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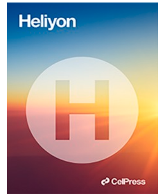

Female BMI has an effect on oocyte gene expression pattern

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A R T I C L E I N F O

Keywords:
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Clinical outcome
Intracytoplasmic sperm injection

A B S T R A C T

Purpose: To explore the mechanisms by which abnormal female BMI affects oocyte quality, particularly whether it involves the alteration of gene expression patterns and how these patterns may impact clinical outcomes.
Methods: In Part 1, we performed a retrospective study to compare the clinical outcomes between the female BMI $\geq$25 kg/m^2 and female BMI $\leq$20 kg/m^2 groups. In Part 2, we performed the transcriptome analyses based on the GSE87201 dataset.
Results: In Part 1, among the clinical outcomes, only the grade 1–2 embryo rate at day 3 of ICSI cycles was significantly different between the two BMI groups; the other outcomes were not. In Part 2, compared with the BMI $\leq$20 kg/m^2 group, the oocyte gene expression pattern of the BMI $\geq$25 kg/m^2 group seemed to result in better oocyte tolerance to exogenous stress, such as intracytoplasmic sperm injection (ICSI). It seemed to explain the result of Part 1 that the BMI $\geq$25 kg/m^2 group had better day-3 embryo quality after ICSI than the BMI $\leq$20 kg/m^2 group.
Conclusions: Abnormal female BMI affects oocyte quality by altering the gene expression patterns of oocytes. While a female BMI $\geq$25 kg/m^2 is known to have certain detrimental effects on ART, our findings suggest that it can also confer some benefits to oocytes.

1. Introduction

The Body Mass Index (BMI) is strongly associated with human health. It also has an effect on female fertility. Abnormal BMI, whether too high or too low, has an adverse effect on female reproductive ability [1].

A recent meta-analysis showed that female obesity is negatively associated with the live birth rate following in-vitro fertilization (IVF) [2]. Another earlier meta-analysis also showed that increased female BMI has a significantly adverse effect on pregnancy outcomes following IVF [3]. Low BMI is a risk factor for female infertility too and is considered to be involved in functional hypothalamic failure [4], a shortened luteal phase, and anovulatory menstrual cycles. Low BMI also has an adverse effect on estrogen metabolism,

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gonadotropin concentration, and follicle growth [5–7].

Although scholars believe that abnormal female BMI can affect the assisted reproductive technology (ART) outcome, how BMI affects the ART outcome remains controversial.

Some investigators believe that the negative impact of abnormal BMI on endometrial receptivity is the main cause of unfavorable ART outcomes [8,9]. In contrast, other investigators think that the main cause is the negative impact on oocyte quality [10,11]. For instance, Cardozo et al. showed that the high BMI of the oocyte donor is associated with an unfavorable ART outcome [12]. They speculated that high BMI has a negative effect on oocyte quality based on the oocyte donation model.

In addition, Valckx et al. showed that the serum metabolite differences are reflected in the follicular fluid between high and normal BMI patients [13], and high BMI may affect oocyte quality through the interaction between the serum and follicular fluid [13]. Low BMI can also affect oocyte quality by affecting gonadotropin concentration and follicle growth [6]. These studies can also support the speculation that an abnormal BMI can affect oocyte quality. However, the speculation still lacks strong support from a clear mechanism.

The mechanisms by which abnormal BMI affects oocyte quality remain unclear, particularly whether it involves the alteration of gene expression patterns and how these patterns may impact clinical outcomes. Our study aims to investigate these questions.

2. Materials and methods

2.1. Part 1: BMI and clinical outcome

2.1.1. Patient selection criteria

This part was a retrospective study. We recruited cycles from September 2015 to September 2021 at our center. The inclusion criteria were set as follows: 1) It was the first in-vitro fertilization-embryo transfer (IVF-ET) treatment cycle in our center. 2) Cycles with female BMI $\geq$25 kg/m^2 and female BMI $\leq$20 kg/m^2 were included in the high and low BMI groups, respectively. To be consistent with Part 2 of this study, we defined the high and low BMI groups as $\geq$ 25 kg/m^2 and $\leq$20 kg/m^2, respectively.

The exclusion criteria were set as follows: 1) It was a gamete donation cycle. 2) 20 kg/m^2 $<$ female BMI $<$25 kg/m^2 3) It was a preimplantation genetic testing (PGT) cycle.

2.2. Stimulation, source of sperm and insemination

The GnRH-agonist prolonged protocol, GnRH-agonist long protocol, GnRH-antagonist protocol, mini-stimulation protocol, luteal phase ovarian stimulation protocol [14], and natural cycle were adopted in this study. Human chorionic gonadotropin was administered when at least one follicle diameter reached 17 mm. Approximately 36 h later, cumulus-oocyte complexes were retrieved using transvaginal ultrasound–guided aspiration.

There were four ways to get the male sperm in this part. The four ways were masturbation, percutaneous epididymal sperm aspiration (PESA), testicular sperm aspiration/extraction (TESA/TESE), and microdissection testicular sperm extraction (MD-TESE), respectively. Oocytes were inseminated by conventional IVF or intracytoplasmic sperm injection (ICSI). In conventional IVF, oocytes were inseminated in 0.5 ml of IVF medium with 150,000 progressive sperm. For some males with masturbation difficulty or severe oligospermia, we prepared and frozen their sperm ahead of time to ensure there was enough sperm on the oocyte pick-up (OPU) day.

2.3. Fertilization check and embryo evaluation

The fertilization check, day-3 embryo assessment, and the definition of grade 1–2 day-3 embryo were the same as previously described by the Istanbul consensus workshop [15]. Day-5/6 blastocysts were scored as per Gardner and Schoolcrafts' system [16]. A blastocyst with $\geq$ 3BB grade was defined as a high quality blastocyst.

2.4. Statistical analyses

We used the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA, version 19.0) for statistical analyses. First, we divided the included cycles according to fertilization method (IVF and ICSI). Then, we compared the main outcomes of IVF cycles, as well as those of ICSI cycles, between the female BMI $\geq$25 kg/m^2 and $\leq$20 kg/m^2 groups. After finding the grade 1–2 embryo rate at day 3 of ICSI cycles was significantly different between the two BMI groups, we performed a multiple linear regression to verify it. The Mann–Whitney test and chi-square test were used for heterogeneous variance data and categorical variables, respectively.

2.5. Part 2: BMI and the gene expression pattern of oocyte

2.5.1. GEO database and analysis method

The GSE87201 dataset (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE87201) is the gene expression profiles of 35 vitrified/warmed MII oocytes from 29 women undergoing oocyte donation (One oocyte data set was excluded due to apparent data quality issues.) [17]. We collected the oocyte data from the GSE87201 dataset according to the donors' BMI. The high and low BMI groups included the oocyte data from the donors whose BMI $\geq$25 kg/m^2 and $\leq$20 kg/m^2, respectively. 20 kg/m^2 $<$ Donor's BMI $<$25 kg/m^2 was excluded from our study.

Due to the limited size of the GSE87201 dataset, we had to define the thresholds for high and low BMI as 25 kg/m^2 and 20 kg/m^2, respectively, to ensure the largest difference between the high and low BMI groups and an equal number of included oocytes. In addition, if a donor had two or more oocytes, we only collected the data of the oocyte whose serial number was one. Finally, each of the two BMI groups collected seven gene expression profiles of oocytes.

We compared the transcriptomes of two BMI groups using GEO2R (the online analysis tool of the GEO database). The following analysis only included protein-coding genes. Differentially expressed genes (DEGs) were determined with a $|\log2\ (\text{fold change})| \geq 0.5$ and a *P*-value <0.05. A volcano plot (GraphPad Prism version 8, La Jolla, CA) and a heat map (https://software.broadinstitute.org/morpheus) visualized the results of DEGs.

Gene Ontology (GO) categories were classified using DAVID (https://david.ncifcrf.gov) to annotate and enrich the DEGs. Due to the low number of differentially expressed protein-coding genes, the Kyoto Encyclopedia of Genes and Genomes (KEGG) was inconclusive.

3. Results

Part 1: The differences in clinical outcomes between the female BMI ≥25 kg/m^2 and ≤20 kg/m^2 groups.

Our study included 902 conventional IVF cycles. The BMI ≥25 kg/m^2 group had 300 cycles, and their female BMI mean was 27.47 ± 2.46 kg/m^2. The BMI ≤20 kg/m^2 group had 602 cycles, and their female BMI mean was 18.65 ± 1.09 kg/m^2.

Table 1 summarizes the clinical outcomes of the IVF cycles with respect to female BMI. All the clinical outcomes showed no significant difference between the two groups (Table 1).

Our study included 353 ICSI cycles. The BMI ≥25 kg/m^2 group had 99 cycles, and their female BMI mean was 26.88 ± 2.31 kg/m^2. The BMI ≤20 kg/m^2 group had 254 cycles, and their female BMI mean was 18.66 ± 1.27 kg/m^2.

Table 2 summarizes the clinical outcomes of the ICSI cycles with respect to female BMI. For the BMI ≥25 kg/m^2 group, only the grade 1–2 embryo rate at day 3 had a significantly higher mean value. The other clinical outcomes showed no significant difference between the two groups (Table 2).

Tables I and II showed that female BMI only had a significant effect on the grade 1–2 embryo rate at day 3 of ICSI cycles. In order to confirm this result, we collected factors that may affect the grade 1–2 embryo rate at day 3 of ICSI cycles and performed simple and multiple linear regressions (Table 3). In the multiple linear regression, the primary infertility, ≥25, GnRH-agonist prolonged protocol, fresh, and masturbation categories were used as references. Multiple linear regression revealed that after adjustment for the confounding factors, the Female BMI still had a significant effect on the grade 1–2 embryo rate at day 3 of ICSI cycles (Table 3). This result was consistent with Table 2.

Part 2: The differences in gene expression pattern of oocyte between the female BMI ≥25 kg/m^2 and ≤20 kg/m^2 groups.

Both the female BMI ≥25 kg/m^2 and female BMI ≤20 kg/m^2 groups included seven donors. The donor BMI means of these two groups were 27.10 ± 1.42 kg/m^2 and 18.44 ± 1.08 kg/m^2, respectively. We compared the gene expression patterns of oocytes between the two BMI groups and found 72 protein-coding genes were the DEGs. For the BMI ≥25 kg/m^2 group, 53 of these genes were significantly up-regulated and the other 19 genes were significantly down-regulated. A heat map and a volcano plot visualize these differentially expressed protein-coding genes (Fig. 1a and b, respectively).

GO analysis was performed to explore the potential impacts of differentially expressed protein-coding genes (Fig. 1c). The significantly different biological process (BP) terms, cellular component (CC) terms, and molecular function (MF) terms were 16, 4, and 2, respectively. Fig. 1c only shows the top 10 BP terms according to *P*-value. Of these 10 BP terms, "cellular amide metabolic process" and "oxidation-reduction process" had the greatest number of genes, and the *P*-value of the latter was smaller than that of the former. In the 4 CC terms, "mitochondrion" had the greatest number of genes and included all the genes of the other 3 terms. In the 2 MF terms, "zinc ion binding" had the smallest *P*-value, and the genes of the 2 MF terms were the same. Therefore, the "oxidation-reduction process" of BP, "mitochondrion" of CC, and "zinc ion binding" of MF were selected for further analysis. Table 4 summarizes the results.

Table 4 shows that most of the alteration of gene expression patterns seemed to improve the oocyte tolerance to exogenous stress, such as ICSI, for the BMI ≥25 kg/m^2 group. Fig. 2 summarizes the biological mechanisms and shows that *APEX2* et al., *ZFAND1* et al., *ZFYVE1*, and *TIGAR* et al. could protect oocytes after ICSI from DNA damage, protein damage, lipid damage, and oxidative stress damage, respectively. Furthermore, *POLG2* et al. are required for mitochondrial function and ATP synthesis. Therefore, the alteration of gene expression patterns might explain why the female BMI ≥25 kg/m^2 group had a better grade 1–2 embryo rate at day 3 than the

Table 1
The clinical outcome comparisons of conventional IVF cycles between the female BMI ≥25 kg/m^2 and female BMI ≤20 kg/m^2 groups.

outcomes	BMI ≥25 kg/m^2 (n = 300)	BMI ≤20 kg/m^2 (n = 602)	*P*-value
MII rate (%)	86.11 ± 20.95	86.12 ± 19.06	*P* = 0.340[a]
2 PN rate (%)	65.67 ± 29.31	67.72 ± 28.02	*P* = 0.197[a]
(1 PN + ≥3 PN) rate (%)	13.14 ± 17.71	15.07 ± 20.90	*P* = 0.224[a]
Grade 1–2 embryo rate at day 3 (%)	54.91 ± 35.10	55.43 ± 34.14	*P* = 0.894[a]
Blastocyst formation rate (%)	57.89 ± 35.45	58.17 ± 35.10	*P* = 0.977[a]
High quality blastocyst rate (%)	15.23 ± 22.13	14.77 ± 21.60	*P* = 0.788[a]
Live birth rate (%)	39/68 (57.35)	65/118 (55.08)	*P* = 0.764[b]

[a] Two-sample Mann–Whitney test. Values are are mean + SD.
[b] Pearson X^2test. Values are number (percentage).

Table 2
The clinical outcome comparisons of intracytoplasmic sperm injection (ICSI) cycles between the female BMI ≥25 kg/m^2 and female BMI ≤20 kg/m^2 groups.

outcomes	BMI ≥25 kg/m^2 (n = 99)	BMI ≤20 kg/m^2 (n = 254)	*P*-value
MII rate (%)	78.55 ± 19.23	78.76 ± 18.40	*P* = 0.951 [a]
2 PN rate (%)	70.47 ± 28.90	74.67 ± 22.58	*P* = 0.548 [a]
(1 PN + ≥3 PN) rate (%)	5.49 ± 11.33	5.85 ± 12.34	*P* = 0.446 [a]
Grade 1–2 embryo rate at day 3 (%)	57.33 ± 37.13	48.04 ± 30.80	*P* = 0.043 [a]
Blastocyst formation rate (%)	43.62 ± 32.75	51.62 ± 34.61	*P* = 0.059 [a]
High quality blastocyst rate (%)	15.23 ± 22.13	14.77 ± 21.60	*P* = 0.373 [a]
Live birth rate (%)	9/18 (50.00)	24/55 (43.64)	*P* = 0.638 [b]

[a] Two-sample Mann–Whitney test. Values are are mean + SD.
[b] Pearson X^2test. Values are number (percentage).

Table 3
Simple and multiple linear regressions on the grade 1–2 embryo rate at day 3 of ICSI cycles.

Characteristics	Simple linear regression Coefficient 95%CI	*P*-value	Multiple linear regression Coefficient 95%CI	*P*-value
Female age	0.004(-0.003; 0.010)	0.263		
Male age	0.003(-0.002; 0.009)	0.267		
Female infertility type				
primary infertility	1			
secondary infertility	0.057(-0.016; 0.129)	0.124		
Female infertility duration (years)	−0.010(-0.022; 0.001)	0.085	−0.019(-0.031; −0.006)	0.003 *[a]
Female BMI				
≥25	1		1	
≤20	−0.093(-0.171; −0.015)	0.020*	−0.107(-0.187; −0.027)	0.009 *[b]
Male infertility type				
primary infertility	1			
secondary infertility	−0.006(-0.085; 0.074)	0.884		
Male infertility duration (years)	−0.007(-0.018; 0.004)	0.195		
Male BMI	0.008(-0.003; 0.018)	0.146		
Ovarian stimulation protocol				
GnRH-agonist prolonged protocol	1			
GnRH-agonist long protocol	−0.054(-0.165; 0.056)	0.332		
GnRH-antagonist protocol	0.022(-0.105; 0.149)	0.730		
Mini-stimulation protocol	0.059(-0.064; 0.182)	0.344		
Luteal phase ovarian stimulation protocol	0.053(-0.064; 0.169)	0.376		
Natural cycle	−0.165(-0.547; 0.218)	0.398		
Ovarian stimulation duration (days)	−0.009(-0.025; 0.007)	0.259		
Number of oocytes retrieval	−0.006(-0.010; −0.002)	0.003*	−0.007(-0.011; −0.003)	0.001 *[a]
Gonadotrophins per oocyte (ampoule [c])	0.002(-0.003; 0.007)	0.413		
MII rate	−0.092(-0.285; 0.101)	0.349		
Sperm source				
fresh	1			
freeze-thaw	0.024(-0.153; 0.200)	0.791		
The way to get sperm				
masturbation	1		1	
PESA	−0.014(-0.133; 0.105)	0.821	0.001(-0.115; 0.117)	0.992 [b]
TESA/TESE	0.107(-0.017; 0.231)	0.091	0.104(-0.017; 0.224)	0.093 [b]
MD-TESE	0.112(-0.120; 0.343)	0.343	0.132(-0.095; 0.358)	0.253 [b]

Multiple linear regression was performed to control for those factors with p values less than 0.1 in simple linear regression. Categorical variables were transformed into dummy variables in these analyses. The dummy variables of each categorical variable in multiple linear regression were under the principle of "in and out together". The primary infertility, ≥25, GnRH-agonist prolonged protocol, fresh, and masturbation categories were used as references.
PESA: Percutaneous epididymal sperm aspiration, TESA/TESE: Testicular sperm aspiration/extraction, MD-TESE: Microdissection testicular sperm extraction.
* Significant difference ($P < 0.05$).
[a] *P*-value of each variable's overall effects after adjusting for the other variables.
[b] *P*-value between each variable's subgroups and reference group.
[c] 75 IU per ampoule.

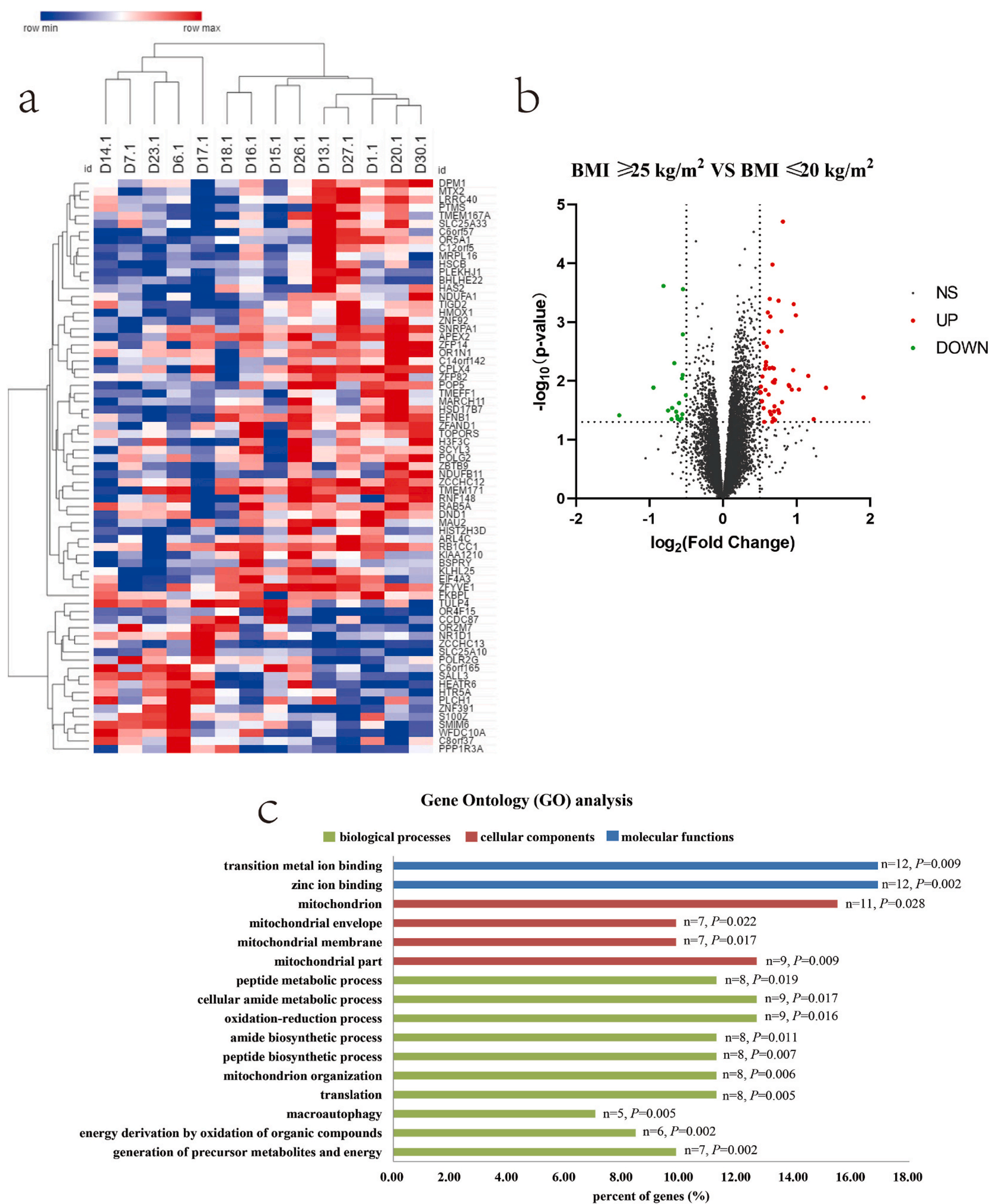

Fig. 1. The characteristics of differentially expressed protein-coding genes
(a) Unsupervised hierarchical clustering based on the characteristics of differentially expressed protein-coding genes. D14, D7, D23, D6, D17, D18, and D15 were the oocyte donors with BMI $\leq$20 kg/m^2. D16, D26, D13, D27, D1, D20, D30 were the oocyte donors with BMI $\geq$25 kg/m^2 (b) A volcano plot depicts significantly upregulated or downregulated oocyte protein-coding genes between the female BMI $\geq$25 kg/m^2 and BMI $\leq$20 kg/m^2 groups. (c) Enriched GO terms based on differentially expressed protein-coding genes. The significantly different biological process (BP) terms, cellular component (CC) terms, and molecular function (MF) terms were 16, 4, and 2, respectively. This figure only shows the top 10 BP terms according to *P*-value. BP, CC, and MF terms are colored green, red, and blue, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
The function analyses of the genes of the oxidation-reduction process, mitochondrion, and zinc ion binding terms.

Gene	Summary	For BMI≥25 kg/m²	BP	CC	MF
TIGAR	Protecting oocytes from DNA damage and oxidative stress damage	up-regulation	O-RP	M	
POLG2	Required for mitochondrial function and synthesis ATP	up-regulation	O-RP	M	
NDUFB11	Required for mitochondrial function or synthesis ATP	up-regulation	O-RP	M	
NDUFA1	Required for mitochondrial function or synthesis ATP	up-regulation	O-RP	M	
SLC25A10	Inhibition might effectively reduce lipid accumulation	down-regulation	O-RP	M	
NR1D1	Transcriptional repressor which coordinates circadian rhythm and metabolic pathways	down-regulation	O-RP		ZIP
HMOX1	Protecting oocytes from oxidative stress damage	up-regulation	O-RP		
HSD17B7	Catalyzes the reduction of estrogens and androgens and regulates the biological potency of these steroids	up-regulation	O-RP		
PPP1R3A	Seems to act as a glycogen-targeting subunit for PP1	down-regulation	O-RP		
APEX2	Protecting oocytes from DNA damage	up-regulation		M	ZIP
MTX2	Required for mitochondrial function or synthesis ATP	up-regulation		M	
MRPL16	Required for mitochondrial function or synthesis ATP	up-regulation		M	
SDHAF4	Protecting oocytes from oxidative stress damage	up-regulation		M	
HSCB	Required for mitochondrial function or synthesis ATP	up-regulation		M	
SLC25A33	Protecting oocytes from oxidative stress damage	up-regulation		M	
ZCCHC12	Transcriptional coactivator in the bone morphogenetic protein (BMP)-signaling pathway.	up-regulation			ZIP
RNF148	Protecting oocytes from protein damage	up-regulation			ZIP
ZNF92	May be involved in transcriptional regulation.	up-regulation			ZIP
ZFAND1	Protecting oocytes from protein damage	up-regulation			ZIP
ZFYVE1	Protecting oocytes from lipid damage	up-regulation			ZIP
TOPORS	Protecting oocytes from DNA damage	up-regulation			ZIP
MARCH11	Protecting oocytes from protein damage	up-regulation			ZIP
PTMS	Parathymosin may mediate immune function	up-regulation			ZIP
BSPRY	May regulate epithelial calcium transport by inhibiting TRPV5 activity.	up-regulation			ZIP
ZCCHC13	Protecting oocytes from protein damage	down-regulation			ZIP

BP: biological process; CC: cellular component; MF: molecular function.
O-RP: oxidation-reduction process; M: mitochondrial; ZIB: zinc ion binding.

BMI ≤20 kg/m² group in ICSI cycles. It seems that female BMI can affect the ART clinical outcomes through altering the gene expression patterns of oocytes.

4. Discussion

We compared ART outcomes and the gene expression patterns of oocytes between the female BMI ≥25 kg/m² and female BMI ≤20 kg/m² groups.

Among the ART outcomes, only the grade 1–2 embryo rate at day 3 of ICSI cycles was significantly different between the two BMI groups; the other outcomes were not. These results conflicted with earlier findings, which showed that overweight women have a lower fertilization rate [18], implantation rate [19] and live birth rate [2]. The conflict was due to the different populations. The earlier studies compared overweight women with normal BMI women, rather than women with a BMI ≤20 kg/m² in our study.

The gene expression patterns of oocytes were different between the two BMI groups. After further analyzing the altered gene expression pattern, we found that the alteration could affect the oocyte quality. This finding was consistent with previous research [20]. In addition, the alteration of gene expression patterns also seemed to explain why the BMI ≥25 kg/m² group had a better grade 1–2 embryo rate at day 3 in ICSI cycles. The relevant analyses were as follows:

Oocytes may have DNA damage after ICSI. Table 4 shows that *APEX2*, *TOPORS*, and *TIGAR* were up-regulated in the BMI ≥25 kg/m² group. These three genes contribute to the repair of DNA damage. *APEX2* is involved in the repair of oxidative DNA damage [21]. Human Apex2 protein repairs 3′-damaged DNA termini and mismatched deoxyribonucleotides at the recessed 3′-termini of a partial DNA duplex through 3′ phosphodiesterase activity and 3′-5′ exonuclease activity, respectively [21,22]. Through SUMO modification of IKK3, *TOPORS* contributes to the antiapoptotic function of NF-kB in response to DNA damage [23]. *TIGAR* protects cells from DNA damage and DNA damage-induced apoptosis [24]. In obese mouse oocytes, forced expression of TIGAR can attenuate ROS production and prevent spindle disorganization and chromosome misalignment during meiosis [25].

ICSI also damages proteins in oocytes. *ZFAND1*, *ZCCHC13*, *RNF148*, and *MARCH11* may be able to protect oocytes from protein damage. *ZFAND1* plays a role in the clearance of cytoplasmic stress granules (SGs). SGs are cytoplasmic assemblies of ribonucleoprotein formed when protein production is suspended after acute exogenous stress, such as ICSI. SGs are important for cellular protein

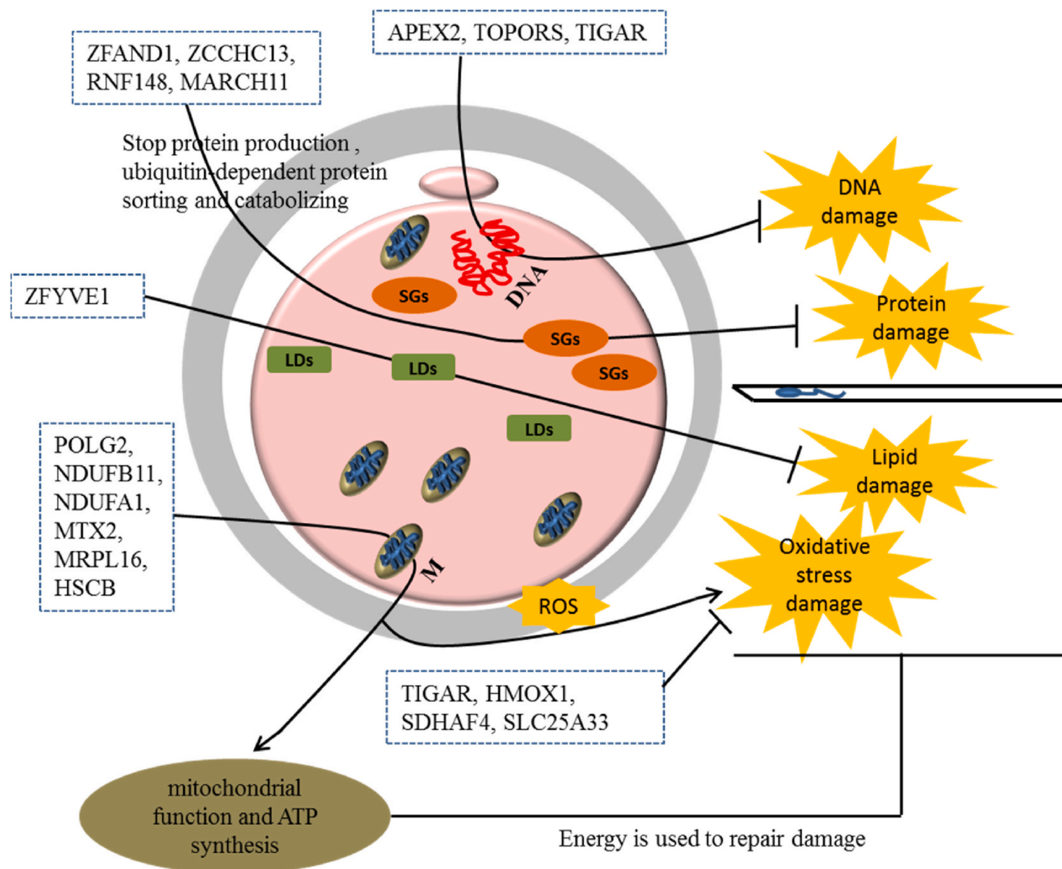

Fig. 2. The changes in gene expression pattern seemed to protect oocytes from the damage of ICSI for the female BMI ≥25 kg/m^2 group *APEX2* et al., *ZFAND1* et al., *ZFYVE1*, and *TIGAR* et al. seemed to protect oocytes after ICSI from DNA damage, protein damage, lipid damage, and oxidative stress damage, respectively. *POLG2* et al. are required for mitochondrial function and ATP synthesis.

homeostasis [26]. Therefore, for the BMI ≥25 kg/m^2 group, the up-regulation of *ZFAND1* might be helpful for the clearance of defective ribosomal products. In addition, after ICSI, the down-regulation of *ZCCHC1*3 might be helpful for the suspension of defective protein production, and the up-regulations of *RNF148* and *MARCH1*1 might be helpful for the sorting and degradation of defective proteins [27].

Lipid homeostasis in oocytes is disturbed after ICSI. *ZFYVE1* contributes to initial lipid droplet (LD) growth and is involved in tethering the endoplasmic reticulum (ER)-LD contact for LD expansion [28]. LD growth contributes to lipid storage and regulates lipid homeostasis [29]. Therefore, for the BMI ≥25 kg/m^2 group, the up-regulation of *ZFYVE1* might be helpful for restoring lipid homeostasis.

Whether it is the repair of DNA damage, the clearance of defective proteins, or the re-establishment of lipid homeostasis, oocytes need energy. Therefore, maintaining mitochondrial function is important. *POLG2*, *NDUFB11*, *NDUFA1*, *MTX2*, *MRPL16*, and *HSCB* are required for mitochondrial function and ATP synthesis. Mitochondrial DNA copy number and adenosine-5′-triphosphate content decrease with the down-regulation of *POLG2* in oocytes [30,31]. *NDUFB11* and *NDUFA1* are accessory subunits of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), which is involved in ATP synthesis [32]. *NDUFA1* is up-regulated after cryoinjury in oocytes [33]. *MTX2* and *MRPL16* are involved in mitochondrial protein synthesis and transport. *HSCB* is involved in the redox reactions of mitochondrial electron transport and other processes. Therefore, for the BMI ≥25 kg/m^2 group, the up-regulation of these genes might be helpful for mitochondrial function and ATP synthesis.

More ATP means more reactive oxygen species (ROS). For the BMI ≥25 kg/m^2 group, the up-regulations of *TIGAR*, *HMOX1*, *SDHAF4*, and *SLC25A33* could protect oocytes from oxidative stress damage. Forced expression of TIGAR can attenuate ROS production in mouse oocytes [25]. *HMOX1* can protect granulosa cells from oxidative damage [34] and has potent anti-inflammatory, antioxidant, and antimetabolic properties [35]. *SDHAF4* and *SLC25A33* can control mitochondrial ROS production [36–38].

In conclusion, for the BMI ≥25 kg/m^2 group, the alteration of gene expression pattern seemed to protect oocytes from the damage of ICSI and result in better embryo quality at day 3. However, the improvement was temporary, lasting only until the cleavage stage. The following clinical outcomes, such as blastocyst formation rate, high quality blastocyst rate, and live birth rate, were not significantly different between the two BMI groups. It was due to a high female BMI also having a negative effect on embryonic development. The negative effect counteracted the beneficial effect brought on by the oocyte's improved tolerance to ICSI.

There were some limitations to our study. First, we did not use the adjusted *P*-value for selecting DEGs. There were too few DEGs to analyze when we used the adjusted *P*-value. Second, Part 1 was a retrospective study. Some confounding factors might have been missed because of the data collection difficulties. Third, due to the limited size of the GSE87201 dataset, we had to define the thresholds for high and low BMI as 25 kg/m^2 and 20 kg/m^2, respectively, to ensure the largest difference between the groups and an equal number of included oocytes. In addition, the oocytes in the GSE87201 dataset were vitrified-warmed oocytes. This might have influenced the gene expression patterns observed. Finally, our results need to be verified by animal experiments. The animal experiments will be done in the future.

5. Conclusion

Our study revealed differences in oocyte gene expression patterns between the female BMI $\geq$25 kg/m^2 and BMI $\leq$20 kg/m^2 groups. These differences appear to influence the clinical outcomes of ART, such as improving the day-3 embryo quality of ICSI cycles. While a female BMI $\geq$25 kg/m^2 is known to have certain detrimental effects on ART, our findings suggest that it can also confer some benefits to oocytes.

Ethical approval

Approval was obtained from the Ethics Committee of First Affiliated Hospital of Fujian Medical University (Approval No.: MRCTA, ECFAH of FMU [2021]194) before the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent has been obtained from each patient or subject after a full explanation of the purpose and nature of all procedures used.

Author contribution statement

Zhiren Liu; Mingting jiang: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Xingting Chen: Analyzed and interpreted the data; Wrote the paper.

Qicai Liu; Guo xinxin; Chen Lin; Yujia Guo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supp. material/referenced in article.

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Declaration of competing interest

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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References

[1] T.K. Jensen, T. Scheike, N. Keiding, I. Schaumburg, P. Grandjean, Fecundability in relation to body mass and menstrual cycle patterns, Epidemiology 10 (1999) 422–428.
[2] N. Sermondade, S. Huberlant, V. Bourhis-Lefebvre, E. Arbo, V. Gallot, M. Colombani, T. Freour, Female obesity is negatively associated with live birth rate following IVF: a systematic review and meta-analysis, Hum. Reprod. Update 25 (2019) 439–451.
[3] V. Rittenberg, S. Seshadri, S.K. Sunkara, S. Sobaleva, E. Oteng-Ntim, T. El-Toukhy, Effect of body mass index on IVF treatment outcome: an updated systematic review and meta-analysis, Reprod. Biomed. Online 23 (2011) 421–439.
[4] D. Unuane, H. Tournaye, B. Velkeniers, K. Poppe, Endocrine disorders & female infertility, Best Pract. Res. Clin. Endocrinol. Metabol. 25 (2011) 861–873.
[5] J. Schneider, H.L. Bradlow, G. Strain, J. Levin, K. Anderson, J. Fishman, Effects of obesity on estradiol metabolism: decreased formation of nonuterotropic metabolites, J. Clin. Endocrinol. Metab. 56 (1983) 973–978.

[6] J. Cai, L. Liu, J. Zhang, H. Qiu, X. Jiang, P. Li, A. Sha, J. Ren, Low body mass index compromises live birth rate in fresh transfer in vitro fertilization cycles: a retrospective study in a Chinese population, Fertil. Steril. 107 (2017) 422–429 e422.
[7] M. Imterat, A. Agarwal, S.C. Esteves, J. Meyer, A. Harlev, Impact of body mass index on female fertility and ART outcomes, Panminerva Med. 61 (2019) 58–67.
[8] J. Bellver, A. Pellicer, J.A. Garcia-Velasco, A. Ballesteros, J. Remohi, M. Meseguer, Obesity reduces uterine receptivity: clinical experience from 9,587 first cycles of ovum donation with normal weight donors, Fertil. Steril. 100 (2013) 1050–1058.
[9] D.A. DeUgarte, C.M. DeUgarte, V. Sahakian, Surrogate obesity negatively impacts pregnancy rates in third-party reproduction, Fertil. Steril. 93 (2010) 1008–1010.
[10] A. Styne-Gross, K. Elkind-Hirsch, R.T. Scott Jr., Obesity does not impact implantation rates or pregnancy outcome in women attempting conception through oocyte donation, Fertil. Steril. 83 (2005) 1629–1634.
[11] B. Luke, M.B. Brown, J.E. Stern, S.A. Missmer, V.Y. Fujimoto, R. Leach, S.W. Group, Female obesity adversely affects assisted reproductive technology (ART) pregnancy and live birth rates, Hum. Reprod. 26 (2011) 245–252.
[12] E.R. Cardozo, A.E. Karmon, J. Gold, J.C. Petrozza, A.K. Styer, Reproductive outcomes in oocyte donation cycles are associated with donor BMI, Hum. Reprod. 31 (2016) 385–392.
[13] S.D. Valckx, I. De Pauw, D. De Neubourg, I. Inion, M. Berth, E. Fransen, P.E. Bols, J.L. Leroy, BMI-related metabolic composition of the follicular fluid of women undergoing assisted reproductive treatment and the consequences for oocyte and embryo quality, Hum. Reprod. 27 (2012) 3531–3539.
[14] J. Llacer, B. Moliner, L. Luque, A. Bernabeu, B. Lledo, J.C. Castillo, J. Guerrero, J. Ten, R. Bernabeu, Luteal phase stimulation versus follicular phase stimulation in poor ovarian responders: results of a randomized controlled trial, Reprod. Biol. Endocrinol. 18 (2020) 9.
[15] E.S.I.G.o.E. Alpha Scientists in Reproductive Medicine, the Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, Hum. Reprod. 26 (2011) 1270–1283.
[16] D.K. Gardner, M. Lane, J. Stevens, T. Schlenker, W.B. Schoolcraft, Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer, Fertil. Steril. 73 (2000) 1155–1158.
[17] M. Barragan, J. Pons, A. Ferrer-Vaquer, D. Cornet-Bartolome, A. Schweitzer, J. Hubbard, H. Auer, A. Rodolosse, R. Vassena, The transcriptome of human oocytes is related to age and ovarian reserve, Mol. Hum. Reprod. 23 (2017) 535–548.
[18] D. Zhang, Y. Zhu, H. Gao, B. Zhou, R. Zhang, T. Wang, G. Ding, F. Qu, H. Huang, X. Lu, Overweight and obesity negatively affect the outcomes of ovarian stimulation and in vitro fertilisation: a cohort study of 2628 Chinese women, Gynecol. Endocrinol. 26 (2010) 325–332.
[19] V.A. Moragianni, S.M. Jones, D.A. Ryley, The effect of body mass index on the outcomes of first assisted reproductive technology cycles, Fertil. Steril. 98 (2012) 102–108.
[20] M.L. Ruebel, M. Cotter, C.R. Sims, D.M. Moutos, T.M. Badger, M.A. Cleves, K. Shankar, A. Andres, Obesity modulates inflammation and lipid metabolism oocyte gene expression: a single-cell transcriptome perspective, J. Clin. Endocrinol. Metab. 102 (2017) 2029–2038.
[21] P. Burkovics, I. Hajdu, V. Szukacsov, I. Unk, L. Haracska, Role of PCNA-dependent stimulation of 3'-phosphodiesterase and 3'-5' exonuclease activities of human Ape2 in repair of oxidative DNA damage, Nucleic Acids Res. 37 (2009) 4247–4255.
[22] P. Burkovics, V. Szukacsov, I. Unk, L. Haracska, Human Ape2 protein has a 3'-5' exonuclease activity that acts preferentially on mismatched base pairs, Nucleic Acids Res. 34 (2006) 2508–2515.
[23] F. Renner, R. Moreno, M.L. Schmitz, SUMOylation-dependent localization of IKKepsilon in PML nuclear bodies is essential for protection against DNA-damage-triggered cell death, Mol. Cell 37 (2010) 503–515.
[24] H.P. Yu, J.M. Xie, B. Li, Y.H. Sun, Q.G. Gao, Z.H. Ding, H.R. Wu, Z.H. Qin, TIGAR regulates DNA damage and repair through pentosephosphate pathway and Cdk5-ATM pathway, Sci. Rep. 5 (2015) 9853.
[25] H. Wang, Q. Cheng, X. Li, F. Hu, L. Han, H. Zhang, L. Li, J. Ge, X. Ying, X. Guo, Q. Wang, Loss of TIGAR induces oxidative stress and meiotic defects in oocytes from obese mice, Mol. Cell. Proteomics 17 (2018) 1354–1364.
[26] A. Turakhiya, S.R. Meyer, G. Marincola, S. Bohm, J.T. Vanselow, A. Schlosser, K. Hofmann, A. Buchberger, ZFAND1 recruits p97 and the 26S proteasome to promote the clearance of arsenite-induced stress granules, Mol. Cell 70 (2018) 906–919 e907.
[27] K. Yogo, H. Tojima, J.Y. Ohno, T. Ogawa, N. Nakamura, S. Hirose, T. Takeya, T. Kohsaka, Identification of SAMT family proteins as substrates of MARCH11 in mouse spermatids, Histochem. Cell Biol. 137 (2012) 53–65.
[28] D. Li, Y.G. Zhao, D. Li, H. Zhao, J. Huang, G. Miao, D. Feng, P. Liu, D. Li, H. Zhang, The ER-localized protein DFCP1 modulates ER-lipid droplet contact formation, Cell Rep. 27 (2019) 343–358 e345.
[29] G. Gao, Y. Sheng, H. Yang, B.T. Chua, L. Xu, DFCP1 associates with lipid droplets, Cell Biol. Int. 43 (2019) 1492–1504.
[30] S.K. Lee, M.H. Zhao, Z. Zheng, J.W. Kwon, S. Liang, S.H. Kim, N.H. Kim, X.S. Cui, Polymerase subunit gamma 2 affects porcine oocyte maturation and subsequent embryonic development, Theriogenology 83 (2015) 121–130.
[31] E. Mahrous, Q. Yang, H.J. Clarke, Regulation of mitochondrial DNA accumulation during oocyte growth and meiotic maturation in the mouse, Reproduction 144 (2012) 177–185.
[32] D.A. Stroud, E.E. Surgenor, L.E. Formosa, B. Reljic, A.E. Frazier, M.G. Dibley, L.D. Osellame, T. Stait, T.H. Beilharz, D.R. Thorburn, A. Salim, M.T. Ryan, Accessory subunits are integral for assembly and function of human mitochondrial complex I, Nature 538 (2016) 123–126.
[33] Z.Y. Zhang, X.L. Yu, M.D. Cai, Y.H. Liu, J.Q. Liu, S.Y. Zhao, X.X. Li, Y.H. Li, Relationship between bovine oocytes developmental competence and mRNA expression of apoptotic and mitochondrial genes following the change of vitrification temperatures and cryoprotectant concentrations, Cryobiology 97 (2020) 110–122.
[34] K. Yu, R.X. Wang, M.H. Li, T.C. Sun, Y.W. Zhou, Y.Y. Li, L.H. Sun, B.L. Zhang, Z.X. Lian, S.G. Xue, Y.X. Liu, S.L. Deng, Melatonin reduces androgen production and upregulates heme oxygenase-1 expression in granulosa cells from PCOS patients with hypoestrogenia and hyperandrogenia, Oxid. Med. Cell. Longev. 2019 (2019), 8218650.
[35] H. Gao, J. Meng, H. Xing, S. Nie, M. Xu, S. Zhang, Y. Jin, T. Sun, H. Huang, H. Zhang, D. Wang, L. Liu, Association of heme oxygenase-1 with the risk of polycystic ovary syndrome in non-obese women, Hum. Reprod. 29 (2014) 1058–1066.
[36] J.G. Van Vranken, D.K. Bricker, N. Dephoure, S.P. Gygi, J.E. Cox, C.S. Thummel, J. Rutter, SDHAF4 promotes mitochondrial succinate dehydrogenase activity and prevents neurodegeneration, Cell Metabol. 20 (2014) 241–252.
[37] C. Favre, A. Zhdanov, M. Leahy, D. Papkovsky, R. O'Connor, Mitochondrial pyrimidine nucleotide carrier (PNC1) regulates mitochondrial biogenesis and the invasive phenotype of cancer cells, Oncogene 29 (2010) 3964–3976.
[38] S. Floyd, C. Favre, F.M. Lasorsa, M. Leahy, G. Trigiante, P. Stroebel, A. Marx, G. Loughran, K. O'Callaghan, C.M. Marobbio, D.J. Slotboom, E.R. Kunji, F. Palmieri, R. O'Connor, The insulin-like growth factor-I-mTOR signaling pathway induces the mitochondrial pyrimidine nucleotide carrier to promote cell growth, Mol. Biol. Cell 18 (2007) 3545–3555.