Original Article

Association between Glucose Consumption and Oocyte Maturation Competence in Mice with Polycystic Ovarian Syndrome

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Abstract

Background: This study evaluated association between glucose uptake by individually cultured oocyte and their maturation competence in mice with polycystic ovarian syndrome (PCOS).

Materials and Methods: In this experimental study, PCOS and non-PCOS cumulus-oocyte complexes (COCs), and cumulus-denuded oocytes (DOs) were cultured individually and categorized in four groups: i. PCOS DOs (n=83), ii. PCOS COCs (n=35), iii. Non-PCOS DOs (n=61) and iv. Non-PCOS COCs (n=62). After the culture period, 50 µl aliquots of the spent drops were used for glucose change analysis using high performance liquid chromatography. Polar NH2 column was used for the study of carbohydrates, acetonitrile with deionized water as the solvent phase and UV as detectors. Oocyte quality (growth differentiation factor 9: *GDF-9*), viability [bcl-2-like protein 4 (*BAX*) and B-cell lymphoma2 (*BCL2*)], in addition to fertilization and embryonic development rates were also evaluated in relation to glucose consumption rate of each oocyte.

Results: Maturation rate was significantly higher in non-PCOS COCs and DOs compared to PCOS COCs (IV: 70.9% vs. II: 45.71%) and DOs (III: 67.2% vs. 1: 53.01%), respectively. There was a significant negative correlation between high glucose intake (38.17 ppm) and *BCL2* gene expression (P=0.03) in PCOS COCs compared to non-PCOS COCs. There was a significant difference in the *GDF-9* gene expression from PCOS DOs (0.66 ± 0.02, P=0.003) and COCs (0.37 ± 0.02, P=0.0001) compared to non-PCOS DOs and COCs, respectively. A negative correlation was also observed between quality of PCOS-DOs and -COCs with glucose intake. Non-PCOS COCs significantly showed higher rate of successful IVF and development compared to PCOS COCs (P=0.01).

Conclusion: Based on the importance of metabolic analysis, the glucose consumption by DOs and COCs in culture medium can be a suitable criterion for their quality assessment. So that, glucose consumption may reflect oocyte maturation competence.

Keywords: Glucose Intake, High Performance Liquid Chromatography, *In Vitro* Maturation, Oocyte Quality, Polycystic Ovarian Syndrome

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Introduction

In vitro maturation (IVM), a modified method of conventional *in vitro* fertilization (IVF), used smaller follicles after little or no exogenous gonadotropin stimulation (1, 2). During IVM, the immature oocytes at the stages of germinal vesicle (GV) or metaphase I (MI) were retrieved. The oocyte maturation and meiosis resumption were followed in the laboratory. Therefore, using gonadotrophin and an ovulation trigger were deleted or minimized during *in vivo* or *in vitro* maturation (1, 3). Additionally, a range of patients was treated in various manners [including follicle-stimulating hormone (FSH) resistance, oocyte donors, candidate for fertility preservation, the presence of severe effects of elevated estradiol, and patients with thrombophilia)] using IVM. Subsequently, there is an emerging interest to treat women with polycystic ovarian syndrome (PCOS) using IVM (1, 2). PCOS is commonly known as an endocrine disorder in the reproductive years of 4-12% of women. Anovulation and infertility are observed in the PCOS women (4). In addition, ovarian hyper-stimulation syndrome (OHSS) might be developed in women with PCOS undergoing IVF cycles to induce more antral follicles (1, 4).

Using animal models have been common, as a valuable resource, to elucidate potential mechanisms of PCOS pathology. The strategies have been introduced to develop animal models of PCOS, such as treatment with androgens, estrogens, progesterone receptor antagonists and genetic manipulations (5). The PCOS mouse models showed that exogenous androgen administration was

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sufficient to produce some symptoms of human PCOS, including hyper-androgenic and PCOS phenotypes [such as increased testosterone and luteinizing hormone (LH)], polycystic ovaries and acyclicity after treatment (6, 7).

Oocyte maturation has two stages: i. Nuclear maturation that is observed as resumption of the first meiosis and extrusion of the first polar body and ii. Cytoplasmic maturation. The other changes within oocyte was defined as cytoplasmic maturation, such as organelles development, accumulation of proteins and mRNA, cytoskeleton reorganization and changes in cellular metabolism. Insufficient cytoplasmic maturation leads to declined developmental potential of *in vitro* matured oocytes. The energy required for progression of all the dynamic processes during oocyte maturation is supplemented via energy metabolism from different substrates such as amino acids, lipids and carbohydrates. Increased glucose metabolism commonly occurs to produce mature oocyte at the metaphase II (MII) stage (6).

The successful fertilization, implantation and ongoing pregnancy influence quality of oocytes (7). So that, studies showed the association of fertilization failure with oocyte abnormalities. One of the most common methods to assay oocyte quality is observation of morphological oocyte characteristics at the light microscopy level (1). However, assessment of oocyte morphology is debatable for embryo selection and prediction of implantation potential. Pre-implantation genetic (PGD) diagnosis is another method for examination of embryo quality. Due to blastomere(s) and/or polar body biopsy as well as possible physical injuries, this is known as an invasive method that lead to a possible reduction of embryo quality (8, 9). Therefore, using a non-invasive oocyte evaluation such as metabolism of spent culture medium could introduce the best oocyte for subsequent fertilization processes.

As mentioned above, energy metabolism is necessary for oocyte maturation. So that, some studies indicated that glucose consumption was mediated gonadotropin-induced meiosis. In addition, the effect of glucose consumption on nuclear maturation of oocyte has been reported in many studies. For example, Xie et al. (6) showed that glucose consumption during IVM led to the release of metabolites from cumulus cells, their absorption by the oocyte and promotion of oocyte maturation. It has also been reported that glucose concentrations at certain level was important for normal mouse ovulation (10) and control of meiotic maturation in mouse cumulus oocyte complexes (11).

There are few studies on the effect of glucose consumption on oocyte maturation (10-13), while there is no data showing the effect of glucose consumption on IVM of oocytes with PCOS. Therefore, due to the importance of oocyte quality analysis and its role on the selection of the best embryo for implantation during assisted reproductive techniques, use of a non-invasive method to analyze oocyte quality is critical in IVF laboratory. Evaluation of maturation medium and changes of its metabolites is suggested as a suitable non-invasive method during *in vitro* oocyte maturation.

To the best of our knowledge, rate of glucose consumption by mouse PCOS oocyte as well as its association with *in vitro* oocyte maturation, viability and quality has not been evaluated. Therefore, the aim of this study was to answer the following questions: i. Is there an association between glucose consumption and IVM of mouse PCOS oocytes? ii. Does the rate of glucose consumption reflect quality of mouse PCOS oocyte and/or its apoptosis?

Materials and Methods

Study of animals

This experimental study was conducted in the University of Guilan (Rasht, Iran) between October 2020 and June 2021. Twenty adult Naval Medical Research Institute (NMRI) female mice (30-35 g, 7-8 weeks old) were used for the present study. Animals were housed in a central animal care room with controlled environment of 22 ± 3°C temperature, 45-55% humidity and 12 hours light/dark cycle. Each four mice were kept in a cage and fed with standard diet and water accessed ad libitum. All chemicals and reagents were purchased from Sigma Aldrich Company (Germany), unless otherwise specified. All investigations were confirmed with the ethical principles of research and they were approved by the Research Ethics Committee for Guilan University of Medical Sciences (IR.GUMS.REC.1399.255).

Polycystic ovarian syndrome induction

All the experimental animals, except control groups (groups III and IV), were administered with estradiol valerate (Aburaihan Co., Iran) at a dose of 40 mg/kg body weight dissolved in 0.5% sesame oil by intramuscular injection once daily for 60 days (14). Vaginal epithelia smears were obtained daily and evaluated by light microscope using Giemsa stain to determine induction of PCOS. So that, the irregular estrous cycle and occurrence of persistent vaginal cornification phase were the symptoms of PCOS induction. With evidence of symptoms, ovaries were also cut through at longest longitudinal dimension and fixed in alcoholic Bouin's solution. After dehydration stage, the ovary was serially sectioned at 5 µm and stained with hematoxylin and eosin. The sections were used for histologic evaluation of PCOS ovaries.

In addition, to confirm the PCOS induction, the blood samples of PCOS mice were collected transcardially. Then, the separated serum was stored at – 20°C to estimate hormones. Levels of serum LH and FSH were evaluated using immunofluorometric techniques. The coefficient of variation for the total trial was 2.9 and 2.6%. Serum testosterone was measured directly through the Coat-A-Count RIA (CA) kit. The inter- and intra-assay coefficients of variation were 12% and 10%, respectively and they were considered with a sensitivity of 4 ng/dl (0.139 nmol/l).

In vitro maturation of oocytes

After confirmation of PCOS induction, the PCOS ovaries were collected and placed in α-minimum essential

medium (α-MEM, Gibco, UK) supplemented with 5% fetal bovine serum (FBS, Gibco, UK). The ovaries were mechanically dissected and oocytes at the germinal vesicle (GV) stage and cumulus-oocyte complexes (COCs) were collected. In this way, only COCs with more than three layers of un-expanded cumulus cells and oocytes greater than 70 µm in diameter with a homogenous cytoplasm were selected. The selected COCs were denuded mechanically by pipetting and cumulus-denuded oocytes (DOs) were also prepared.

After three times washing, each PCOS DOs and COCs was cultured (one PCOS DOs/drop or one PCOS COCs/drop) in the α-MEM supplemented with 5% FBS, 0.23 mM sodium pyruvate, 75 mU/ml of follicle-stimulating hormone, 7.5 IU/ml human chorionic gonadotropin, 50 µg/ml penicillin and 50 µg/ml streptomycin (experimental group). Non-PCOS DOs and COCs was also cultured individually (one Non-PCOS oocyte or COCs/drop as control group). Therefore, groups were culture as following: i. PCOS DOs (n=83), ii. PCOS COCs (n=35), iii. Non-PCOS DOs (n=61) and iv. Non-PCOS COCs (n=62). Maturation rate of each DOs and COCs was examined 24 hours after culture in maturation medium at 37°C under 5% CO_2 in humidified air.

In vitro fertilization and embryo culture

Sperms were collected from the caudal epididymis of male mice (n=8, 10-12 weeks old) and capacitated for 1 hour in Hams'F10 medium, at 37°C and 5% CO_2. PCOS and non-PCOS COCs as well as the matured DOs, *in vitro*, were inseminated with capacitated sperm in α-MEM supplemented with 10% of FBS for 4-5 hours, at 37°C and 5% CO_2. Subsequently, two pronucleus (2PN) zygotes were cultured in α-MEM medium with 10% FBS and incubated at 37°C and 5% CO_2. Only embryos with normal morphology from 2-cell, 8-cell to blastocysts were collected and studied.

Measurement of glucose intake

High performance liquid chromatography (HPLC, Waters, USA) method was used to study glucose changes in the culture medium. One of the advantages of the HPLC method is that it detects the smallest changes in the amount of culture medium's glucose. At the end of each culture period (24 hours after DOs and COCs culture), 50 µl of the culture medium was taken from each dish to measure glucose level to measure glucose level. Therefore, the samples were included as the following: i. Pre-IVM culture medium, ii. Culture medium of matured PCOS DOs, iii. Culture medium of matured PCOS COCs, iv. Culture medium of matured non-PCOS Dos, and v. Culture medium of matured non-PCOS COCs. HPLC system in this study used UV detection made at 195 nm with column temperature of 50°C. The utilized column was NH_2 column (250 mm×4.6 mm). Ratio of the used acetonitrile and deionized water was 80 to 20%. A guard column was attached to the inlet of the column of prevent clogging (15).

RNA isolation and quantitative reverse transcription polymerase chain reaction

Twenty-four hours after culture period, the matured DOs and COCs (experimental and control groups) were collected for RNA extraction. While the spent medium was also used to measure glucose intake using HPLC method at the same time. Extraction of total RNA was performed using RNeasy Mini Kit (Roche Molecular Bio Chemicals, Germany) and stored at -80˚C. Complementary DNA (cDNA) was synthesized by the cDNA kit (Thermo Scientific, EU) as directed by the manufacturer's instructions at 42˚C for 60 minutes, and stored at -20˚C.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to quantify mRNA transcript levels of *BAX*, *BCL2* and GDF9 genes. Primer pairs for amplifying these genes were designed using GenBank at NCBI. The primer sequences are shown in Table 1. In this study, housekeeping gene was Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Real time thermal cycler (Applied bio systems, USA) was used for analyzing gene expression. QuantiTect SYBR Green RT-PCR kit (Applied Bio systems, USA) was also employed for amplifying the targeted genes. Amplification of reference and target genes was performed in the same run, for each sample. The protocol of qRT-PCR was programmed as: the holding step at 95˚C for 5 minutes, cycling step at 95˚C for 15 seconds, 58˚C for 30 seconds and 72˚C for 15 seconds, which was followed by a melt curve step at 95˚C for 15 seconds, 60˚C for 1 minute, and 95˚C for 15 seconds. Determining relative quantitation for target genes was performed using ΔΔCt method. All experiments of qRT-PCR were done five times.

Table 1: Primer sequences used for quantitative reverse transcription polymerase chain reaction

Genes	Primer pair sequence (5′-3′)	Annealing temperature (°C)	Size (bp)
GDF-9	F: CACCGTACTCATTCACCCT	57.5	19
	R: CACCTGGTCTTTTGTGCAT	57.0	19
BAX	F: CACTGGACTTCCTCCGTGA	57.0	19
	R: CTCCAGCCACAAAGATGGTCA	57.2	21
BCL2	F: GCGGATATACCTTCTTCCCT	56.4	22
	R: ATTCTGGTGTTTCCCCGTTG	57.2	20
GAPDH (endogenous)	F: CAAGGTCATCCATGACAACTTTG	61.3	23
	R: GTCCACCACCCTGTTGCTGTAG	59.6	22

Statistical analysis

All experiments were repeated five times and data were expressed as mean ± standard deviation (SD). The χ^2, One-Way ANOVA and Tukey's post-hoc tests have been used to analyze differences among the groups and gene expression. Statistical analysis was performed using SPSS version 20 (IBM, USA). P<0.05 was considered statistically significant.

Results

PCOS ovaries evaluation

The irregular estrous cycles were confirmed in the PCOS mice and restricted to estrous stages upon estradiol valerate treatment. Histological examinations showed that number of pre-antral follicles was increased in the PCOS mice. In addition, the atretic and cystic follicles were observed in these mice (n=6 ovaries) and their ovaries contained fewer corpora luteal. Evaluation of steroid hormones showed that serum testosterone and luteinizing hormone levels were increased in the estradiol valerate-treated mice (P=0.04) at 60 days. Serum FSH level was not changed after treatment with estradiol valerate.

In vitro maturation of PCOS oocytes and glucose intake

Overall rate of *in vitro* DOs and COCs matured in the different groups are shown in Table 2. The results indicated that 67.2% of non-PCOS DOs and 53.01% of PCOS DOs had the first polar body. In addition, 70.9% of non-PCOS COCs and 45.71% of PCOS COCs developed to MII stage. Maturation rate was significantly (P=0.001) higher in the group of non-PCOS COCs compared to PCOS COCs. In addition, significant difference was observed in the maturation rate of non-PCOS DOs compared to PCOS DOs (P=0.04). Simultaneously, proportion of GV and GVBD oocytes was higher in the group with PCOS COCs (Table 2).

The measured glucose levels in the MEM-α culture medium after IVM are shown in Table 2. Level of glucose in the MEM-α culture medium was 957.75 ppm. It should be mentioned that this level of glucose was detected in the culture medium before IVM (pre-IVM medium). Glucose measurement of medium culture after IVM indicated that level of glucose intake was significantly lower in the non-PCOS DOs and PCOS DOs compared to the PCOS COCs (P=0.001) and non-PCOS COCs (P=0.03). An increase was also observed in the rate of glucose intake in the PCOS COCs compared to non-PCOS COCs (P=0.001). There was no significant difference in the rate of glucose intake between non-PCOS DOs and POCs DOs groups (P=0.29). But non-PCOS DOs consumed more glucose than PCOS DOs. Maximum rate of the glucose intake for each oocyte was observed in PCOS COCs (38.17 ppm, Fig.1).

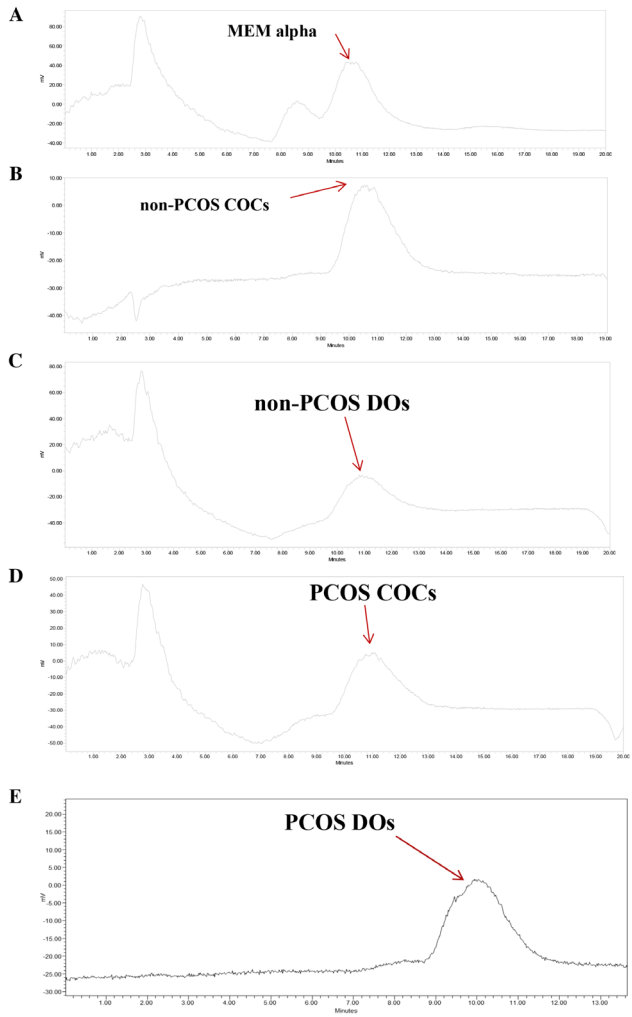

Fig.1: Distribution of relative amounts of glucose in the used culture medium from each group. **A.** MEM-α, **B.** Non-PCOS COCs, **C.** Non-PCOS DOs, **D.** PCOS COCs, and **E.** PCOS DOs. Maximum point of each curve (arrows) indicates the amount of glucose in each utilized culture medium. Obviously, amount of glucose in the used culture medium is inversely related to the amount of glucose consumed by the DOs/COCs. Rate of glucose consumption is higher in PCOS COCs (P=0.001). MEM-α; Modification of minimum essential medium-α, PCOS; Polycystic ovarian syndrome, COCs; Cumulus-oocyte complexes, and DOs; Cumulus-denuded oocytes.

Table 2: Association of glucose consumption with *in vitro* DOs/COCs maturation rate

Groups	Number	GV (%)	GVBD (%)	M II (%)	Deg. oocytes (%)	Average of glucose intake/each oocyte (ppm)	No. of replicates
Normal DOs	61	7 (11.47)	8 (13.11)	41 (67.2)[a]	5 (8.19)	25.05 ± 0.05	18
Normal COCs	62	6 (9.6)	7 (11.29)	44 (70.9)[**]	5 (8.06)	29.8 ± 0.05	16
PCOS DOs	83	10 (12.04)	20 (24.09)	44 (53.01)	9 (10.84)[a]	23.18 ± 0.06	25
PCOS COCs	35	5 (14.2)	3 (8.57)	16 (45.71)	11 (31.42)[*]	38.17 ± 0.07[**]	16

Data are presented as mean ± SD or n (%). DOs; Cumulus-denuded oocytes, COCs; Cumulus-oocyte complexes, PCOS; Polycystic ovarian syndrome, GV; Germinal vesicle, MII; Metaphase II, GVBD; Germinal vesicle breakdown, Deg. Oocytes; Degenerated oocytes, No; Number. There is significant difference in maturation rate of non-PCOS COCs compared to PCOS COCs. Higher degenerated oocytes were observed in PCOS COCs. *; P<0.05, **; P<0.001 vs. COCs, [a]; P<0.05 versus DOs by one-way ANOVA.

In vitro maturation- *in vitro* fertilization outcomes

To evaluate effects of glucose intake by PCOS and non-PCOS DOs/COCs on the development competence, their fertilization and development rate were analyzed among the groups (groups I-IV). In comparison with PCOS COCs, non-PCOS COCs significantly showed higher rate of successive IVF and development to 2-cells, 8-cells and blastocyst stages (P=0.01). As shown in Table 3, non-PCOS DOs had also significantly higher development rate compared to PCOS DOs (P=0.03).

BAX and *BCL2* mRNA content and glucose intake status in PCOS oocytes

Expression level of two apoptosis marker genes (*BAX* and *BCL2*) are observed in Figure 2A-D. Our results showed equal expression levels of *BAX* gene in non-PCOS and PCOS groups. According to the data obtained from qRT-PCR assay and statistical analysis, it can be concluded that there is no significant difference in the rate of *BAX* expression gene between non-PCOS and PCOS groups (P=0.21). Therefore, statistical analysis revealed no significant difference at the expression level of *BAX* gene and glucose intake between non-PCOS and PCOS groups (Fig.2A, C).

Level of *BCL2* gene expression in the different groups of non-PCOS and PCOS DOs/COCs was also measured. A significant negative correlation (CR=-0.8) of glucose intake (38.17 ppm) and *BCL2* gene expression (0.605, P=0.0005) in PCOS COCs was detected (Fig.2D). Minimal expression level of this gene was observed for *BCL2* in PCOS COCs compared to non-PCOS COCs (P=0.0005). There is not significant difference in the expression level of *BCL2* gene between PCOS and non-PCOS DOs (P=0.058, Fig.2C). It can be calculated that this minimal expression level of *BCL2* gene can be correlated to more presence of atretic oocytes in the PCOS COCs than the other groups.

GDF-9 mRNA content and glucose intake status in PCOS oocytes

In this study, expression of gene related to oocyte quality (*GDF-9*) was investigated by qRT-PCR in non-PCOS and PCOS groups. The results showed a decline in *GDF-9* gene expression level of PCOS COCs and DOs compared to non-PCOS COCs and DOs, respectively. According to the data obtained and statistical analysis, it can be concluded that there was a negative correlation in the quality of DOs and COCs and glucose intake among the non-PCOS and PCOS groups. In this way, the non-PCOS DOs and COCs of control group had higher level of *GDF-9* expression than PCOS groups. Mean expression levels of *GDF-9* gene in non-PCOS DOs and COCs groups were 1.00 ± 0.08 and 1.24 ± 0.02, respectively. Whereas, the mean values of *GDF-9* gene expression in the PCOS DOs and COCs groups were 0.66 ± 0.02 and 0.37 ± 0.02, respectively. Statistical analysis revealed a significant difference at PCOS DOs (P=0.0002) and COCs (P=0.0001) groups compared to non-PCOS DOs and COCs, respectively (Fig.3).

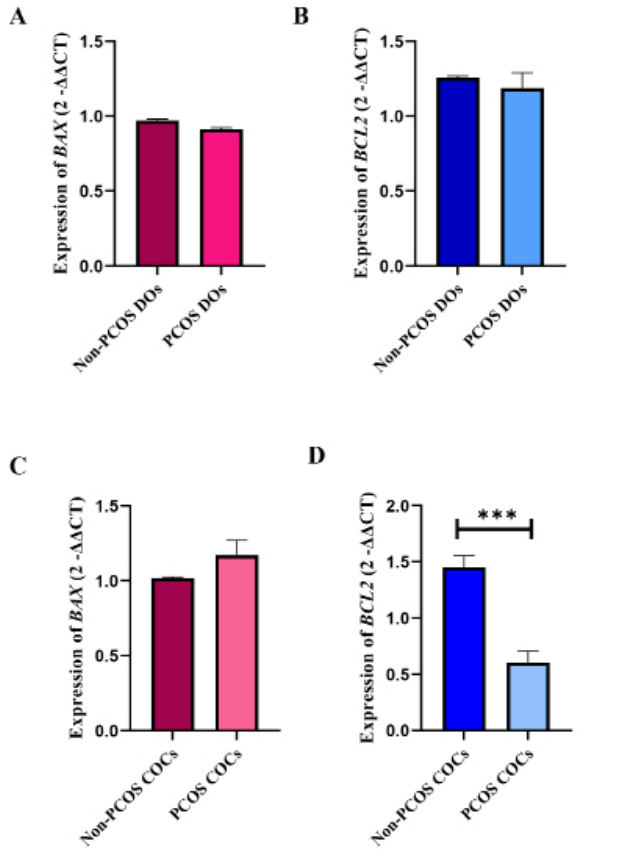

Fig.2: Relative expression of *BAX* and *BCL2* genes in the PCOS and non-PCOS groups. **A, B.** Non-PCOS and PCOS DOs and **C, D.** Non-PCOS and PCOS COCs. Statistical analysis shows significant difference of *BCL2* gene expression in PCOS COCs compared to non-PCOS COCs. PCOS; Polycystic ovarian syndrome, COCs; Cumulus-oocyte complexes, Dos; cumulus-denuded oocytes, and ***; P=0.0005.

Table 3: *In vitro* fertilization and embryo development outcomes

IVF outcomes	Non-PCOS DOs	Non-PCOS COCs	PCOS DOs	PCOS COCs
No. of MII oocytes	41	32	49	24
Fertilization rate (%)	58.23 ± 1.33[a]	67.48 ±3.2**	51.11 ± 2.01	43.62 ± 2.4
2-cell rate (%)	43.13 ± 2.31[b]	54.349 ± 2.2**	30.52 ± 1.41	29.13 ± 2.26
8-cell rate (%)	34.2 ± 2.24[a]	41.25 ± 1.37*	22.94 ± 2.12	21.95 ± 3.05
Blastocyst rate (%)	30.91 ± 2.12[a]	38.29 ± 2.07**	20.97 ± 2.54	18.87 ± 2.18

Data are expressed as mean ± SD and percentage. IVF; *In vitro* fertilization, PCOS; Polycystic ovarian syndrome, COCs; Cumulus-oocyte complexes, Dos; cumulus-denuded oocytes, MII; Metaphase II, No; Number, *; P<0.05, **; P<0.01 vs. PCOS COCs, [a]; P<0.05, and [b]; P<0.01 vs. PCOS DOs by one-way ANOVA. There is a significant difference in fertilization and embryo development rate of oocytes in the non-PCOS COCs compared to the PCOS COCs as well as the non-PCOS DOs compared to the PCOS DOs.

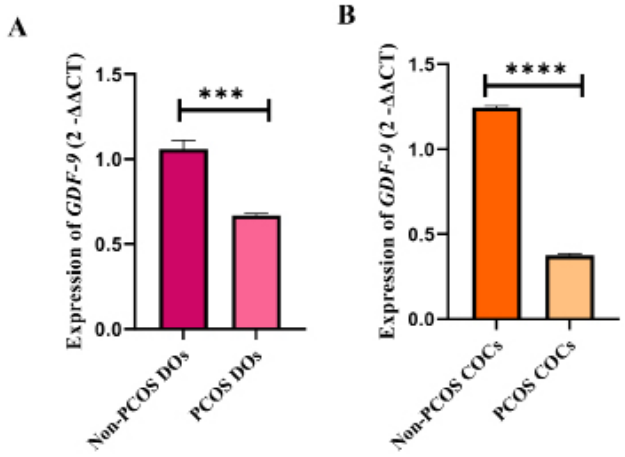

Fig.3: Relative expression level of *GDF-9* gene in the non-PCOS and PCOS groups. The level of *GDF-9* gene expression was significantly decreased in the **A.** PCOS DOs and **B.** COCs compared to non-PCOS DOs and COCs, respectively. PCOS; Polycystic ovarian syndrome, COCs; Cumulus-oocyte complexes, Dos; Cumulus-denuded oocytes, ***; P=0.0002, and ****; P=0.0001.

Discussion

In the present study, relative abundance of genes potentially involved in oocyte quality, apoptosis markers and maturation competence of PCOS DOs and COCs was analyzed. Then, association of oocyte parameters with glucose intake from IVM culture medium was studied.

During the first part of the present study, a lower maturation competence and higher degeneration rate were observed in the PCOS COCs. In addition, the PCOS COCs showed decreased expression of anti-apoptotic marker (*BCL2* gene). In the second part, analyzing glucose consumption revealed that each PCOS COCs had a significant increase in glucose intake. Therefore, the results suggest that glucose consumption rate of each oocyte could introduce a non-invasive method to predict oocyte maturation competence and its quality. Attempts to identify potential biomarkers, to determine oocyte quality using metabolic pathways, have been reported in the literature. Although, they differ from this study in various aspects, such as evaluation of PCOS oocytes and using a non-invasive method to evaluate each oocyte through amount of the consumed glucose.

Given that the crosstalk between oocytes and granulosa cells is necessary for their survival and showing quality of oocyte growth, therefore, analysis of the used culture media for DOs and COCs IVM provides valuable information about the usage of different metabolites by oocytes (16, 17). Metabolism of oocytes and embryos has mainly been studied in antral follicles and mature oocytes. So that, metabolism of COCs plays an important role in oocyte quality (17). However, little information is known about metabolism of immature oocyte, especially oocyte with PCOS. Of note, to the best of our knowledge, this is the first study in which the rate of glucose intake by PCOS oocyte was studied during IVM.

In a study, McLennan et al. (18) reported that glucose intake played an important role in determination of the most suitable oocyte during IVM. It was also indicated that changes in the glucose concentration from culture medium affected cytoplasmic maturation of oocytes. Furthermore, it was well revealed that glucose was necessary for oocyte maturation and COCs expansion (19). In the other studies, it was reported that maturation process of bovine and porcine oocytes needed metabolite, such as glucose and fatty acids (20). It was documented that maturation of mouse COCs to the MII stage did not happen due to the lack of energy supply (6). This is in agreement with our study, whereby glucose intake was detected by both COCs and DOs. In addition, difference of glucose intake between normal and PCOS COCs or DOs was observed. Role of glucose consumption on the aging prevention of DOs and COCs has also been reported during IVM (21). Therefore, in the present study, it was shown that glucose consumption among PCOS oocytes was significantly increased, in comparison with the normal types. It seems that presence of cumulus cells plays an important role in glucose intake. In addition, glucose intake in the PCOS COCs was increased due to the increase in the thickness of theca layers, oocyte volume, glycolysis process and glucosamine synthesis by cumulus cells to proliferate. On the other hand, there was not significant difference in the glucose intake among PCOS DOs and non-PCOS COCs or DOs. Therefore, it was inferred that abnormal glucose uptake and maturation of PCOS oocytes was occured by cumulus cells. So that, it has been reported that bi-directional communication between the cumulus cells and oocyte facilitated glucose transport into the oocyte. Glucose transport has been demonstrated as a gradient through cumulus cell-corona radiate-oocyte by gap junction during bovine COC culture. It has been found that the cumulus cells metabolized the glucose and provided the metabolites for oocyte (22).

Due to the static IVM conditions, presence of a supra-physiological concentration of glucose in the culture medium led to improved nuclear maturation and developmental competence of oocytes (22). However, to the best of our knowledge, there is currently limited data on the influences of PCOS on the oocyte developmental competence and its association with glucose consumption. The results of present study showed different behavior of cumulus cells in the mouse PCOS COCs during IVM. The glucose consumption by PCOS cumulus cells was increased in comparison with intact COCs. However, the glucose intake by PCOS DOs was lower than non-PCOS DOs. Therefore, another possible explanation for the differences of glucose intake was related to oocyte quality (low expression of *GDF-9*). The lower capacity of PCOS DOs for intake and using glucose led to the accumulation of absorbed glucose in the follicular fluid and cumulus cells of PCOS COCs. Further evidences of metabolic cooperation between oocyte and cumulus cell should be provided to established the oocyte improve glucose metabolism in cumulus cells via influencing their transcriptome (17). So that, oocyte-secreted growth factors regulated metabolism of cumulus cells. Therefore,

simultaneous evaluation of the other metabolites and oocyte-secreted factors can also help understand the exact metabolism of oocytes and/or follicles which this is one of the most important limitations in this study.

Conclusion

It is obvious that PCOS oocytes have poor maturation capacity than normal oocytes, due to poor folliculogenesis and the incidence of follicular apoptosis. So that, developmental oocyte competence may influence culture condition and metabolites consumption, especially glucose consumption. Therefore, glucose consumed by the COCs and DOs can be utilized for evaluation of oocyte quality and developmental capacity during IVM and pre-implantation processes and effects on the success of fertilization and subsequent embryo development.

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Authors' Contributions

F.K., F.Gh.; Contributed to conception and design. F.K., Z.Z.; Contributed to all experimental work, data and statistical analysis, and interpretation of data. F.Gh., Z.Z.; Were responsible for overall supervision. F.Gh.; Drafted the manuscript, which was revised by Z.Z. All authors read and approved the final manuscript.

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