The correlation between cumulus oophorous cells and their gene expression with human embryo potential serves as a prognostic non-invasive indicator for ICSI success

Mahmoud Soliman lews[a], Ahmed Esmat Abdellah[b], Ahmed Talat Galal Ahmed[c], Ahmed Hashem Abdellah[a], Abeer Madkour Mahmoud[b]

[a] Department of Obstetrics and Gynecology, Faculty of Medicine, Qena University, Qena, Egypt.

[b] Department of Human Anatomy and Embryology, Faculty of Medicine, Qena University, Qena, Egypt.

[c] Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Abstract

Background: Cumulus cells (CCs) are not only solid structural cells that surround the oocyte, but also CCs play a major role in the differentiation and maturity of the oocyte. The gene expression profile of CCs reflects the actual activity of these cells.

Objectives: This study aimed to correlate the expression level of specific genes produced by CCs with pregnancy incidence in patients undergoing Intra-Cytoplasmic Sperm Injection (ICSI). The selected genes were chosen for their roles: G6DP in glucose metabolism, BCL2 in regulating apoptosis, and GREM1 and HAS2 in CCs expansion.

Patients and methods: Oocytes were collected from fifty patients who underwent ICSI. The G6DP, BCL2, HAS2, and GREM1 gene expression levels were assessed in CCs from the denuded oocytes. Embryo development was monitored, and ICSI results were correlated with the expression level of the previously mentioned genes.

Results: All genes (BCL2, HAS2, and GREM1), except for G6DP, showed significant expression levels in pregnant women with successful ICSI compared to those with failed ICSI: HAS2 (P = 0.015), GREMLIN (P = 0.008), and BCL2 (P = 0.035). However, the grade and quality of grade I embryos did not differ significantly (P = 0.081).

Conclusion: Gene expression analysis of HAS2, GREMLIN, and BCL2 in CCs can be used as a prognostic non-invasive indicator of ICSI success.

Key words: Cumulus cells; Gene expression; ICSI.

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***Correspondence**: ahmed.esmat@med.svu.edu.eg

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Introduction

Several studies were conducted based on the use of gene expression as a non-invasive indicator for cell behavior. Many of them were focused on CCs` gene expression profile, a non-invasive prognostic tool to predict the genetic competence of the oocyte **(Rohn et al., 2025)**.

Understanding oocyte genetic integrity may help in evaluating its further activity after fertilization and embryo development, which may help us in choosing proper embryos for effective embryo transfer policy **(Marchais et al., 2022)**.

Embryo selection with a higher potential of implantation has been described as one of the major challenges in infertility treatment with assisted reproductive technologies (ARTs) **(Illingworth et al., 2024)**.

Due to the crucial role of CCs in the follicle development and growth of oocytes, several studies have shown that oocyte quality was determined by CC viability. Hence, the Identification of the expression profile of genes using quantitative PCR (q-PCR) as previously published has a significant relation correlating cumulus gene expression with oocyte maturity, fertilization, embryonic development, implantation, and pregnancy **(Elias et al., 2025)**.

Removal of CCs before IVF in bovine samples significantly reduced the cleavage rate through the loss of a factor secreted by these cells **(Choi et al., 2015)**.

Previous studies on transcriptomes in human CCs reported that, in 611 differentially expressed genes in CCs from early and non-early cleavage embryos, 24% were overexpressed in the early cleavage in CCs. These genes were involved in several signaling pathways, including cell cycle, survival and death signaling, chemokine and cytokine signaling, angiogenesis, and lipid metabolism **(Prabhu et al., 2017)**.

The gene expression of CCs was described as cellular functions. Glucose metabolism is one of the crucial properties of living cells. Among multiple genes included in the glycolytic pathway is the G6DP **(Chen et al., 2022)**.

CCs have great expansion capacity, which is thought to be regulated by multiple genes, of which HAS2 and GREM1 were selected for our study.

Cell expansion and differentiation are directed by multiple paths that aim at regulating cell apoptosis to maintain a proper mass of cells around the oocytes for its integrity **(Gao et al., 2022)**.

BCL2 is a prominent member among molecules that regulate apoptosis, and its expression is found to be increased in CCs obtained from mature and fertilized oocytes **(Dehgham et al., 2020)**, and that was the basis of selecting its expression level to be studied.

Patients and methods

Type of the study: A prospective observational study.

Sample size: 50 cases

The following simple formula would be used for calculating the adequate sample size in prevalence study:

$$n = \frac{Z^2 p(1-p)}{d^2}$$

where n is the sample size, Z is standard normal variant (at 5% type 1 error ($P<0.05$) it is 1.96, d (absolute error or precision) =0.05, the level of confidence usually aimed for is 95%, the result was 18 women we raised the sample to 25 women to get more informative results.

Study setting: The study was held from April 2023 to April 2024 at the Assisted Reproduction Unit, Obstetrics and Gynecology Department, Qena Faculty of Medicine, Qena University. Females aged between 20 and 45 years, with a body mass index (BMI) ≤ 30 kg/m2, no gynecological problems (e.g. fibroid, endometriosis, uterine polyp, hydrosalpinx or adenomysis), and males with mild to moderate oligozoospermia or asthenozoospermia were included: however, abnormal sperm morphology; (globozoospermia and pin-point sperm) and azoospermia; (whether

obstructive or non-obstructive) were excluded.

Ovarian stimulation protocol:

Fifty couples were selected from those who were planning to undergo ICSI. Antagonist protocol was applied to all patients. It involves administration of HMG (human menopausal gonadotropin) as in-vitro fertilization menotropin (IVF-M), LG Chem Ltd, Korea 75-150 IU (75 IU FSH+75 IU LH) and recombinant FSH (r-FSH) in the form of Gonal–F, Merck 75,300 IU. Serial transvaginal ultrasound (TVUS) monitoring was done to evaluate ovarian follicles growth. Upon reaching a size of 14mm, ovarian follicles underwent pituitary down-regulation through administration of gonadotrophin releasing hormone antagonist (GnRH); specifically, cetrotide at a dosage of 0.25 mg. Ovulation trigger was subsequently achieved by administrating 500 micrograms of recombinant human chorionic gonadotropin (R-HCG), known as Ovitrelle S.C (Cheng et al., 2025).

Hormonal assay protocol:

Venous blood samples were collected on day 2 of the menstrual cycle. 10 ml of blood was drawn from the elbow using a disposable syringe and drained into a clean gel plastic tube. The sample was centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded, and the pellet was placed in a 1.5 ml Eppendorf tube and stored at -20°C. Hormone analysis (E2, LH, FSH, prolactin, TSH, and AMH) was performed using a Rayto analyzer.

Cumulus Cells (CCs) and oocyte retrieval:

CCs were separated from the oocyte with strippers after brief exposure to hyaluronidase (80 UI/ml, Origio, USA) at 37 C. After oocyte recovery, cumulus cells were washed in cold phosphate buffer saline (Dubelcco's medium) (Gibco, Invitrogen, Paris, France), and then centrifuged at 200 g for 10 min. The supernatant was removed, and the pellet was resuspended in cell lysis buffer of the Absolutely RNA Nanoprep kit (Stratagene Europe, Amsterdam, The Netherlands) before storage in liquid nitrogen at -196 C. Labelling allowed individual follow-up of the whole process **(Attia et al., 2025)**.

Denuded oocytes were kept in a culture dish containing Global culture media (Global multipharm, Canada) where they were incubated for one hour before ICSI at 37◦C.

ICSI and embryo development assessment:

ICSI was performed using the male sample under inverted microscope (RI systems, Japan). Semen samples were processed using simple was technique. Oocytes were incubated in LABOTECT incubator in specific culture dishes from Global Multipharm at 37℃ and pH of 7.2.

The morphological characteristics of the oocyte and embryo were recorded on an individual basis. At the time of ICSI (Day 0), the oocytes were first classified into three categories based on nuclear status: (i) mature oocyte with the first polar body (metaphase II, MII), (ii) immature oocyte at the germinal vesicle (GV) stage, and (iii) immature oocyte without the first polar body called metaphase I (MI). On Day 1, fertilization was investigated under an inverted microscope. Embryos were monitored for the next four days (till day 5), where embryo grading could be recorded. Embryos graded according to shape of blastomeres and percent of degeneration into three grades.

Grade I embryos had equal blastomeres and degeneration of less than 25%, and grade II embryos had equal blastomeres and degeneration of less than 50%, while grade III embryos had unequal blastomeres or degeneration of more than 50% **(Kocur et al., 2025)**.

Quantitative Real -Time PCR (qRT–PCR):

The expression levels of the selected genes were assessed using the reverse transcription-quantitative polymerase chain reaction (qRT-PCR) technique, which is a highly sensitive method for measuring steady-state mRNA levels. To validate the expression of the target gene, a quantitative real-time qRT-PCR SYBR Green assay was employed.

Primer sequences for the genes were designed and synthesized by Alpha DNA Ltd. (Canada), then lyophilized and stored at -20°C. The mRNA levels of the endogenous control gene GAPDH were amplified and used to normalize the mRNA levels of the target gene. **(Table 1)**

Table 1. The primer sequences, product length, code number and temperature of studied genes

Gene	Sequence	Product length (bp)	Code number	Temp
HAS2	**F: GCCTCATCTGTGGAGATGGT R: ATGCACTGAACACACCCAAA**	**182**	**NM_005328**	**54**℃
GREMLIN	**F: TGCTGGAGTCCAGCCAAGA R: GCACCAGTCTCGCTTCAGGTA**	**65**	**NM_013372**	**54**℃
BCL2	**F: ACCGTCGTGACTTCGCAGAG R: GGTGTGCAGATGCCGGTTCA**	**239**	**NM_009741.1**	**84**℃
G6DP	**F: CTGATCCTGGGTCGCTTCAT R: ACGTACATGGGCACAAAACCA**	**68**	**NM_174602.2**	**30**℃
GADPH	**F: GTCTCCTCTGACTTCAACAGCG R: ACCACCCTGTTGCTGTAGCCAA**	**111**		

RNA extraction:

RNA was extracted from all samples using TransZol Up Plus RNA Kit, EasyScript®, One-Step gDNA Removal and cDNA Synthesis SuperMix TransStart®, and Top Green qPCR Super Mix provided by TransGen biotech (China), as follows:

- One milliliter of the denuding media containing CCs was subjected to centrifugation for one minute at 12,000 rpm. The supernatant was removed, and the resulting pellet was resuspended in 1000 µl of TransZol Up.
- The samples were stored overnight at -23ºC. For every milliliter of TransZol Up Reagent, 200 µl of chloroform was added. The mixture was gently vortexed for 30 seconds and allowed to incubate for three minutes at room temperature.
- Subsequently, the tube was centrifuged at 10,000 rpm for 15 minutes within a temperature range of 2-8 ºC. This process resulted in the formation of three distinct layers: a lower pink organic phase, an interphase, and a colorless upper aqueous phase that contained the RNA.
- The upper aqueous phase constituted approximately 50–60% of the total volume of TransZol Up.
- The colorless upper phase containing RNA was transferred to a new RNase-free tube to prevent DNA contamination from the interphase, allowing for the retention of a portion of the aqueous phase.
- An equal volume of 96–100% ethanol was added, which may result in visible precipitates. The mixture was gently inverted to ensure thorough mixing. The resulting precipitates and solution were then transferred to a spin column and centrifuged at 12,000 rpm for 30 seconds at room temperature.
- The flow-through was discarded, and if the lysate volume exceeded the spin column's capacity, this step was repeated. Subsequently, 500 µl of CB9 was added to the spin column, followed by another centrifugation at 12,000 rpm for 30 seconds at room temperature, after which the flow-through was discarded.
- The previous step was repeated once more.
- Next, 500 µl of WB9 was added to the spin column, ensuring that ethanol was included, and centrifuged at 12,000 rpm for 30 seconds

at room temperature, with the flow-through discarded.

- The previous step was repeated once.
- The column matrix was then centrifuged at 12,000 rpm for 2 minutes at room temperature to remove any residual ethanol, followed by air-drying for several minutes.
- The spin column was placed in a clean 1.5 mL RNase-free tube, and after adding 50-200 µl of RNase-free water, it was incubated for 1 minute at room temperature.
- The RNA was eluted by centrifuging at 12,000 rpm for 1 minute. To enhance yield, it is advisable to repeat the elution steps.
- The extracted RNA was subsequently stored at -20°C.

Gene expression Calculation:

Changes in the expression of mature RNAs assessed using the relative cycle threshold (2-ΔΔCt) method, as established by Livak and Schmittgen (2001). This method calculates the ratio of gene expression levels between the control and test groups. Values above 1 indicate an increase in gene expression and values. between 0 and 1 suggest a decrease, and a fold change of 1 implies no difference. The expressions of the target genes are normalized by setting appropriate thresholds to ensure accurate Ct values derived from the qRT-PCR instrument.

1. ΔCT

The difference in CT values (ΔCt), referred to as the 'normalized raw data', for each target gene and the housekeeping gene was determined by subtracting the chosen normalization factor from the Ct value of each gene of interest.

ΔCt (control)=CT (gene)-CT(HKG)

ΔCt (patient)=CT (gene)-CT(HKG)

Finally, the expression ratio calculated according to the formula:

2-ΔCT = Normalized expression ratio

2. ΔΔCT

The calculation of the Double Delta Ct Value (ΔΔCt) for the genes of interest involved subtracting the ΔCt value of each test group from that of the control group, represented as follows: ΔΔCt = ΔCt(patient) - ΔCt(control). To ascertain the expression fold change for each gene, the value of 2 raised to the power of -ΔΔCt utilized, reflecting the Relative Fold Change. Thus, the findings articulated as a fold change in the expression level of the target gene, normalized against the endogenous control (housekeeping gene) and relative to the calibrator, which is the target gene in control subjects (Stephenson, 2016). In conclusion, the fold-change value in gene expression was determined using the equation: Fold change = $2^{(-\Delta\Delta Ct)}$ Normalized expression ratio.

Ethical approval code: The study protocol was approved by the Institutional Research Committee at the Faculty of Medicine, South Valley University, Qena, Egypt, (approval number: **SVU-MED-ANA001-2-24-2-567)**. Written consent was obtained from the couples who participated in the study.

Statistical analysis

conducted using SPSS v26 (IBM Inc., Chicago, IL, USA). The Shapiro-Wilks test evaluated data normality. Qualitative variables presented as frequencies and percentages. Quantitative parametric data described as mean and standard deviation (SD) and compared via independent sample t-test. Quantitative non-parametric data presented as median and interquartile range (IQR) and compared by the Wilcoxon test. Qualitative variables presented as frequency and percentage and compared by the chi-square test. Pearson's correlation measured the strength and direction of association between continuous variables, with a significance level set at $P < 0.05$.

Results

Demographic data and previous trials of the studied females

The age ranged from 21 to 41 years. BMI ranged from 22.1 to 37.8 kg/m^2. And all patients had primary infertility, **(Table 2)**.

Table 2. Demographic data and previous trials of the studied females

Age (years)	**Mean ± SD**	32.8 ± 7.11
	Range	21 – 41
BMI (kg/m^2)	**Mean ± SD**	28.7 ± 4.05
	Range	22.1 - 37.8
Type of infertility	**Primary**	50 (100%)
Previous trials	**Median**	0
	IQR	0 – 1

Semen analysis of the studied males: The median (IQR) of normal form was 0.25(0.25 - 0.5) % **(Table 3)**.

Hormones of the studied patients: LH ranged from 2.33 to 8.9 IU/L. FSH ranged from 3.4 to 9.5 IU/L **(Table 4)**.

E2 at D2, E2 at OPU day, trigger, and protocol of the studied patient:

E2 at D2 ranged from 22.2 to 73.4 pg/ml with a mean value (± SD) of 39 (± 12.89) pg/ml. The median (IQR) of E2 at OPU day was 1976.05(1660.4 - 2587.53) pg/ml. All patients had the trigger of HCG. The protocol used was antagonist in all patients **(Table 5)**.

MII injected, fertilization, endometrial thickness, embryo transfer, and B-HCG of the studied patient: 20 (40%) patients had positive B-HCG **(Table 6, Fig 1).**

Table 3. Semen analysis of the studied males:

Semen concentration (million/ml)	**Median**	17.5
	IQR	5 - 23.75
Progressive motility (%)	**Median**	15
	IQR	10 - 25
Normal form (%)	**Median**	0.25
	IQR	0.25 - 0.5

Table 4. Hormones of the studied females

LH (IU/L)	Mean ± SD	4.4 ± 1.32
	Range	2.33 - 8.9
AMH (ng/ml)	**Mean ± SD**	2 ± 1.12
	Range	0.65 - 4.1
PRL (ng/ml)	**Mean ± SD**	15.4 ± 5.04
	Range	6.9 - 26.2
FSH (IU/L)	**Mean ± SD**	5.9 ± 1.47
	Range	3.4 - 9.5
TSH (IU/L)	**Mean ± SD**	1.7 ± 0.7
	Range	0.77 - 3.82

LH: Luteinizing hormone, AMH: Anti-Müllerin hormone, PRL: Prolactin, FSH: Follicle-stimulating hormone, TSH: Thyroid-stimulating hormone.

Table 5. E2 at D2, E2 at OPU day, trigger and protocol of the studied patient

E2 on D2 (pg/ml)	**Mean ± SD**	39 ± 12.89
	Range	22.2 - 73.4
E2 on OPU day (pg/mL)	**Median**	1976.05
	IQR	1660.4 - 2587.53

Trigger	**HCG**	50 (100%)
Protocol	**Antagonist**	50 (100%)

E2 at D2: Estradiol at day two, E2 at OPU: Estradiol at ovum pick-up day, HCG: Human chorionic gonadotropin.

Table 6. MII injected, fertilization, endometrial thickness, embryo transfer and B-HCG of the studied patient

Metaphase II (M II) injected	Median	5.5
	IQR	2.25 - 16.5
Fertilization (zygote)	**Median**	3
	IQR	1 – 7
Endometrial thickness (mm)	**Mean ± SD**	1.1 ± 0.31
	Range	0.72 - 1.95
Positive B-HCG no (%)		20 (40%)

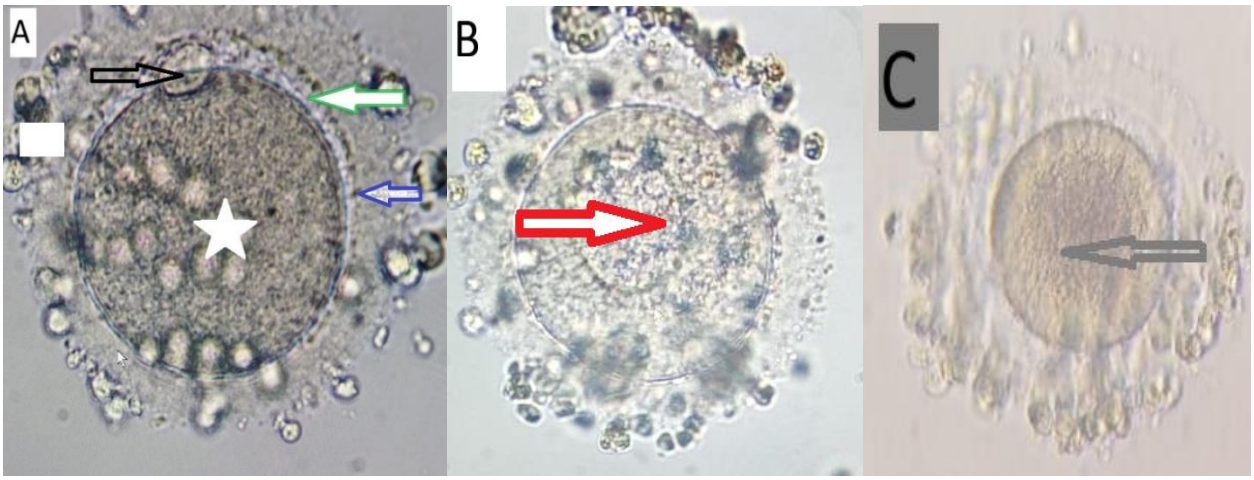

Fig 1. M II oocytes with different morphological criteria showing:
A: Good morphological quality with remnant of CCs (white rectangle), first polar body (black arrow), clear cytoplasm (white star), zona pellucida (blue arrow), clear peri-vitelline space (green arrow). **B**: Bad morphological quality with vacuolated cytoplasm (orange arrow). **C:** Bad morphological quality with excess smooth endoplasmic reticulum (grey arrow)

Correlation between gene expressions and ICSI parameters among pregnant females:

As shown in **Table 7**, **Fig 2;** the expression profile of all genes revealed a significant correlation with fertilization rate (p=0.037) and positive correlation with grade I embryos (p=0.009) and grade II embryos (p=0.033). On the contrary side all of them showed a negative correlation with abnormal oocytes (p= 0.009) **(Table 6).**

Table7. Correlations between gene expressions with ICSI parameters among pregnant females

Parameters		**HAS2**	**GREM1**	**BCL2**	**G6DP**
Total oocytes	**r**	**0.155**	**0.144**	**0.146**	**0.105**
	p	**0.431**	**0.464**	**0.459**	**0.593**
Metaphase II oocytes	**r**	**0.160**	**0.170**	**0.096**	**0.141**
	p	**0.415**	**0.386**	**0.626**	**0.475**
Fertilization rate	**r**	**0.395**	**0.444**	**0.496**	**0.217**
	p	**0.037 ***	**0.018 ***	**0.007***	**0.268**

Total embryos	**r**	**0.239**	**0.278**	**0.220**	**0.092**
	p	**0.221**	**0.152**	**0.261**	**0.642**
Grade I embryos	**r**	**0.482**	**0.532**	**0.497**	**0.226**
	p	**0.009***	**0.004***	**0.007***	**0.247**
Grade II embryos	**r**	**0.404**	**0.487**	**0.505**	**0.222**
	p	**0.033***	**0.009***	**0.006***	**0.256**
Grade III embryos	**r**	**0.115**	**0.124**	**0.159**	**0.114**
	p	**0.228**	**0.292**	**0.314**	**0.564**

*: significant; r: Pearson's correlation coefficient.

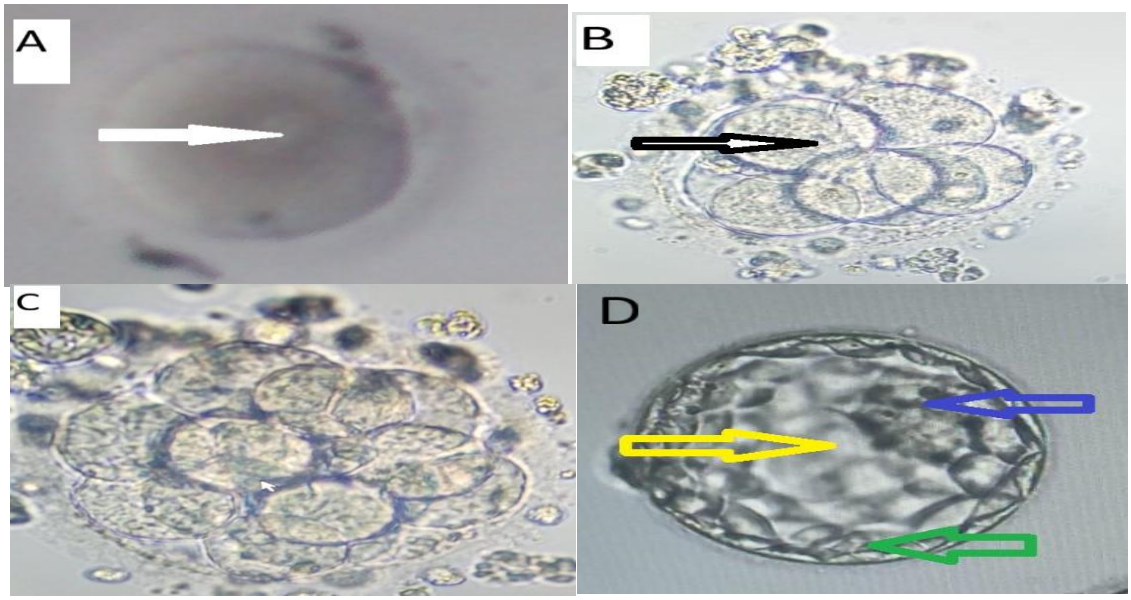

Fig 2. Embryos with different developmental stages.

A: zygote with 2 pro-nuclei (white arrow). **B**: cleavage stage embryo, 8 cells stage, with equal balstomeres (black arrow). **C**: morula stage embryo with compaction. **D**: blastocyst with good sized inner cell mass (blue arrow), distended blastocoele (yellow arrow), intact outer cell mass (green arrow).

Relation between embryo quality and grading between pregnant and non-pregnant women: There is no statistical difference between both groups regarding grade and quality of embryos **(Table 8)**.

Relation between age and (fertilization and endometrial thickness) of the studied patients:

Fertilization and endometrial thickness were significantly higher in female group with an age <30 than age >30 group (P < 0.05) **(Table 9)**.

Relation between age and genes' expression of the studied oocytes: the HAS2, GREMLIN, BCL2, and GAPDH gene levels were significantly higher in in the female group aged <30 than in the group aged >30. However, the G6DP gene level was not significantly different between both groups **(Table 10)**.

Table 8. embryo grading in pregnant and non-pregnant women

Variables	Pregnant	Non-pregnant	P-value
Grade I embryo	2.57 ± 0.57	3.98 ± 0.50	0.081 NS
Grade II embryo	1.11 ± 0.32	1.15 ± 0.27	0.916 NS
Grade III embryo	0.54 ± 0.19	0.85 ± 0.24	0.378 NS

NS: Not significant; independent sample t-test

Table 9. Fertilization and endometrial thickness in relation to age of the studied groups

Variables		Age < 30 years	Age > 30 years	P-value
Fertilization (zygote)	Median	9	2	<0.001*
	IQR	3.5 - 11	1 - 3.5	
Endometrial thickness (mm)	Mean ± SD	1.32 ± 0.24	1.04 ± 0.3	0.015*
	Range	1.1 - 1.73	0.72 - 1.95	

*: significant; independent sample t-test

Table 10. Relation between age and genes' expression of the studied oocytes

Variables		Age < 30 years	Age > 30 years	P-value
HAS2	Median	90.1	60	<0.001*
	IQR	87.35 - 126.05	46.05 - 80.05	
GREMLIN	Median	160.7	96.4	<0.001*
	IQR	150.7 - 224.52	81.99 - 121.1	
BCL2	Median	98	60	<0.001*
	IQR	83.56 - 108	40.85 - 79.9	
G6DP	Mean ± SD	29.72 ± 4.38	30.29 ± 4.03	0.719
	Range	23.97 - 36.16	23.12 - 39.76	
GAPDH	Mean ± SD	33.87 ± 2.5	29.66 ± 2.64	<0.001*
	Range	31.3 - 39.76	23.78 - 33	

*: significant; independent sample t-test

Discussion

In the current study, samples obtained from CCs were examined for the expression of selected genes. The expression profiles of these genes were correlated to the pregnancy status, which can be used as a non-invasive predictor of ICSI outcome, which matches the aim of the study conducted by **Tanghe et al., 2002.**

The intra-follicular environment beside the part of the follicle's wall known as cumulus cells (CCs), has crucial role in the growth and development of oocytes. Identification of the mechanisms by which CCs affect the growth of oocytes and protect them from damaging systemic diseases is a crucial step in the management of infertility, as described by **(DaBroi et al., 2018).**

CCs that are in direct contact with the oocyte, forming what is known as COC (cumulus oocyte complex), have a crucial role in keeping the oocyte in communication with the CCs **(Feuerstein et al., 2007)**.

Russell et al. (2006) reported that hyaluronic acid synthase (HAS2) is necessary for both differentiation and CCs expansion and thus correlates with the process of embryogenesis. Hyaluronic acid (HA) is a major component of the intercellular matrix between CCs, so adequate production of HA is important for CCs expansion and subsequent oocyte maturation **(Ploutarchou et al., 2015)**.

HAS2 is the main source of hyaluronan that is the main component of the extracellular matrix that binds oocyte and CCs together, enabling the oocyte to complete its

maturation and resume division **(Dabbagh et al., 2022)**.

Gebhardt et al. (2011) reported that Gremlin (GREM1) expression in CCs correlates with birth weight at 35 weeks of gestation and found to be high in oocytes with higher developmental capacity, leading to enhanced implantation rates and improved embryo developmental.

In this study we found that its expression level in pregnant women was significantly higher than those who not achieved pregnancy.

Both HAS2 and GREM1 are members of the growth factor superfamily, Growth Differentiation Factor 9 (GDF9), which is considered the first potential marker for the oocyte's quality **(Peng et al., 2010)**.

CCs are living potential cells that undergo apoptosis. The balance between CCs expansion and CCS apoptosis is the determinant factor for achieving ovulation with subsequent healthy or Poor-quality oocytes **(DaBroil et al., 2018)**.

B-cell lymphoma (BCL2) is a prominent member among molecules that regulate apoptosis, and its expression increased in CCs obtained from mature and fertilized oocytes **(Dehghan et al., 2020)**.

Another study found that altered expansion found that altered expression of BCL2 in patients with polycystic ovaries affects the developmental capacity of oocytes **(Nikmard et al., 2022)**.

Glucose is an essential metabolite for CCs like all other cells in the human body. It is an essential component for energy production, cellular activities, and maturation of the nucleus. Oocytes are known to have poor glucose uptake capacity, and thus they depend on CCs as intermediates to supply them with elements utilized by the oocyte **(Richani et al., 2021)**.

The glycolytic pathway is the main path for glucose metabolism with subsequent production of lactate and pyruvate that are transferred to the oocytes, where they are used.

A study described that applying silencing of the Glucose 6 Phosphate Dehydrogenase (G6DP) gene of CCs had lowered oocyte maturity compared to the control **(Xie et al., 2018)**.

Selecting an embryo with a higher affinity of implantation is the most challenging step in the process of ICSI. Recent criteria for selection depend on morphological elements of oocytes and embryos. Early cleavage of embryos, degree of fragmentation, and blastocyst formation are the major morphological criteria of selection **(Assou et al., 2010)**.

Oocyte quality is influenced by various factors that affect both the nucleus and cytoplasm maturation through complex intra-follicular mechanisms, subsequently impacting embryo development **(Dabbagh et al., 2022)**.

The crucial role of CCs in the proper development of follicles and maturity of the oocyte highlights several studies to understand the possible mechanisms by which CCs play their role. Therefore, several studies correlating cumulus gene expression with oocyte maturity, fertilization, embryonic development, implantation, and pregnancy have been conducted.

The current study referred to all the previously mentioned data concerning the different activity pathways of the CCs to select those genes (HAS2, GREM1, BCL2, G6DP) to be studied depending on their importance in oocyte maturity regulation.

Our study followed the expression pattern of the previously mentioned genes and correlated those patterns with incidence of pregnancy, which may be a prognostic non-invasive tool for the prediction of ICSI success.

In the current study, HAS2, GREM1, and BCL2 showed significant differences between pregnant and non-pregnant females

included in the study. The expression profiles of the previously mentioned genes were high in the positive B-HCG compared to patients with negative B-HCG.

There was a statistical difference between pregnant and non-pregnant females regarding embryo quality and embryo grading.

In the current study, all patients included were complaining of primary infertility, which may reflect the fact that couples are facing more pressure in the surrounding society to get their first baby, on the other hand; couples in our society who are not interested in getting their second baby using ICSI may be due to the high cost of the procedure.

The findings indicate that most patients had no prior in vitro fertilization trials, with only a few having undergone one ICSI trial. The data also suggests a significant prevalence of overweight or obese individuals, highlighting this condition as a potential risk factor for infertility in the population.

This finding runs in line with the finding of **Dağ et al.,** indicating that overweight and obese women are more often susceptible to experiencing reproductive issues like abnormal menstruation and infertility. Moreover, **Zhu et al. (2022)** highlight the detrimental effects of overweight and obesity on human reproductive health.

Moreover, elevated BMI may contribute to inflammation linked to factors such as oxidative stress, psychological stress, and lifestyle factors **(Skonieczna et al., 2024)**. In general, obesity is common in women due to multiple environmental and genetic elements, like calorie-dense diets and lack of activity.

In the current study, most patients exhibited male factor infertility, mainly teratozoospermia. Factors like paternal age, environmental toxins, and tobacco abuse increase infertility risks. These factors may interfere with spermatogenesis, a sensitive biochemical process that affects the blood-testis barrier and leads to sperm mutations **(Sharma et al., 2015)**.

Shadyab et al. (2017) reported that the mean age of women ranged between 35 and 37.5 years. Another one found that the mean age was 27-34 years **(Fikrie et al., 2024)** which is compatible with the findings in our study.

In the current study, the number of retrieved oocytes was insignificantly different between both groups, which may be against the finding explained by AMH level among females' reproductive ages.

In the current study, the fertilization rate was higher among patients below 30 years old than in those who were above 30 years.

Endometrial thickness was higher in patients below 30 years old than the other group.

In the current study, the expression profile of HAS2, GREM1, and BCL2 genes was high in females below 30 years old, while G6DP gene expression was insignificantly different between both groups.

Conclusion

A large cohort of CCs must be analysed before any future use. Our findings support the use of gene expression analysis of the CCs as a non-invasive method for embryo selection in ICSI, with the gene expression profiles of HAS2, GREM1, and BCL2 serving as predictive indicators of ICSI success.

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