCOS could be part of ongoing research and might still need to be fully elucidated Upregulation of *NDUFB6* and *ATP5H* as subunits of the first and the last parts of the mitochondrial respiratory chain (complex I and V) without significant difference in the investigated subunits of complex II (*SDHA* and *SDHB*) and *COX7C* subunit of complex IV in our study might highlight their crucial role in response to PCOS condition.

NDUFA3, the other gene of complex *I* that was evaluated in this study, had approximately the same transcriptional levels in the control and the IS groups. *NDUFA3,* as a component of the mitochondrial respiratory chain complex I, is involved in transferring electrons from *NADH* to ubiquinone, a critical step in generating ATP. Some recent studies have reported the downregulation of *NDUFA3* in the skeletal muscle and respiratory complexes of women with PCOS. This downregulation is associated with mitochondrial dysfunction, impacting energy production at the cellular level (1).

SDHA and *SDHB* are integral components of *SDH*, connect the Krebs cycle to the ETC, play an essential role in the overall process of OXPHOS and contribute to the electron flow of the respiratory chain. Elevated expression of *SDHA* has been observed in the context of PCOS (34). However, specific information on the expression of *SDHB* in PCOS is not readily available.

COX7C is a vital component of the cytochrome c oxidase complex, serving as the final enzyme in the mitochondrial ETC. Numerous studies have shown that *COX7C* is downregulated in women with PCOS. This downregulation has been linked to mitochondrial dysfunction observed in PCOS, leading to consequences

for cellular energy production. The reduced expression of *COX7C* in PCOS is associated with disruptions in mitochondrial function, potentially playing a role in the metabolic imbalances characteristic of the syndrome (1). Different expression patterns of these genes in our investigation might also be related to the specific pathways of DNA methylation, which may influence transcriptional changes of these genes (35).

In our study, the expression pattern of SDHA was similar to SDHB, with almost no difference between the control and the sensitive groups. Both groups had higher expression in the resistant group than in other groups; however, the differences were insignificant. Unlike our result, downregulation of this gene was reported in samples from PCOS-like rats (36). On the other hand, previous research has shown that the upregulation of TXNIP considerably increased SDHA expression and ROS production (37). Also, *NCF2* can increase ROS in the cell by activating*NOX2* (38). The preservation of the cellular redox state and the prevention of OS depend on *TXNIP*. Produced ROS in the cells can lead to activation of *IL-1β* through binding of *TXNIP* to the *NLRP3* inflammasome (39). Therefore, the upregulation of SDHA from human CCs in our study might be due to the interaction of this gene with over-expression of TXNIP in the IR group. Also, a recent study has shown that upregulation of SDHB leads to high levels of ROS and mitochondria dysfunction (40), which is considered one of the leading causes of IR.

Despite previous studies that recognized the roles of specific genes in mitochondrial function and PCOS development, further research is still required to investigate how these genes lead to specific symptoms of PCOS and infertility.

Generally, in women with PCOS, impaired mitochondrial function is linked to insulin resistance, contributing to metabolic dysregulation in the pathophysiology of the condition (19). The current study investigated the expression of OXPHOS genes in the CCs of PCOS patients, which might impact follicle development, ovulation, and eventual pregnancy. Abnormal metabolic markers, such as resistance to insulin and different patterns of mitochondrial-related gene expression, might be involved in the developing follicles in PCOS patients. Proper mitochondrial biogenesis and normal insulin response can facilitate folliculogenesis, egg maturation, and fertility.

Our study had limitations certainly in the available small sample size. The inherent variability of biological systems due to potential confounding factors, such as lifestyle factors, dietary habits, and genetic variations, could influence the results that may rival or exceed experimental differences between groups. Although differences between biological replicates maybe large, sufficient numbers may allow more minor observed differences to be discerned. Considering the small population of donor patients that met our study design and dividing them into three subgroups with few participants, the mean variations in large sample studies might be different than what we have calculated in our data analyses.

On the other hand, we performed a comparative analysis of gene expression between IR, IS, and control groups, offering insights into the differences in OXPHOS gene expression in these groups. We specifically examined the OXPHOS gene expression in the CCs of the PCOS patients using real-time PCR as a reliable technique for gene expression quantification, which, to the best of our knowledge is the first such specific study. The findings of our research could improve understanding of the molecular factors associated with pathophysiology of PCOS in reproduction.

Conclusion

In general, this investigation and previous studies have presented the aberrant mitochondrial activity and the abnormal expression of OXPHOS genes in PCOS patients that were certainly linked to IR. We found upregulation of *NCF2*, *TXN1P*, *UCP2,* and *ATP5H* as genes with known roles in PCOS and diabetes in PCOS patients with IR. *NDUFB6* was the only investigated gene with overexpression in IS group compared to controls and IR patients. Therefore, any alteration in the transcriptional profile of OXPHOS genes in PCOS patients might reduce their total reproductive potential.

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Authors' Contributions

B.M., S.O.R.M., M.B.; Contributed to manuscript drafting, Revising, and Performed the statistical analyses. B.M., P.E.-Y., F.H.; Collected the data. M.B., M.E., P.E.-Y., F.H.; Assisted in the study design and Supervision. B.M.; Conducting the project. All coauthors approved the final version to be published and agreed to be accountable for all aspects of the work.

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