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Interference of Bisphenol A on Cumulus Cells Development and Number of Retrieved Mature Oocytes in Unexpected Poor Ovarian Response Women: A Prospective Cohort Study

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Abstract

Objective: This study aimed to investigate the relationship between follicular fluid Bisphenol A (BPA) concentrations with alterations in the expressions of *NOTCH1-3, CASPASE 3/7, HLA-G*, and *ICAM-1* genes and the number of retrieved mature oocytes (MII oocyte) in the cumulus cells of infertile poor ovarian response stimulates women.

Materials and Methods: In this prospective cohort study, 80 infertile unexpected poor ovarian response (POR) subjects were selected on the basis of subgroup 1a of the POSEIDON classification. They were divided into two groups: group 1 consisted of 40 women, each with a higher number of metaphase II (MII) oocytes (G1, 3-4 oocytes retrieved), while group 2 comprised of 40 women, each with a lower number of MII oocytes (G2, ≤2 oocytes retrieved). The expressions of the studied genes were evaluated by quantitative-real time polymerase chain reaction (PCR). The concentration of BPA in follicular fluid was measured with HPLC.

Results: The expression levels of *NOTCH1-3, HLA-G*, and *ICAM-1* genes were significantly lower in G2 than G1 ($P<0.05$). Meanwhile, *CASPASE 3/7* expression levels were higher in unexpected POR patients in G2 compared to G1 ($P<0.05$). There was a significant direct correlation between the levels of *NOTCH1-3*, *HLA-G* and *ICAM-1* gene expressions and there was also a significant inverse correlation ($P<0.05$) between the levels of *CASPASE 3/7*, with the number of MII oocytes and embryo development between the two groups. The concentration of BPA in the follicular fluids of G2 was higher compared to G1 ($P<0.05$).

Conclusion: A higher concentration of BPA was associated with a lower number of mature oocytes and oocyte quality in these patients. Also, alterations of *NOTCH1-3, CASPASE 3/7, HLA-G*, and *ICAM-1* transcript levels in unexpected POR women were associated with BPA concentration.

Keywords: Embryo Development, Oocyte, Reproductive System

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Introduction

Poor response to ovarian stimulation in most cases refers to women with impaired ovarian reserve or poor ovarian response (POR) to exogenous gonadotropin stimulation (1). Indeed, POR is a challenge in the field of reproductive infertility. For a complete description of POR patients, several guidelines have been developed based on tests of ovarian reserve and checking the outcomes of previous *in vitro* fertilization (IVF) cycles (2). Four parameters -namely, age, antral follicle count (AFC), anti-müllerian hormone (AMH), and ovarian response -should be investigated for the diagnosis of POR patients in IVF cycles. The new classification for POR criteria is included in the POSEIDON stratification. One of the side effects of POR is irregularity of oocyte maturation (3).

POR is defined as impaired reproduction ability that leads to infertility (4). In classification groups of POR, unexpected POR subjects are very valuable for infertility studies. This subgroup exhibits normal ovarian reserve, with adequate AMH levels >1.2 ng/ml and AFC >5. However, they experience issues with ovarian response during controlled ovarian stimulation (COS), resulting in the retrieval of ≤4 oocytes on the ovum pickup day (5). Consequently, these individuals are classified as unexpectedly young (<35 years) POR. Understanding

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the cause of unexpected POR in young women has attracted enormous interest from infertility researchers. Polycarbonate is a kind of plastic that contains Bisphenol A (BPA) (6). Warm, acidic, or alkaline conditions affect and break polycarbonate materials. Also, BPA can attach to food and drink, and it may enter the body (7). BPA can act like sex hormones by causing disarray in the endocrine system function and it can affect hormonal signaling pathways (8). A recent study showed that a simple chromatographic method can determine BPA concentration in follicular fluids as well as in blood. In low amounts, BPA may interfere with the expression of genes that are responsible for oocyte maturation, as well as affect the ovaries, uterus, and other reproductive organ functions (9). BPA plays a key role in regulating ovarian response, oocyte maturation, the reproductive system, and gene expression processes (10). The role of alterations in the expression of reproduction genes in the pathophysiology of POR is still unclear (11).

The Notch pathway is a conserved signaling system that is essential in many developmental cell processes. The Notch pathway regulates embryonic stage and postnatal ovary development. The main established functions of the Notch pathway are follicle development, the meiotic process, and ovarian angiogenesis. Disruption of the Notch pathway may affect the reproductive process, oocyte maturation, and embryo quality (12).

Caspases, an evolutionarily conserved family of cysteine proteases, are valuable for the process of apoptosis (13). A recent study suggested that apoptosis of cumulus cells is correlated with the quantity and quality of MII oocytes, embryo development disorder, and poor blastocyst outcomes (14). Members of the Caspase family, especially Caspase-3 and -7, regulate the stereotypical events that occur during cell suicide. Caspase-3 is important for chromatin condensation, DNA fragmentation, and nuclear collapse. Also, Caspase-7 has an important role in the demolition phase of apoptosis. This gene is essential for producing reactive oxygen species (ROS) and separating cells from the extracellular matrix (15).

The *HLA-G* gene encodes sHLA-G protein. HLA-G is a tolerogenic molecule that controls cells of both innate and adaptive immune systems and is considered a marker for oocyte maturation and embryo quality. Secretion of HLA-G is associated with higher rates of pregnancy, implantation, oocyte maturation, and embryo quality (16).

Intercellular Adhesion Molecule 1 (ICAM-1) is a potential marker of embryo quality. In this respect, recent studies suggest that ICAM-1 is a biochemical marker for oocyte quality. Indeed, ICAM-1 release is much higher in immature oocytes compared to mature oocytes. As a result, it has a key role in oocyte maturation. ICAM-1 encodes a cell surface glycoprotein, which is expressed in endothelial and immune cells (17).

A network of proteins including *NOTCH1-3, CASPASE 3/7, HLA-G,* and *ICAM-1* gene regulates the differentiation of oocytes during oogenesis (18).

In the present study, we aimed to assess the relationship between BPA concentration in follicular fluid and alterations in the expression of *NOTCH1*-3, *CASPASE 3*/7, *HLA-G,* and *ICAM-1* genes in cumulus cells of POR patients (19). Also, we assessed the association between BPA concentration with oocyte and embryo quality in infertile women with POR following COS.

Materials and Methods

Patient population and ovarian stimulation

This prospective cohort study was carried out at Royan Institute, Tehran, Iran from March 2019 to March 2020. The participants consisted of 80 patients with unexpected POR, selected based on subgroup 1a of the POSEIDON classification. In addition, they had a history of IVF failure with a family history of POR. The participants were divided into two groups: group 1, which included 40 women with a higher number of mature oocytes (G1, ≤4 oocytes retrieved), and group 2, a lower number of mature oocytes (G2, ≤2 oocytes retrieved), which also included 40 women. All participants underwent intracytoplasmic sperm injection (ICSI). All patients were younger than 35 years and their partners had normal spermogram results. Women with a history of operation in the uterus and ovaries, endometriosis, and those with a body mass index (BMI) higher than 25 kg/m^2 were excluded from this study. All participants were included in this study after giving informed consent. The ethnicity of the participants included: Lur, Baluch, Turkmen, Azari, Tabari (Mazandarani), Gilak, Talesh, Arab, Isfahan, Kurdish, and Fars.

The Ethics Committee at Royan Institute, Iran (IR.ACECR.ROYAN.REC.1394.150) approved this project on the day of ovarian puncture. All methods of data collection were performed in accordance with the Declaration of Helsinki. All study participants provided informed consent before inclusion in the study.

The follicular fluid sample of each woman was collected in a special tube (without BPA compound, Kaneka Medex, Japan) and then stored at -70°C until the HPLC and gene expression analysis.

For ovary stimulation, gonadotropin-releasing hormone (GnRH) flexible antagonist protocol was used for all patients (19).

Retrieval of cumulus cells

Ovum pick-up was done 36 hours after human chorionic gonadotropin (hCG) injection and then cumulus-oocyte

complexes (COCs) were collected from each patient.

Metaphase II oocytes, fertilization evaluation, and embryo quality

Cumulus cells were collected from each mature oocyte. Retrieved oocytes were classified as mature in the MII stage and as immature in the metaphase I (MI) or germinal vesicle (GV) stage. During the ICSI process, 10 minutes after denudation, MII oocytes were injected and incubated until transfer day into the uterus. Fertilization was evaluated 16-20 hours after the ICSI process according to Veeck's morphological grading system. Embryos were classified into two groups: i. Containing oocytes exhibiting normal fertilization with two pronuclei and ii. Containing oocytes exhibiting abnormal fertilization with more than two pronuclei (19). Based on the embryos' grades, patient age, and previous assisted reproductive technology (ART) cycles, we selected two embryos for the transfer process.

Follicular fluids bisphenol A analysis

Total BPA (conjugated and free) in follicular fluid was measured using high-performance liquid chromatography (HPLC), the LODs of BPA in follicular fluid was 1.14 ng/ml (20).

RNA extraction, cDNA synthesis, and quantitative real time polymerase chain reaction

The total RNA of cumulus cells was extracted by Trizol (TRI, Sigma-Aldrich, USA) (20).

Next, cDNA was synthesized from total RNA using the AidTM H-Minus First Strand cDNA synthesis (Fermentas, German) (20).

NOTCH1-3, *CASPASE 3*/*7*, *HLA-G,* and *ICAM-1* gene were chosen as the target genes and the *18srRNA* gene was selected as the reference gene. Primers were designed by Primer Express 3.0 software. Primer sequences are provided in Table 1. Real-time quantitative polymerase chain reaction (PCR) was carried out by a Step OnePlus™ instrument (Applied Biosystems, USA). The reaction contained 2 µl cDNA, 10 µl of Power SYBR Green Master Mix (Applied Biosystems, USA), and 1 µl (500 nM primer) of forward and reverse primers (20).

Data analysis

The categorical variables were presented as numbers (%) and continuous variables as mean ± SD. Chi-square analysis was used for qualitative data and in-between demographic and clinical characteristics comparisons were performed by independent t test. Univariate and backward multiple linear regression at significant levels were calculated between the associations of BPA concentration, gene expression, and embryos by PAST software (PAST4, UK). $P<0.05$ were considered statistically significant.

Also, an assessment of the gene expression alterations between two groups of unexpected POR patients was done via model base clustering and ordination methods, and linear discrimination analysis (LDA) by PAST 4 software. Heat map analysis for visualizing gene expressions in all samples was performed by package in R 4.2.

Table 1: Real time gene expression primer sequences

Gene	Primer sequence (5′-3′)	Size (bp)
NOTCH-1	F: AGAAGATGCTCCAGCAACACA	84
	R: GCAAGTCTCCTACAAAACACGG	
NOTCH-2	F: AATGAGTGTCTGAGTGAACCCT	85
	R: GACTCCATCAAATCCTGCCTG	
NOTCH-3	F: CTCATGGTATCTGCACCAACCT	84
	R: GGGTCACAGTCATTGATGTCCT	
CASPASE-7	F: AGGACCACCGCATCTCTACAT	84
	R: CCAAGTCTGGCTCGTTCTCA	
CASPASE-3	F: AAGCGAATCAATGGACTCTGG	87
	R: CAAGTTTCTGAATGTTTCCCTGAG	

Standard curves were used to evaluate primer efficiency. For real-time PCR data analysis, the ΔΔCT method was exploited to determine the relative expression of target genes between groups. The fold change (FC) was calculated as $2^{-\Delta\Delta CT}$.

Results

Demographic data, clinical outcomes and ethnicity of patients

The demographic and clinical characteristics of participants are shown in Table 2. No statistically significant differences were found between the G1 and G2 unexpected POR patient groups in terms of BMI, days of ovarian stimulation, the dose of gonadotropins, and infertility duration. The average basal AMH levels (mIU/ml) and AFC were significantly higher in G1 compared to G2 (P<0.05, Table 2). The mean concentration of BPA in follicular fluid was significantly higher in G2 than in G1 (5.23 ± 1.23 ng/mL vs. 1.77 ± 1.49 ng/mL, P<0.0001, Table 2). No statistically significant difference was found between the ethnicity of G1 and G2 groups (Fig.1).

The number of follicles, fertilization rate, number of good quality embryos, number of embryos, and number of clinical pregnancy rates (fetal heart detection by ultrasound) were significantly higher in G1 compared to G2 (P<0.05, Table 3). Meanwhile, the number of transferred embryos did not differ between groups (P>0.05, Table 3).

Table 2: Clinical characteristics and outcome in G1 and G2 groups of unexpected POR patients

Patient character	G2 group	G1 group	P value
Age (Y)	29.60 ± 3.77	29.66 ± 4.26	0.948
BMI (kg/m^2)	24.2 ± 1.6	24 ± 1.4	0.803
Duration of infertility (Y)	7.4 ± 1.66	4.66 ± 1.22	0.501
AMH (mIU/ml)	4.1 ± 1.9	7.01 ± 1.8	0.004*
AFC	0.8 ± 0.2	3.2 ± 0.2	0.002*
BPA concentration (ng/mL)	5.23 ± 1.23	1.77 ± 1.49	0.001*

Variables are presented as mean ± SE. P values were determined by GLM procedure with a significance level of P<0.05. GLM; Generalized linear model, POR; Poor Ovarian Response, BMI; Body mass index, AMH; Anti müllerian hormone, AFC; Antra follicle count, and *; Significance values.

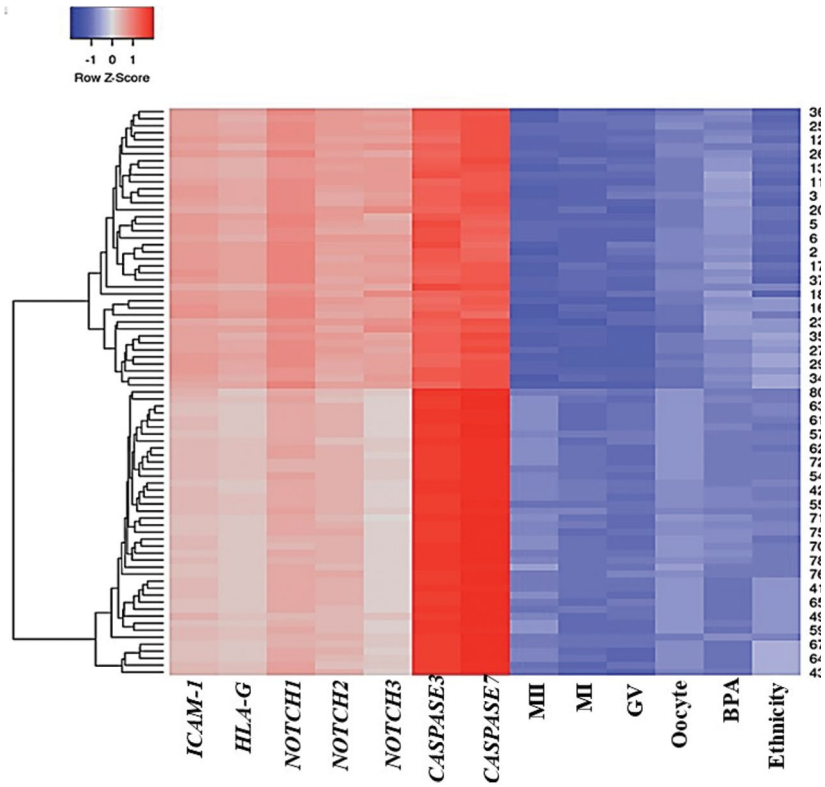

Fig.1: Heat map based on gene expression profile, oocyte stages, Bisphenol (BPA) concentration, and ethnicity.

Gene expression profile

The relative gene expression of *NOTCH1, NOTCH2, NOTCH3, HLA-G,* and *ICAM-1* were significantly lower in G2 compared to G1 (P<0.05). In contrast, *CASPASE-3* and *CASPASE-7* expression levels were higher in G2 compared to G1 (P<0.05, Fig.2).

The heat map analysis data demonstrates a FC in counts of mRNA molecules in the G1 group versus G2. Each line represents data for an individual patient. Based on clustering, the two main clusters stand distantly from each other and show differences in the expression level of genes as well as oocyte characteristics and BPA concentration (Fig.1).

The LDA plot also grouped individuals based on gene expression levels, number of MI, and BPA concentration (Fig.3). Based on the analysis, the two groups studied (G1 and G2) were grouped separately. Therefore, these genes together may affect the number of oocytes produced and their maturation.

In regard to the interaction of two genes in oocyte number release, a model-based pair-wise clustering was performed. Some of these results are provided in Figure S1 (See Supplementary Online Information at www.celljournal.org). For instance, the combination of *ICAMP1* gene expression data with *HLAG* and *NOTCH1* separated samples into two distinct groups (Fig.S1B, C, See Supplementary Online Information at www.celljournal.org).

The pair-wise correlations between genes expressed showed a significant positive and negative correlation between variants in samples (Fig.S2, Table S2, See Supplementary Online Information at www.celljournal.org).

Demographic data such as the number of MII oocytes, and embryo qualities were significantly associated with levels of *NOTCH1*-3, *CASPASE 3*/*7*, *HLA-G,* and *ICAM-1* gene expression. Also, BPA concentration was significantly associated with levels of *NOTCH1*-3, *CASPASE 3*/*7*, *HLA-G,* and *ICAM-1* gene expression (Fig.S2, See Supplementary Online Information at www.celljournal.org). Downregulation of *NOTCH1*-3, *HLA-G,* and *ICAM-1* and upregulation of *CASPASE 3*/*7* gene expression led to a lower number of MII oocytes and embryos.

A high level of BPA concentration was associated with the downregulation of *NOTCH1*-3, *HLA-G,* and *ICAM-1* gene and protein expression and the upregulation of *CASPASE 3*/*7* gene and protein expression in the G1 group.

Table 3: Cycle characteristics and IVF/ICSI outcomes in G1 and G2 groups of unexpected POR patients

Patient character	G2 group	G1 group	P value
Number of follicles	0.9 ± 0.22	3.89 ± 0.36	0.001*
Number of oocytes retrieved	0.8 ± 0.56	3.73 ± 1.21	0.002*
Metaphase I oocyte	1.8 ± 0.66	0.89 ± 0.21	0.002*
GV	2.8 ± 0.96	0.63 ± 0.33	0.003*
Degenerated/dead	3.8 ± 0.22	0.92 ± 0.37	0.001*
Regular fertilization rate, % (of 2PN/MII oocytes)	0.6 ± 5.9	3.51 ± 1.3	0.030*
Total embryos	0.8 ± 0.2	2.2 ± 0.2	0.002*
Number of good quality embryos	0.33 ± 0.42	2.87 ± 013	0.001*
Number of ET	1.52 ± 0.32	0.35 ± 0.69	0.003*
No. clinical pregnancy	0.70 ± 0.33	2.73 ± 0.43	0.020*

Variables are presented as mean ± SE. P values were determined with a significance level of P<0.05. IVF; *In vitro* fertilization, ICSI; Intracytoplasmic sperm injection, POR; Poor ovarian response, GV; Germinal vesicle, MII; Metaphase II oocytes, 2PN; Two pronuclear, and ET; Embryos transferred.

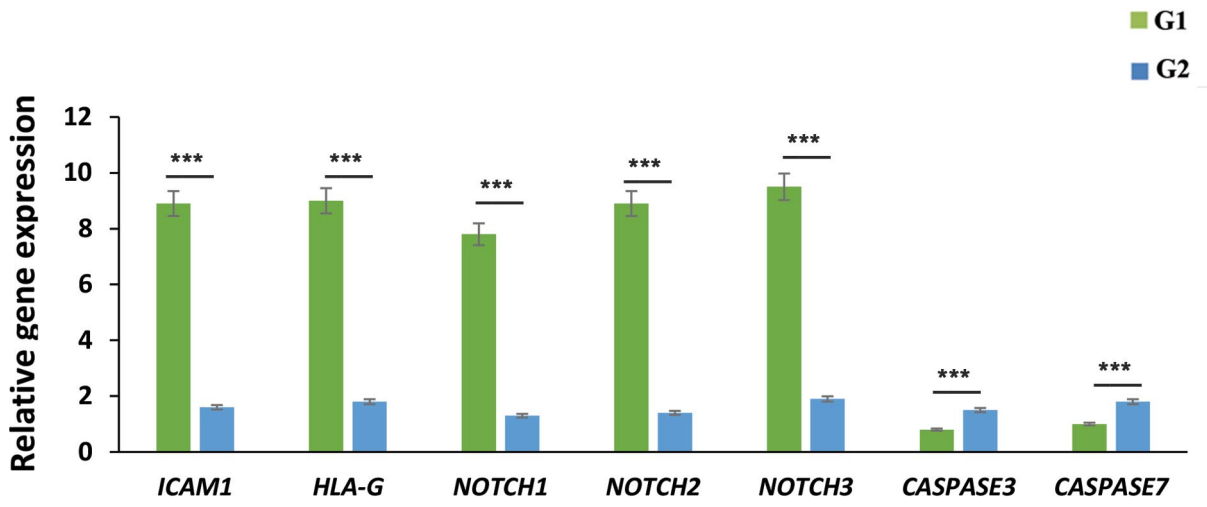

Fig.2: Comparison of *NOTCH1, NOTCH2, NOTCH3, CASPASE-3, CASPASE-7, HLA-G* and *ICAM-1* genes expression of real time quantitative PCR analysis between G2 group and G1 group of unexpected POR patients. Data are presented as the mean ±SE. Different letters (*NOTCH1, NOTCH2, NOTCH3, CASPASE-3, CASPASE-7, HLA-G* and *ICAM-1*) in superscript on the same column indicate significant differences. PCR; Polymerase chain reaction, POR; Poor ovarian response, and ***; P<0.05.

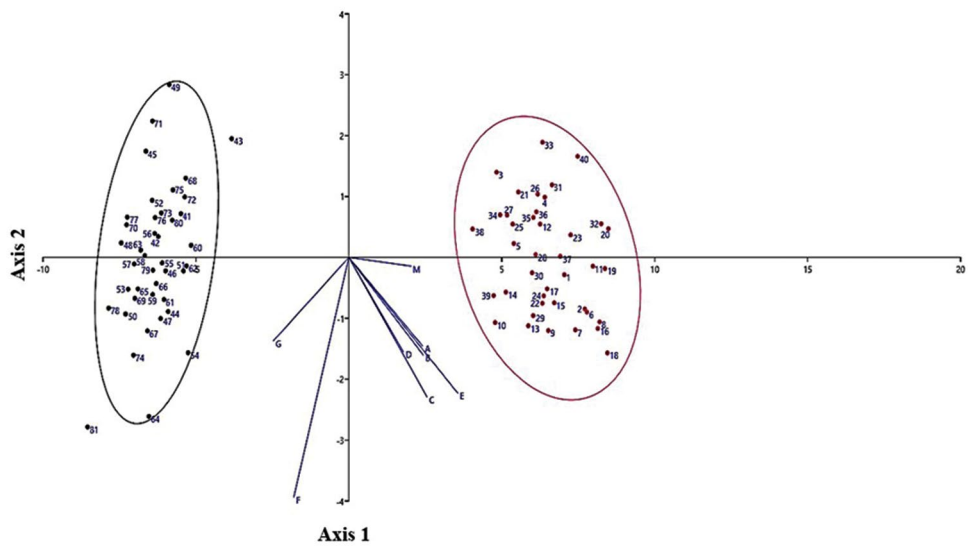

Fig.3: LDA ordination based on gene expression of two groups studied. G2-black dots and G1-red dots. A; *NOTCH1,* B; *NOTCH2,* C; *NOTCH3,* D; *CASPASE-3,* E; *CASPASE-7,* F; *HLA-,* G; *ICAM-1* gene expression, and M; BPA concentration. LDA; linear discrimination analysis and BPA; Bisphenol A.

Protein networking was performed for the 7 genes studied (Fig.S3, See Supplementary Online Information at www.celljournal.org). K means clustering formed 3 distinct clusters including 12 related proteins. ICAM1, VCAM1, CASP3, and CASP7 proteins are grouped in cluster 1 (red colour). DLL4, JAG1, MAML1, NOTCH1, NOTCH2, and NOTCH3 clustered together (green colour) while HLAG and LILRB1 proteins made a separate group (blue colour). Based on protein-protein interaction (PPI) enrichment, the network has significantly more interactions than expected (P<8.61e-07) with an average local clustering coefficient of 0.841. Node annotation and their scores are provided in Table S1 (See supplementary Online Information at www.celljournal.org). The highest scores are calculated between NOTCH 1, 2, 3, and ICAMP1 proteins (>0.7).

KEGG pathways showed that 6 of 12 proteins in the network annotated to Notch signaling cell differentiation, and apoptosis pathways with the highest significance in the enrichment of the network (P<1.24e-10). However, the HLAG protein in the network was distantly grouped from the other genes studied.

Discussion

The results of this study showed that *NOTCH1*-3,

HLA-G, and *ICAM-1* transcripts were significantly lower in the G2 group than the G1 group, while *CASPASE 3*/*7* expression levels were higher in G2. Also, BPA concentrations were higher in the G2 group compared to the G1 group. BPA concentration can affect the transcript profile alterations in cumulus cells from unexpected POR patients. Furthermore, based on our data, the G2 group demonstrated significantly lower numbers of good-quality oocytes and clinical pregnancy rates compared to the G1 group. As a result, BPA can disturb the function of ovulation and MII maturation in unexpected POR patients. Indeed, BPA is effective in the reproductive system. According to a study in reproductive medicine on POR patients, no accurate markers or clinical methods were identified to predict the reduced ovarian reserve or POR with exogenous gonadotropins. A recent study showed a relationship between infertility and a decline in the number of follicles in POR patients. Oocyte quality is important for female reproduction. Oocytes of POR women have poor quality and it is possible that the oocytes may not develop into embryos (21).

The studies reported that intrinsic defects in the oocyte are one of the primary reasons related to infertile POR patients. Recent studies reported that cumulus cells-oocyte complex cross-talk plays a pivotal role in achieving oocyte quality (22).

According to a study, some genes expressed in the cumulus cells affect oocyte maturation (22). Furthermore, the evaluation of gene expression in cumulus cells can be considered as genetic markers for the association between oocyte quality and infertility (23).

Our findings showed that BPA may be considered a disruptive important marker in oocyte maturation, embryo development, and decreases in clinical pregnancy rate. In recent studies, increasing interest has been paid to the relationship between BPA concentration and infertility disorders. BPA has several biological effects such as reproductive disorders (23). A study on mouse oocytes showed that BPA is an established environmental endocrine disruptor and it can interfere with the female germ cell development. BPA, even in small amounts, can impair GnRH secretion in the hypothalamus and promote pituitary proliferation (24).

As mentioned above, BPA disturbs the function of reproduction-related gene expression. Moreover, studies have reported that altered gene expression of cumulus cells might have a key role in women with POR pathogenesis (24). Accordingly, in the present study, we focused on G1 versus G2 group. Chromatography is an analytical technique used to separate a mixture of chemical substances into their individual compounds. In this study, chromatography was used for the evaluation of BPA concentration in the follicular fluid of POR women (25).

Evaluation of gene expression is an efficient measure for understanding the etiology of infertile women with POR patients and for studying the expression profile for *NOTCH1*-3, *CASPASE 3*/*7*, *HLA-G,* and *ICAM-1* gene in the cumulus cells of infertile women with POR. In this study, we found that *NOTCH1*-3, *HLA-G,* and *ICAM-1* transcripts were significantly lower in the G2 group compared to the G1 group. Our data indicate that a high level of BPA concentration is the main contributor to oocyte maturity disorder and infertility in unexpected POR female patients. Also, a study by Ricci et al. (25), confirmed our data.

Recent investigation has demonstrated that alterations of *NOTCH1*-3, *HLA-G,* and *ICAM-1* transcripts can be effective on the quality and quantity of oocytes (25). The Notch pathway is conserved in metazoan organisms. It also plays a pivotal role during the cell developmental process (26). The Notch pathway is a contact-dependent signaling system that is used as genetic markers associated with oocyte quality and other developmental processes. A recent study showed that the Notch pathway is active in the embryonic and postnatal ovary and is important in many processes such as follicle development, meiosis, ovarian vasculogenesis, and steroid hormone production (26). The Notch pathway is active in the ovarian process and its disruption affects events including meiotic spindle assembly, follicle assembly and growth, and granulosa cell proliferation. Therefore, disruption of the Notch pathway can be because of ovarian pathologies. This disruption in the Notch pathway is associated with abnormal folliculogenesis and these aberrations result in reduced fertility. Efforts to enhance knowledge of cellular interactions are important since the Notch signaling pathway plays a significant role in ovarian pathologies and its deeper understanding provides critical insights for improving reproductive system functions (27).

Studies have shown that *ICAM1* and *HLA-G* gene expression disorders may affect embryo development and oocyte quality (28). One major finding of the present paper is that the reduction of the *ICAM1* and *HLA-G* gene expression in unexpected POR patients who have the highest amount of BPA (G2 group) is the main cause of oocyte and embryo development disorders.

A recent study showed that there is a significant relationship between the production of the *ICAM-1* transcript and oocyte quality. A recent study showed that ICAM-1 has an essential role in oocyte maturation and grading. They showed that the increased rate of ICAM-1 molecules can affect embryo development. As a result, ICAM-1 can be a key role marker in oocyte quality and embryo development (28).

Moreover, a recent study has shown that an increased rate of *HLA-G* antigen transcripts affects the rate of implantation, embryo development, and oocyte quality. Also, another study demonstrated that the low production of *HLA-G* transcripts in cumulus cells was related to

the lower number of MII oocytes (29). Assessment of the presence of HLA-G molecules in early embryos and cumulus cells of oocytes is a useful tool for improving pregnancy rates in assisted reproduction techniques (ART). So, the evaluation of the *HLA-G* transcripts might be considered a prognostic marker to identify the embryo's developmental potential (30).

On the contrary, *CASPASE-3* and *CASPASE-7* expression levels were higher in the G2 group. Caspase-3 and -7 are two pro-apoptotic genes in the cumulus cells that are associated with embryo quality and clinical pregnancy rates. It is of note that an increase in the incidence of the apoptotic process is associated with embryo quality and clinical pregnancy rates. Caspase-3 and -7 are two reliable biomarkers for the evaluation of the correlation between the incidence of apoptosis and the number of MII oocytes (30). Recent investigation has demonstrated that the increase in apoptotic signals in the granulosa cells of oocytes is related to the lower number of MII oocytes, embryo development disorder, and reduced fertilization rate (31). This study indicated that apoptotic signals from cumulus cells are transferred to the oocyte via gap junctions and have a strong effect on the oocyte developmental process. On the other hand, a study (32) indicated that exposure to BPA for pregnant women is very harmful because BPA can disrupt the normal differentiation of male germ cells early in life.

Protein network analysis showed that Notch1, Notch2, Notch3, ICAM-1, Caspase-3, and Caspase-7 are connected in a pathway (32). Notch regulates apoptosis through extensive networks, involving cell cycle, growth, and survival pathways. NOTCH 1, 2, and 3 proteins regulate the expression of oocyte and embryo development related genes via the interaction of these proteins with apoptosis proteins such as Caspase-3 and Caspase-7 (31, 32). For the effective genes to be expressed during the development of the oocyte and embryo, these proteins must work together correctly. Also, protein network analysis showed ICAM-1 is related to Notch1, Notch2, Notch3, Caspase-3, and Caspase-7, while HLAG protein was distantly grouped from the other genes studied. Although ICAM-1 and HLA-G are not clustered with other proteins studied, they have important roles in controlling gene expression during oogenesis, oocyte maturation, and embryo development.

Conclusion

Our data indicate that significant differences in the expression levels of *NOTCH1*-3, *CASPASE 3*/7, *HLA-G,* and *ICAM-1* genes in unexpected POR are related to BPA concentration. BPA may have a role in the aberrant expression profile in cumulus cells from unexpected POR. Furthermore; the presence of BPA can interfere with the number of MII oocytes and embryo quality in unexpected POR women.

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Authors' Contributions

Z.N., S.A.; Data curation, Writing original draft preparation, and Supervision. Z.N., A.M.; Conceptualization, Methodology, and Software. S.A.; Visualization, Investigation, Software, and Validation. M.K.; Data curation and Writing Reviewing and Editing. All authors read and approved the final manuscript.

References

1. Noli SA, Ferrari S, Ricci E, Reschini M, Cipriani S, Dallagiovanna C, et al. The role of diet in unexpected poor response to ovarian stimulation: a cross-sectional study. Reprod Biomed Online. 2020; 41(5): 874-883.
2. Borgatti M, Rizzo R, Canto MB, Fumagalli D, Renzini MM, Fadini R, et al. Release of sICAM-1 in oocytes and in vitro fertilized human embryos. PLoS One. 2008; 3(12): e3970.
3. Baker VL, Gracia C, Glassner MJ, Schnell VL, Doody K, Coddington CC, et al. Multicenter evaluation of the Access AMH antimüllerian hormone assay for the prediction of antral follicle count and poor ovarian response to controlled ovarian stimulation. Fertil Steril. 2018; 110(3): 506-513. e3.
4. Zhou W, Fang F, Zhu W, Chen ZJ, Du Y, Zhang J. Bisphenol A and ovarian reserve among infertile women with polycystic ovarian syndrome. Int J Environ Res Public Health. 2016; 14(1): 18.
5. Santangeli S, Maradonna F, Olivotto I, Piccinetti CC, Gioacchini G, Carnevali O. Effects of BPA on female reproductive function: The involvement of epigenetic mechanism. Gen Comp Endocrinol. 2017; 245: 122-126.
6. Ding ZM, Jiao XF, Wu D, Zhang JY, Chen F, Wang YS, et al. Bisphenol AF negatively affects oocyte maturation of mouse in vitro through increasing oxidative stress and DNA damage. Chem Biol Interact. 2017; 278: 222-229.
7. Vanorny DA, Mayo KE. The role of Notch signaling in the mammalian ovary. Reproduction. 2017; 153(6): R187-R204.
8. Jukic AM, Calafat AM, McConnaughey DR, Longnecker MP, Hoppin JA, Weinberg CR, et al. Urinary concentrations of phthalate metabolites and bisphenol A and associations with follicular-phase length, luteal-phase length, fecundability, and early pregnancy loss. Environ Health Perspect. 2016; 124(3): 321-328.
9. Akın L, Kendirci M, Narin F, Kurtoglu S, Saraymen R, Kondolot M, et al. The endocrine disruptor bisphenol A may play a role in the aetiopathogenesis of polycystic ovary syndrome in adolescent girls. Acta Paediatr. 2015; 104(4): e171-e177.
10. Liu Y, Pan Z, Wu Y, Song J, Chen J. Comparison of anti-Müllerian hormone and antral follicle count in the prediction of ovarian response: a systematic review and meta-analysis. J Ovarian Res. 2023 Jun 27;16(1):117.
11. Domingo-Domenech J, Vidal SJ, Rodriguez-Bravo V, Castillo-Martin M, Quinn SA, Rodriguez-Barrueco R, et al. Suppression of acquired docetaxel resistance in prostate cancer through depletion of Notch- and hedgehog-dependent tumor-initiating cells. Cancer Cell. 2012;373-388 :(3)22 .
12. Borman ED, Foster WG, Greenacre MK, Muir CC, deCatanzaro D. Stress lowers the threshold dose at which bisphenol A disrupts blastocyst implantation, in conjunction with decreased uterine closure and e-cadherin. Chem Biol Interact. 2015; 237: 87-95.
13. Crawford BR, Decatanzaro D. Disruption of blastocyst implantation by triclosan in mice: impacts of repeated and acute doses and combination with bisphenol-A. Reprod Toxicol. 2012; 34(4): 607-613.
14. Fiori F, Parpinel M, Morreale F, Pellegrini N. The Update of the italian food composition database of gluten-free products and its application in food-based dietary guidelines menus. Nutrients. 2022; 14(19): 4171.
15. Jeong HG, Kim SK, Lee JR, Jee BC. Correlation of oocyte number with serum anti-Müllerian hormone levels measured by either Access or Elecsys in fresh in vitro fertilization cycles. Clin Exp Reprod Med. 2022 Sep;49(3):202-209. doi: 10.5653/cerm.2022.05211. Epub 2022 Aug 31.
16. Caserta D, Ciardo F, Bordi G, Guerranti C, Fanello E, Perra G, et al. Correlation of endocrine disrupting chemicals serum levels and

white blood cells gene expression of nuclear receptors in a population of infertile women. Int J Endocrinol. 2013; 2013: 510703.
17. Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-Müllerian hormone and antral follicle count as biomarkers of ovarian response. Hum Reprod Update. 2015 Nov-Dec;21(6):698-710.
18. Salehi E, Aflatoonian R, Moeini A, Yamini N, Asadi E, Khosravizadeh Z, et al. Apoptotic biomarkers in cumulus cells in relation to embryo quality in polycystic ovary syndrome. Arch Gynecol Obstet. 2017; 296(6): 1219-1227.
19. Nishikawa M, Iwano H, Yanagisawa R, Koike N, Inoue H, Yokota H. Placental transfer of conjugated bisphenol A and subsequent reactivation in the rat fetus. Environ Health Perspect. 2010; 118(9): 1196-1203.
20. Aftabsavad S, Noormohammadi Z, Moini A, Karimipoor M. Effect of bisphenol A on alterations of ICAM-1 and HLA-G genes expression and DNA methylation profiles in cumulus cells of infertile women with poor response to ovarian stimulation. Sci Rep. 2021; 11(1): 9595.
21. Pandian Z, McTavish AR, Aucott L, Hamilton MP, Bhattacharya S. Interventions for 'poor responders' to controlled ovarian hyper stimulation (COH) in in-vitro fertilisation (IVF). Cochrane Database Syst Rev. 2010; (1): CD004379.
22. Bahrami N, Nazari A, Afshari Z, Aftabsavad S, Moini A, Noormohammadi Z. Gene expression and demographic analyses in women with the poor ovarian response: a computational approach. J Assist Reprod Genet. 2023; 40(11): 2627-2638.
23. Polyzos NP, Devroey P. A systematic review of randomized trials for the treatment of poor ovarian responders: is there any light at the end of the tunnel? Fertil Steril. 2011; 96(5): 1058-1061. e7.
24. Rochester JR. Bisphenol A and human health: a review of the literature. Reprod Toxicol. 2013; 42: 132-155.
25. Ricci E, Noli S, Cipriani S, La Vecchia I, Chiaffarino F, Ferrari S, et al. Maternal and paternal caffeine intake and ART outcomes in couples referring to an italian fertility clinic: a prospective cohort. Nutrients. 2018; 10(8): 1116.
26. Brentnall M, Rodriguez-Menocal L, De Guevara RL, Cepero E, Boise LH. Caspase-9, Caspase-3 and Caspase-7 have distinct roles during intrinsic apoptosis. BMC Cell Biol. 2013; 14: 32.
27. Eskew AM, Bedrick BS, Chavarro JE, Riley JK, Jungheim ES. Dietary patterns are associated with improved ovarian reserve in overweight and obese women: a cross-sectional study of the Lifestyle and Ovarian Reserve (LORe) cohort. Reprod Biol Endocrinol. 2022 Feb 19;20(1):33.
28. Farimani M, Nazari A, Mohammadi S, Anvari Aliabad R. Evaluation of intra-ovarian platelet-rich plasma administration on oocytes-dependent variables in patients with poor ovarian response: A retrospective study according to the POSEIDON criteria. Reprod Biol Endocrinol. 2021 Sep 8;19(1):137.
29. Huo X, Chen D, He Y, Zhu W, Zhou W, Zhang J. Bisphenol-A and female infertility: a possible role of gene-environment interactions. Int J Environ Res Public Health. 2015; 12(9): 11101-11116.
30. Miao M, Yuan W, Zhu G, He X, Li DK. In utero exposure to bisphenol-A and its effect on birth weight of offspring. Reprod Toxicol. 2011; 32(1): 64-68.
31. Li Y, Liu S, Gao F, Peng Z, Zhang J, Li S, et al. BPA interferes with granulosa cell development and oocyte meiosis in mouse preantral follicles. Exp Biol Med (Maywood). 2023; 248(13): 1145-1158.
32. Nagels HE, Rishworth JR, Siristatidis CS, Kroon B. Androgens (dehydroepiandrosterone or testosterone) for women undergoing assisted reproduction. Cochrane Database Syst Rev. 2015; 2015(11): CD009749.