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RESEARCH

A bridge between infertility and anxiety: a novel role for genes

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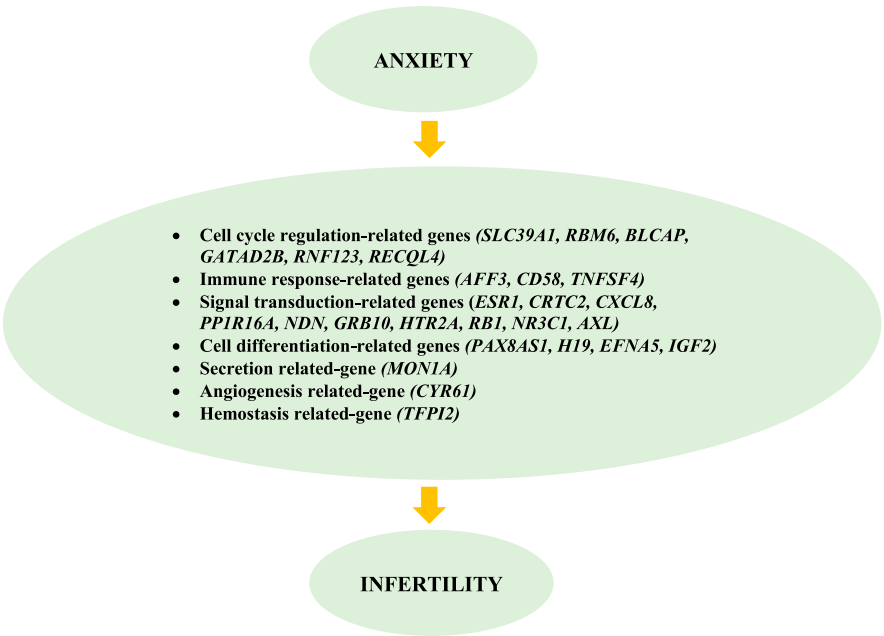
*(Deceased)

Graphical Abstract



Abstract

Psychological imbalance is hypothesized to negatively affect reproductive function and vice versa; however, the underlying mechanisms of this relationship remain poorly understood. Therefore, this study aimed to investigate the association between infertility and anxiety and/or depression by examining underlying genetic factors. The study was conducted in several stages: i) psychological assessment of women undergoing assisted reproductive technology (ART) due to couple infertility using the Hospital Anxiety and Depression Scale (HADS), ii) collection of endometrial samples

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before ART procedures, iii) isolation and cultivation of human endometrial-derived mesenchymal stromal cells (EnSCs), iv) analysis of 26 EnSC genes using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and v) statistical analysis. A total of 32 women were included in the study. Twelve participants reported considerable symptoms of anxiety, and one showed symptoms of depression. Nineteen women conceived after ART. Gene expression analysis suggested altered expression of genes involved in cell cycle regulation, transcription and translation, immune response, signal transduction, cell differentiation, secretion, angiogenesis, and hemostasis according to anxiety status. These mechanisms may influence, or at least reflect, pregnancy outcomes following ART. The findings suggest a potential relationship between anxiety symptoms and gene activity in the endometrium of women undergoing ART. However, the relatively small sample size limits the strength of the conclusions; therefore, the results should be interpreted as preliminary. Further studies with larger cohorts are required to confirm these observations and to better understand the interaction between psychological well-being and molecular mechanisms involved in fertility.

Lay summary

Infertility affects many couples worldwide and can be emotionally challenging. People undergoing fertility treatment often experience stress or anxiety, but it is still unclear how emotional well-being may influence reproductive health. In this study, we explored whether anxiety might be linked to biological processes involved in fertility. Women undergoing assisted reproductive treatment completed a questionnaire about anxiety and depression symptoms. We also analyzed small tissue samples from the lining of the uterus, which is important for embryo implantation and pregnancy. Our findings suggested that women who reported higher anxiety levels showed differences in some biological processes compared with women who reported lower anxiety levels. Some of these differences were also observed in women who did not become pregnant after treatment. Although the study involved a small number of participants, the results suggest that emotional well-being may be connected to reproduction and highlight the importance of psychological support during fertility treatment.

Keywords: infertility; assisted reproductive technology; anxiety; endometrium; stromal cells; genes

Introduction

Infertility is a disease of a female or a male reproductive system and is defined as the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse (Practice Committee of the American Society for Reproductive Medicine). Women older than 35 years or couples with known risk factors for infertility should be considered for evaluation after 6 months of unsuccessful attempts to conceive (Practice Committee of the American Society for Reproductive Medicine).

Fertility disorders affect millions of people of reproductive age worldwide and extend their impact to families and communities. An estimated 15% couples of reproductive age have fertility problems (Cui 2010). According to the World Health Organization (WHO), fertility issues affect about 48.5 million couples and 186 million individuals globally (World Health Organisation 2004, Martinez *et al.* 2012, Mascarenhas *et al.* 2012). Moreover, experts believe that the rate of infertility is increasing, and reproductive health issues are becoming more challenging. This is evidenced by the steady growth in the use of assisted reproductive technology (ART) over the past four decades, with an annual increase of approximately 5–10% (Fortune Business Insights 2019).

Infertility is an issue that affects many aspects of people's lives, as individuals and as a couple. Alongside physical, social, and economic impact on couples, there is a significant impact on their mental health, from the moment of the diagnosis up until the conclusion of their treatment (Ying *et al.* 2016, Yazdani *et al.* 2017). Studies suggest that up to 60% of infertile individuals report psychiatric symptoms and experience significantly higher levels of anxiety and depression than fertile individuals (De Berardis *et al.* 2014). Nearly 41% of infertile women have experienced depression, and even 87% of infertile women also have anxiety (Ramezanzadeh *et al.* 2004).

On the other hand, it is reported that negative emotions and mental illness itself could increase the incidence of fertility disorders and undesirable pregnancy outcomes, such as recurrent implantation failure and miscarriage (Louis *et al.* 2011, Lynch *et al.* 2014, Geraghty *et al.* 2015, Litzky & Marsit 2019*a*, Wu *et al.* 2021). Literature findings on epigenetic effects of the prenatal environment provide some evidence suggesting that the expression of imprinted genes associated with infertility may be due to underlying anxiety and depression that have been found to be more frequent in the ART individuals

(Litzky & Marsit 2019*a*). Although clear conclusions are still lacking, these findings remain significant, as analyses of endometrial gene expression levels have demonstrated changes across different phases of the menstrual cycle (Díaz-Gimeno *et al.* 2011, Haouzi *et al.* 2012, Ruiz-Alonso *et al.* 2012, von Grothusen *et al.* 2014), in response to ovarian stimulation protocols used in ART (Horcajadas *et al.* 2008, Macklon *et al.* 2008, Haouzi *et al.* 2009), during the embryo implantation process (Altmäe *et al.* 2010, Koot *et al.* 2016), and in the presence of gynecological pathologies (Fung *et al.* 2018).

Therefore, this study aimed to investigate the potential association between anxiety symptoms and gene expression patterns in endometrial stromal cells in women undergoing ART procedures.

Materials and methods

Participant recruitment and enrollment

In this prospective cohort study, we enrolled 32 women undergoing ART procedures due to couple infertility at Vilnius University Hospital Santaros Klinikos, Obstetrics and Gynecology Center, Santaros Fertility Center. Protocols were approved by the Ethics Committee of Biomedical Research of Vilnius Region, No. 158200-18/7-1049-550. All participants received written and oral information, and they all signed informed consent before the study.

The inclusion criteria were as follows: i) woman's age at the time of enrollment between 18 and 45 years; ii) the minimum duration of infertility of 1 year; iii) infertility due to fallopian tube pathology, male factor infertility, or unexplained infertility; and iv) provision of written informed consent to participate in the study.

The exclusion criteria were as follows: i) a confirmed oncological disease diagnosed within the previous three years; ii) active smoking or substance abuse, including alcohol or other addictive substances; iii) medical contraindications to pregnancy; iv) uncontrolled endocrine or other medical conditions, such as hyperprolactinemia or thyroid disorders; and v) a history of diagnosed mental disorders.

Structure of a study

The study was performed in stages: i) psychological assessment of anxiety and depression for women using the Hospital Anxiety and Depression Scale (HADS); ii) endometrial samples collection from studied women; iii) human endometrial-derived mesenchymal stromal cells (EnSCs) isolation from collected endometrium samples and cultivation; iv) EnSC gene expression analysis by reverse transcription-quantitative polymerase chain reaction (RT-qPCR); v) statistical analysis of results of measured anxiety, outcomes of performed ART procedures, and gene expression profile; and vi) conclusions.

Psychological assessment

Anxiety levels were measured before the ART cycle with the HADS (Zigmond & Snaith 1983). The HADS is a widely used self-rating scale worldwide developed to assess psychological distress in non-psychiatric patients. The scale comprises 14 items: seven questions for anxiety and seven questions for depression. Each item is rated on a four-point Likert scale ranging from 0 (absence) to 3 (extreme presence) with maximum scores of 21 for anxiety and depression. Participants with HADS' standard scores ≥ 8 were considered at risk for clinical anxiety or depression. Higher scores indicate greater levels of anxiety and depression. A cutoff score of 8 or more for anxiety has a specificity of 0.78 and a sensitivity of 0.90, and for depression a specificity of 0.79 and a sensitivity of 0.83 (Bjelland *et al.* 2002). In this study, HADS' depression subscale's Cronbach's alpha was 0.694 and anxiety subscale's 0.799.

Endometrial sample collection

Endometrial tissue samples were collected during routine clinical procedures from 32 women who had been assessed using the HADS. The samples were obtained by a reproductive medicine specialist using a 3 mm Pipelle (endometrial sampling catheter) during an endometrial scratching procedure performed on the inner uterine lining. The procedure was carried out once on day 20–22 of the cycle prior to *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) procedure.

EnSCs isolation and cultivation

In this study, to isolate EnSCs from the endometrial sample, the specimen was transferred to a sterile Petri dish, washed 3 times with sterile phosphate buffered saline (PBS) (Gibco, Thermo Fisher Scientific, USA) and cut with a sterile scalpel into 1–2 mm pieces. Further procedures were performed as described earlier (Valatkaitė *et al.* 2021). Isolated EnSCs were cultivated at 37°C in a humidified 5% carbon dioxide (CO_2) atmosphere. Growth medium was replaced every three days.

Gene expression analysis by RT-qPCR

We analyzed 26 genes by RT-qPCR: cell cycle regulation-related genes *(SLC39A1, RBM6, BLCAP, GATAD2B, RNF123, RECQL4)*, immune response-related genes *(AFF3, CD58, TNFSF4)*, signal transduction-related genes *(ESR1, CRTC2, CXCL8, PPP1R16A, NDN, GRB10, HTR2A, RB1, NR3C1, AXL)*, cell differentiation-related genes *(PAX8AS1, H19, EFNA5, IGF2)*, secretion-related genes *(MON1A)*, angiogenesis *(CYR61)*, and hemostasis-related gene *(TFPI2)*.

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RT-qPCR samples were prepared using commercial kits, according to the manufacturer's instructions. Total ribonucleic acid (RNA) was purified using TRIzol reagent (Invitrogen, USA), complementary deoxyribonucleic acid (cDNA) was synthesized using LunaScript® RT SuperMix Kit (New England Biolabs, USA), and qPCR was performed using Luna® Universal qPCR Master Mix (New England Biolabs, USA) on the RotorGene 6000 system (Corbett Life Science, QIAGEN, Germany). Primer sequences (Metabion international AG, Planegg/Steinkirchen, Germany) are presented in Additional file 1 (see section on Supplementary materials given at the end of the article). mRNA levels were normalized to *GAPDH* expression. Relative gene expression was calculated using the ΔΔCt method.

Statistical analysis

Statistical analysis of gene expression levels was performed using GraphPad Prism, version 8.0.1, for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). Data are represented as mean ± SD, with gray data points indicating outliers. Group differences were analyzed using the non-parametric Mann–Whitney U test. Participants were divided into four groups according to anxiety status and pregnancy outcome: No anxiety/Conceived, Anxiety/Conceived, No anxiety/Not conceived, and Anxiety/Not conceived. Pairwise comparisons were performed between the following groups: No anxiety/Conceived vs No anxiety/Not conceived; Anxiety/Conceived vs Anxiety/Not conceived; No anxiety/Conceived vs Anxiety/Conceived; and No anxiety/Not conceived vs Anxiety/Not conceived. Statistical significance was defined as $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***). Outliers were identified using the ROUT method ($Q = 5\%$).

Results

Descriptive characteristics of the study population

The age of the population ranged from 23 to 41 years old (average: 33.4 years) with a minimum of 1-year experience of infertility (range: 2–19, average: 6.7 years). More than half of the women had a normal BMI (62.5%, $n = 20$), while 37.5% ($n = 12$) were outside the normal range. Nevertheless, clinical, laboratory, and instrumental evaluations did not indicate any impairment of reproductive health.

Analysis of medical histories revealed that half of the study population (50%, $n = 16$) had comorbidities, the most frequent of which was endometriosis (25%, $n = 8$). In addition, less than one-fifth of the women were diagnosed with other diseases which were not classified as reproductive health or endocrine diseases (18.8%, $n = 6$).

Regarding reproductive characteristics, more than a third of women (40.6%, $n = 13$) had not previously been treated for infertility, and this corresponded with indications for the ART procedure immediately after the clinical evaluation of the couple. The great majority of women were childless (87.5%, $n = 28$).

More detailed descriptive characteristics of women are presented in Additional file 2.

During the study, 12 women (37.5% of the study population) expressed considerable symptoms of anxiety and only 1 woman showed symptoms of depression with the minimum limit – the total score 8. However, this participant also reported clinically relevant anxiety symptoms, with a total score of 13. Overall, the psychological assessment of the women showed that anxiety scores varied from 0 to 13 with 6.19 ± 3.42 mean and a mode of 4, while depression scores varied from 0 to 8 with 2.13 ± 2.06 mean and a mode of 1. Based on these results of the HADS, further attention was paid to anxiety in the psychological assessment study.

Analysis of pregnancy outcomes revealed that 59.4% ($n = 19$) of the study population achieved pregnancy following ART, while 40.6% ($n = 13$) did not achieve pregnancy.

Based on the scores of the HADS and the outcome after ART procedure, women were divided into four groups accordingly: women who did not present any considerable symptoms of anxiety and conceived (No anxiety/Conceived group; $n = 10$); women who presented considerable symptoms of anxiety and conceived (Anxiety/Conceived group; $n = 9$); women who did not present any considerable symptoms of anxiety and did not conceive (No anxiety/Not conceived group; $n = 10$); and women who presented considerable symptoms of anxiety and did not conceive (Anxiety/Not conceived; $n = 3$).

Analysis of gene expression profiles in relation to women's psychological well-being and ART outcomes

Based on the division of study population into four different women's groups – No anxiety/Conceived, Anxiety/Conceived, No anxiety/Not conceived, Anxiety/Not conceived – we analyzed the expression levels of 26 genes in EnSCs isolated from endometrial tissue samples of women, accordingly.

Cell cycle regulation and transcription/translation-related genes

Studies of the expression of cell cycle regulation-related genes revealed that expression levels of *SLC39A1*

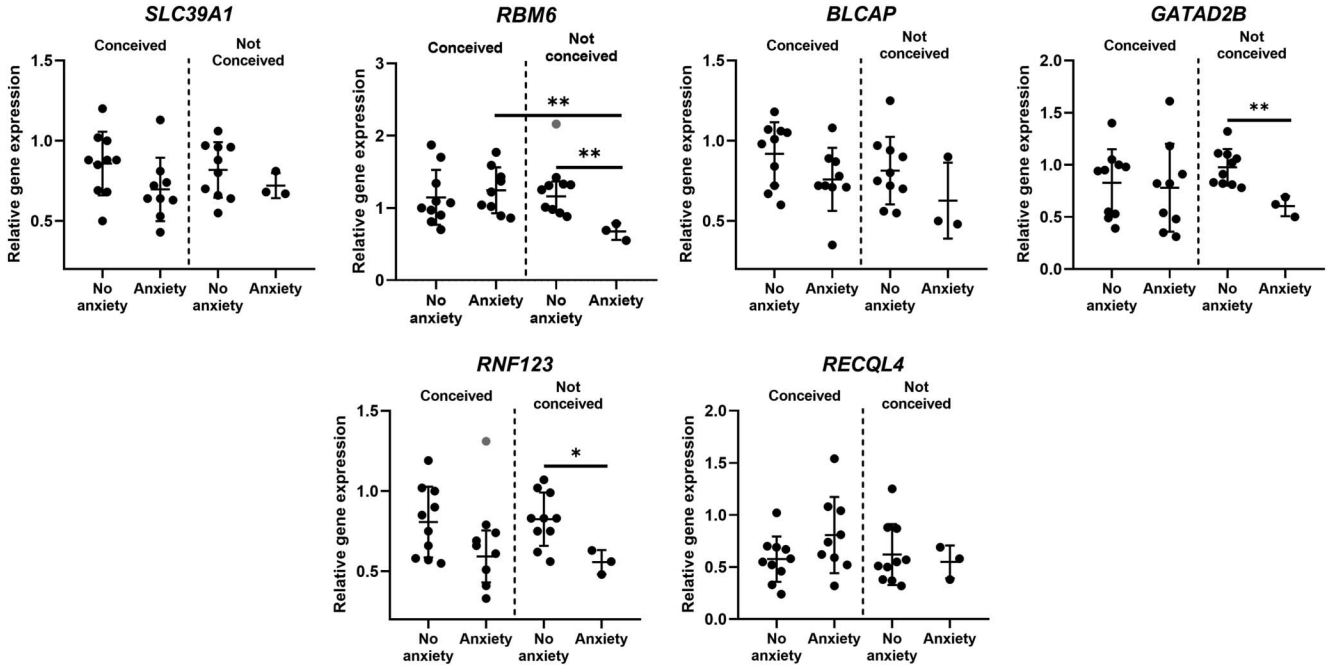

Figure 1

Expression of cell cycle regulation- and transcription/translation-related genes – (A) *SLC39A1*, (B) *RBM6*, (C) *BLCAP*, (D) *GATAD2B*, (E) *RNF123*, and (F) *RECQL4*. Results are presented as mean ± standard deviation; gray data points indicate outliers based on ROUT (Q = 5%). Study groups: No anxiety/Conceived group (n = 10), Anxiety/Conceived group (n = 9), No anxiety/Not conceived group (n = 10), and Anxiety/Not conceived group (n = 3). $P \leq 0.05$ (*), $P \leq 0.01$ (**) based on Mann–Whitney U test.

(Fig. 1A), *RBM6* (Fig. 1B), *BLCAP* (Fig. 1C), *GATAD2B* (Fig. 1D), *RNF123* (Fig. 1E), and *RECQL4* (Fig. 1F) did not differ significantly between the No anxiety/Conceived and Anxiety/Conceived groups, although varying tendencies were observed: *SLC39A1* (Fig. 1A), *BLCAP* (Fig. 1C), and *RNF123* (Fig. 1E) tend to be downregulated in participants who had expressed considerable symptoms of anxiety, whereas *RECQL4* (Fig. 1F) tends to be upregulated and *RBM6* (Fig. 1B) and *GATAD2B* (Fig. 1D) levels remained stable. In contrast, gene expression analysis revealed significantly lower expression levels of *RNF123* (Fig. 1E), *GATAD2B* (Fig. 1D), and *RBM6* (Fig. 1B) in both the Anxiety/Not conceived and the No anxiety/Not conceived groups. A significant drop in the expression level of *RBM6* was noted in the Anxiety/Not conceived group compared to the Anxiety/Conceived group (Fig. 1B). A similar tendency was observed for the remaining genes, although the differences were not statistically significant.

Immune response-related genes

Analysis of immune response-related gene expression (*AFF3*, *CD58*, and *TNFSF4*; Fig. 2A, B, C) showed significantly higher *AFF3* expression and significantly lower *TNFSF4* expression in women who did not conceive and did not report anxiety symptoms compared with women who conceived without anxiety symptoms. A tendency toward lower *TNFSF4* expression was also observed in women who did not conceive and reported anxiety symptoms compared with women who conceived, regardless of their psychological well-being. In addition, *CD58* expression tended to decrease in women who conceived despite considerable anxiety symptoms and in women who did not conceive (Fig. 2B).

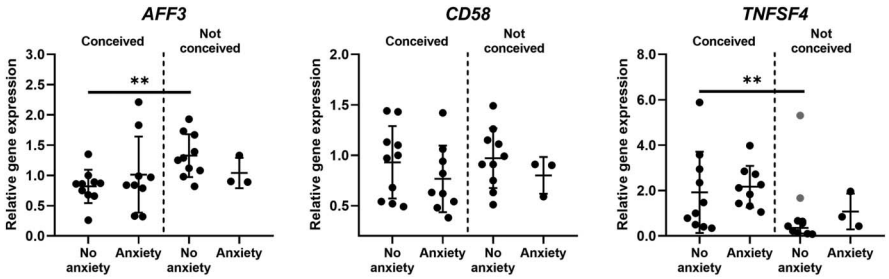

Figure 2

Expression of immune response-related genes – (A) *AFF3,* (B) *CD58*, and (C) *TNFSF4*. Results are presented as mean ± standard deviation; gray data points indicate outliers based on ROUT (Q = 5%). Study groups: No anxiety/Conceived group (n = 10), Anxiety/Conceived group (n = 9), No anxiety/Not conceived group (n = 10), and Anxiety/Not conceived group (n = 3). $P \leq 0.01$ (**) based on Mann–Whitney U test.

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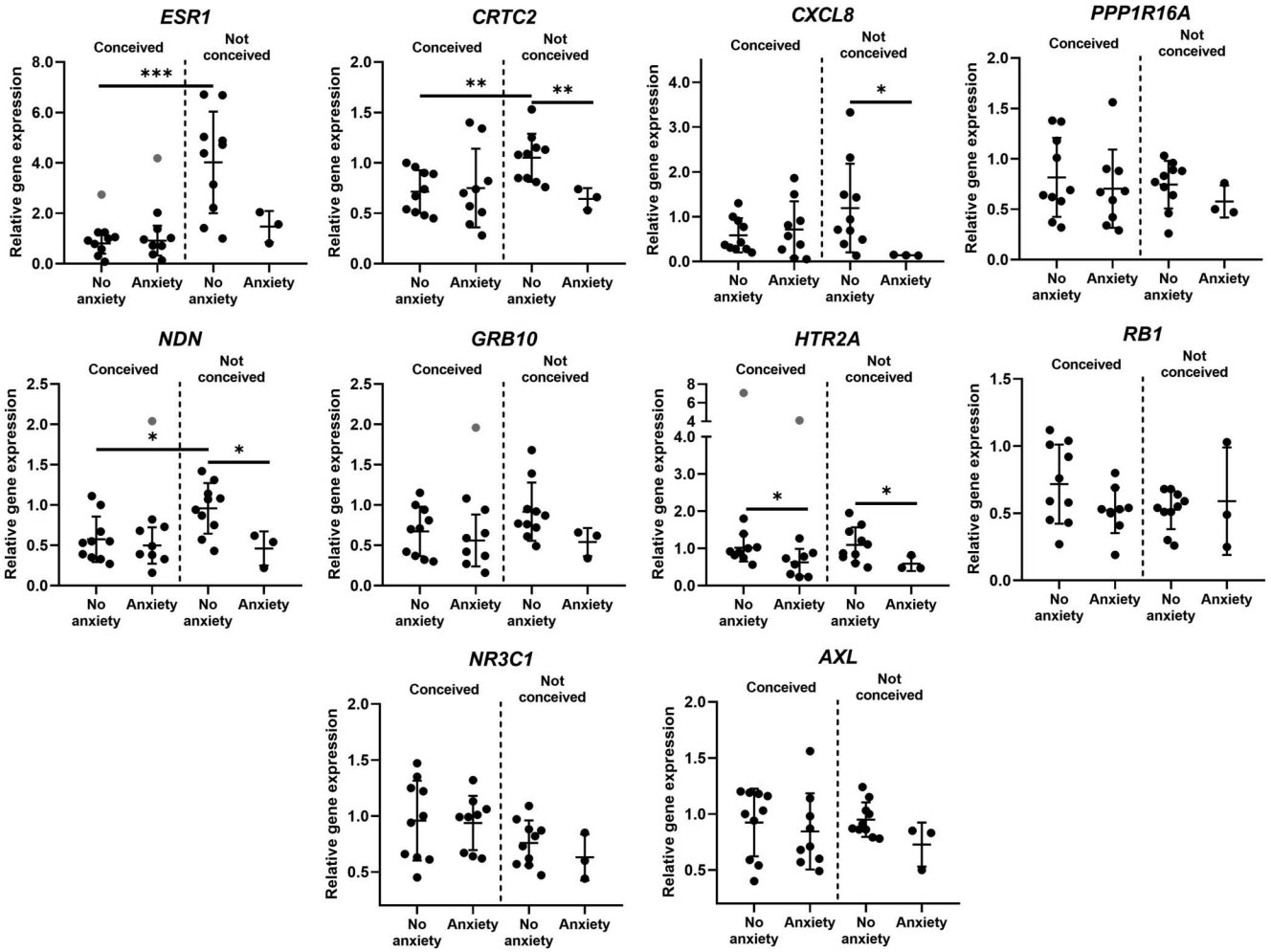

Figure 3

Expression of signal transduction-related genes – (A) *ESR1*, (B) *CRTC2*, (C) *CXCL8*, (D) *PPP1R16A*, (E) *NDN*, (F) *GRB10*, (G) *HTR2A*, (H) *RB1*, (I) *NR3C1*, and (J) *AXL*. Results are presented as mean ± standard deviation; gray data points indicate outliers based on ROUT (Q = 5%). Study groups: No anxiety/Conceived group (n = 10), Anxiety/Conceived group (n = 9), No anxiety/Not conceived group (n = 10), and Anxiety/Not conceived group (n = 3). $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***) based on Mann–Whitney U test.

Signal transduction-related genes

Analysis of signal transduction-related gene expression suggested that anxiety symptoms may be associated with downregulation of several investigated genes in EnSCs, with the effect appearing more pronounced in women who did not conceive compared with conceived women. There was a significant drop in the expression of *CRTC2* (Fig. 3B), *HTR2A* (Fig. 3G), *CXCL8* (Fig. 3C), and *NDN* (Fig. 3E) in EnSCs of not conceived participants with anxiety. Nevertheless, the expression of *ESR1* (Fig. 3A), *CRTC2* (Fig. 3B), and *NDN* (Fig. 3E) was significantly higher in EnSCs of the No anxiety/Not conceived group than the No anxiety/Conceived group although it tended to decrease due to the effects of anxiety, except *ESR1* (Fig. 3A), which remained in a stable level in both groups of conceived women and the Anxiety/Not conceived group.

While in the analysis of gene expression profile, women's anxiety and ART outcomes did not demonstrate strong correlations, overall, the efficiency of signal transduction-related genes *ESR1* (Fig. 3A), *CRTC2* (Fig. 3B), *CXCL8* (Fig. 3C), *PPP1R16A* (Fig. 3D), *NDN* (Fig. 3E), *GRB10* (Fig. 3F), *HTR2A* (Fig. 3G), *RB1* (Fig. 3H), *NR3C1* (Fig. 3I). and *AXL* (Fig. 3J) in EnSCs may at least show tendencies or reflect women's psychological well-being and results of conception.

Cell differentiation-related genes

Analysis of cell differentiation-related gene expression (*PAX8AS1*, *EFNA5*, *IGF2*, and *H19*; Fig. 4A, B, C, D) showed a tendency toward lower expression in the Anxiety/Not conceived group compared with both the No anxiety/Conceived group and Anxiety/Conceived

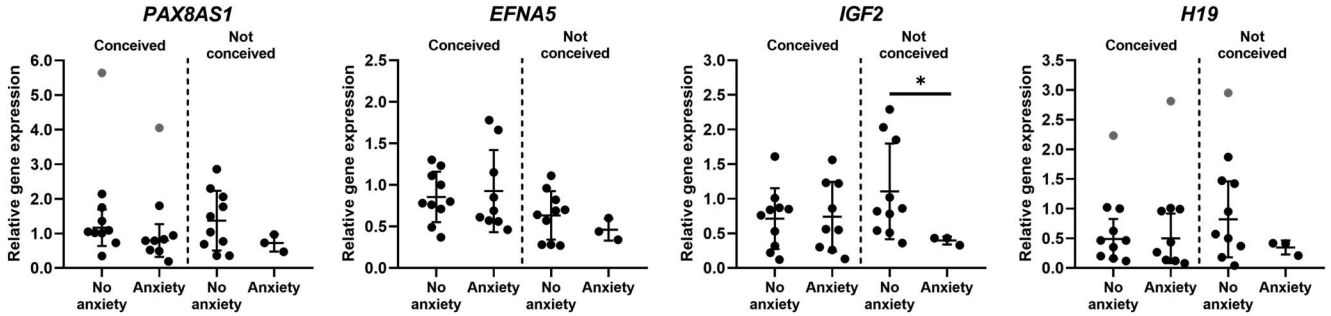

Figure 4

Expression of cell differentiation-related genes – (A) *PAX8AS1*, (B) *EFNA5*, (C) *IGF2*, and (D) *H19*. Results are presented as mean ± standard deviation; gray data points indicate outliers based on ROUT (Q = 5%). Study groups: No anxiety/Conceived group (n = 10), Anxiety/Conceived group (n = 9), No anxiety/Not conceived group (n = 10), and Anxiety/Not conceived group (n = 3). $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***) based on Mann–Whitney U test.

groups. Expression of *PAX8AS1* tends to decrease due to the effects of anxiety in EnSCs of both investigated groups (Fig. 4A). Expression of *IGF2* (Fig. 4C) also significantly decreased in EnSCs of women who did not conceive and experienced anxiety; on the other hand, *EFNA5* (Fig. 4B) is slightly upregulated in conceived women who had expressed considerable symptoms of anxiety, while the expression level of *IGF2* (Fig. 4C) remains consistent. The data suggest that anxiety symptoms may be associated with reduced expression of differentiation-related genes, as women who did not conceive showed lower expression of genes related to decidualization.

Secretion-related genes

Analysis of the secretion-related gene *MON1A* showed no significant differences in gene expression in EnSCs between women who conceived and those who did not conceive (Fig. 5A).

Angiogenesis- and hemostasis-related genes

Analysis of the angiogenesis-related gene *CYR61* revealed significantly higher expression in the No anxiety/Not conceived group compared with the No anxiety/Conceived and Anxiety/Conceived groups (Fig. 5B). However, the lowest value was observed in the Anxiety/Not conceived group.

Analysis of the hemostasis-related gene *TFPI2* revealed that EnSCs in the No anxiety/Conceived group showed significantly higher expression of this gene than those in the No anxiety/Not conceived group (Fig. 5C). Furthermore, *TFPI2* expression levels were lower in the groups of women who did not conceive compared with those who conceived, regardless of psychological well-being. In addition, *TFPI2* expression was lower in the Anxiety/Not conceived group compared with the No anxiety/Conceived group.

The theoretical analysis of the interaction network of proteins, coded by investigated genes, was performed to deepen the understanding of the correlation between investigated genes, anxiety, and infertility on the biological function level (Fig. 6). A table of genes, respected proteins, and their function is presented in Additional file 3.

Interaction network analysis revealed that selected genes were, indeed, researched by other authors in the context of infertility and stress, validating our selected gene pool (see Additional file 4). Proteins coded by investigated genes were proven to be involved in cellular response to lipid, hormone stimulus, oxygen-containing compound, endogenous stimulus, organic substance, positive regulation of multicellular organismal process, and anatomical structure morphogenesis.

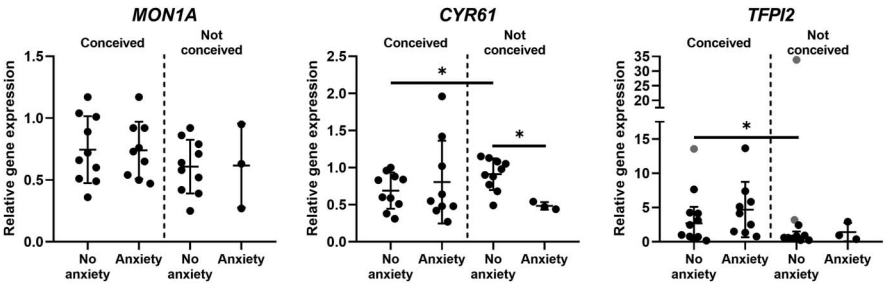

Figure 5

Expression of secretion-, angiogenesis-, and hemostasis-related genes – (A) *MON1A*, (B) *CYR61*, and (C) *TFPI2*. Results are presented as mean ± standard deviation; gray data points indicate outliers based on ROUT (Q = 5%). Study groups: No anxiety/Conceived group (n = 10), Anxiety/Conceived group (n = 9), No anxiety/Not conceived group (n = 10), and Anxiety/Not conceived group (n = 3). $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***) based on Mann–Whitney U test.

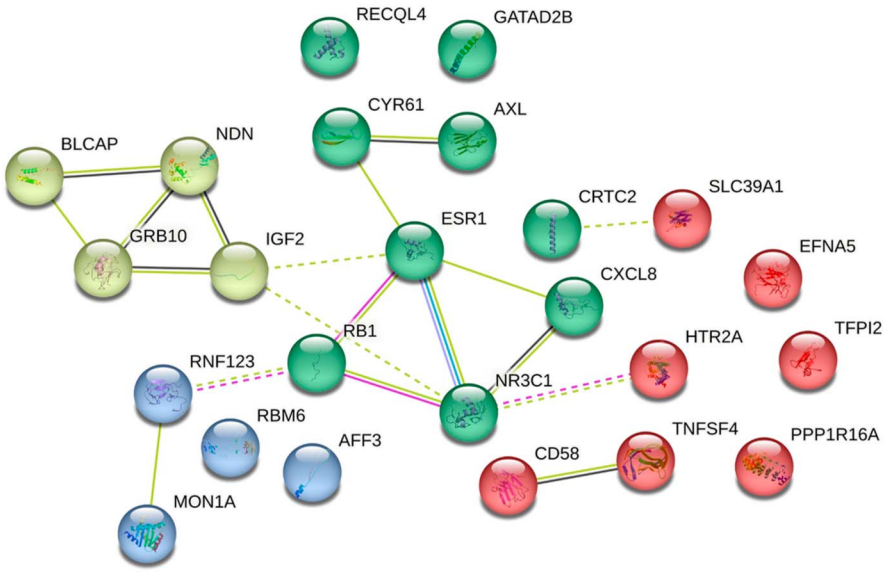

Figure 6

Theoretical interaction networks of the proteins coded by the analyzed genes are inherent in anxiety, depression, and infertility. The interaction networks were identified using the STRING database (Hall 2018). Four clusters were created based on means clustering. Lines between nodes represent evidence of the associations: green line represents co-mentioning in PubMed abstracts; black line – co-expression; pink line – experimentally determined connection; blue line – data from curated databases; and purple line – protein homology.

Discussion

The WHO classifies infertility as the fifth highest serious global disability in populations younger than 60 years old (Hall 2018). Over the past 40 years, significant advances in infertility treatment have been developed, including improved education, advanced medical procedures, and third-party reproductive technologies (Ombelet *et al.* 2008). Despite the progress that has been made over the years, studies estimate that approximately 30–40% of women remain childless 5 years after initial infertility diagnosis (Pinborg *et al.* 2009). For this reason, more research and new insights are essential.

Considering the previously proven psychological impact on the physical health, conception and pregnancy development (Bjelica & Kapor-Stanulović 2004) in the general population and increasing evidence of its significance for the reproductive health, including the infertile couples' population (Louis *et al.* 2011, Lynch *et al.* 2014, Geraghty *et al.* 2015, Litzky & Marsit 2019, Wu *et al.* 2021), the deeper understanding of these mechanisms and their practical usage in the reproductive medicine field could become particularly worthwhile, rather than attributing negative feelings solely as a consequence of fertility problems. Results of earlier studies lead us to a hypothesis that emotions and mental conditions may have a different impact on ART outcomes through gene expression profile.

In this study, analysis of gene expression in EnSCs from women undergoing ART suggested a potential relationship between anxiety symptoms and pregnancy outcomes. First, this was observed when analyzing women's psychological well-being, conception rates following ART procedures, and the expression of genes related to cell cycle regulation and transcription/translation. The expression of the *SLC39A1* and *GATAD2B* genes is associated with zinc metabolism in the organism. *SLC39A1* is a member of the zinc-iron permease family and is located in the cell membrane, acting as a zinc uptake transporter (National Center for Biotechnology Information (NCBI) (2022*d*)) and *GATAD2B* encodes a zinc finger protein transcriptional repressor (National Center for Biotechnology Information (NCBI) 2022*b*). Zinc deficiency caused by the absence or mutation of genes could cause growth retardation, immune dysfunction, metabolic disorders, and, also, infertility through the negative impact on the early stages of oocytes maturation, progression, and completion of meiosis, reducing the ability of the oocyte cells to divide and be fertilized (Jeong & Eide 2013, Jeon *et al.* 2015, American Physiological Society 2018, Hester & Diaz 2018). Moreover, zinc deficiency has been associated with depression, emotional instability, increased anxiety and aggression, irritability, and deficits in social behavior, impaired memory, and capacity to learn (Hagmeyer *et al.* 2014). These findings may help explain the reduced expression of *SLC39A1* and *GATAD2B* in women with considerable anxiety symptoms, particularly in those who did not conceive, as well as the upregulation of these genes in both groups of women without anxiety. The *RBM6* gene expression analysis revealed significantly lower expression in women who did not conceive and reported anxiety symptoms compared with both women who did not conceive without anxiety symptoms and those who conceived despite reporting anxiety symptoms. Nevertheless, the function of *RBM6* in psychological health and women's infertility remains poorly understood and requires further investigation. *RBM6* may also be related to oocyte development, given the established role of the *RBM5 gene*, another member of

the *RBM* family that acts as a male germ cell splicing factor essential for spermatid differentiation and male fertility through its involvement in RNA splicing (O'Bryan *et al.* 2013). These findings suggest a potential association between anxiety symptoms and alterations in gene expression patterns related to endometrial function.

Analysis of the expression of immune response-related genes (*AFF3*, *CD58*, and *TNFSF4*) showed a slight downregulation of *CD58* in women reporting anxiety symptoms, particularly in those who did not conceive. These results suggest that *CD58* and *TNFSF4* may contribute to fertility disorders in women by affecting cytotoxic mechanisms and disrupting cellular growth, differentiation, and immune responses in the endometrium (Prefumo *et al.* 2002, Sengupta *et al.* 2017). In particular, because it is known that *TNFSF4* is important in the interaction of antigen-presenting T lymphocytes and the adhesion of T lymphocytes to the endothelium. Moreover, *AFF3* expression was significantly higher in women who did not conceive and did not report anxiety symptoms compared with those who conceived without anxiety symptoms. These results could be explained by the function of *AFF3* as a tissue-restricted nuclear transcriptional activator (National Center for Biotechnology Information (NCBI) 2022*a*). Overall, studied genes should be investigated in the future for a clearer view of their role in fertility disorders and the field of psychological well-being.

Analysis of signal transduction-related gene expression revealed a tendency toward lower expression of several genes (*ESR1*, *CRTC2*, *CXCL8*, *PPP1R16A*, *NDN*, *GRB10*, *HTR2A*, *RB1*, *NR3C1*, and *AXL*) in women who did not conceive and reported anxiety symptoms compared with conceived women without anxiety symptoms. These findings may reflect possible biological mechanisms linking psychological stress and reproductive function. It is well known that infertility etiology is closely related to displayed alterations in signal transducer activity (McCallie *et al.* 2017). Signal transduction also plays a significant role in psychological well-being. For example, *HTR2A* codes one of the most abundantly expressed serotonin 2A receptor (5-HT2A) in the brain, with high levels in the cerebral cortical areas, hippocampus, nucleus accumbens, and caudate nucleus (Barnes & Sharp 1999). This gene controls post-synaptic serotonin signaling and is a trigger for severe psychological disorders such as various forms of psychosis, schizophrenia, obsessive-compulsive disorder, and depression (National Center for Biotechnology Information (NCBI) 2026). In addition, *HTR2A* polymorphism is individually associated with male infertility without detectable allelic or genotype interaction (Cortés-Rodriguez *et al.* 2018). Moreover, *NR3C1*, which encodes the glucocorticoid receptor, may also play an important role, as it functions both as a transcription factor binding to glucocorticoid response elements in nuclear and mitochondrial DNA and as a modulator of other transcription factors (National Center for Biotechnology Information (NCBI) 2022*c*). At homeostatic levels, glucocorticoids regulate the timing of the onset of puberty, mediate the release of sex steroids, and integrate immune regulation of conception and pregnancy progression, suggesting that both stress-induced and physiological levels of glucocorticoids are necessary for fertility (Whirledge & Cidlowski 2013). So, as a primary mediator of basal and stress-related homeostasis, it is not surprising that stress-induced levels of glucocorticoids have been linked to reduced fecundity (Whirledge & Cidlowski 2013). It could also be very interesting to explore the role of estrogen. On the one hand, estrogen plays significant role in the pathogenesis of reproductive health disorders by promoting endometriotic tissue cell survival, maintenance, and differentiation. Estrogen activates a wide range of tissue- and organ-specific physiological responses through binding to its receptor ESR1, which is predominantly expressed in the thecal layer and regulates uterine processes that prepare the endometrium for embryo attachment and implantation (Matsuzaki *et al.* 1999). Thus, the expression of the *ESR1* gene could possibly have a direct impact on woman's fertility and ART procedure results (Bulun 2009, Burney & Giudice 2012). On the other hand, *ESR* polymorphisms have been associated with endometriosis-related infertility and with women undergoing ART procedures who experience implantation failure or an increased risk of adverse outcomes, such as ovarian hyperstimulation syndrome, which may negatively affect conception (Paskulin *et al.* 2013, de Mattos *et al.* 2014). Moreover, it is important to note that *ESR1* codes estrogen receptor alpha (ERα), which generally exerts an anxiogenic effect in combination with estrogen receptor beta (ERβ), and the G-protein-coupled estrogen receptor 30, known as GPR30, which influences emotional responses, focusing on the hypothalamic–pituitary–adrenal axis and the oxytocinergic and serotonergic systems (Borrow & Handa 2017). These tendencies were observed in the study, although further research is required.

Analysis of cell differentiation-related genes (*PAX8AS1*, *EFNA5*, *IGF2*, and *H19*) revealed that the expression of these genes tended to be lower in women who did not conceive and reported considerable anxiety symptoms compared with women who conceived without anxiety symptoms. These findings suggest that reduced differentiation potential may be associated with lower chances of pregnancy and that these consistent associations could be related to women's psychological well-being. The relevance of these genes in infertility may be explained by their role in embryo implantation and embryogenesis. First, the reduced expression of *PAX8AS1* in women who did not conceive and reported

considerable anxiety symptoms may be explained by the role of this gene, which encodes a transcription factor involved in cell growth and differentiation during embryonic development (Poleev *et al.* 1992). Moreover, results of significant downregulation of this gene are presented in combination with *IGF2* gene in the IVF compared with the subfertile group (Litzky *et al.* 2017). Second, decreased signaling of *EFNA5* could cause a partially impaired response to human chorionic gonadotropin (hCG), which includes attenuated ovarian expression of progesterone receptor (PGR), prostaglandin-endoperoxide synthase 2 (PTGS2), ADAM metallopeptidase with thrombospondin type 1 motif 4 (ADAMTS4), tumor necrosis factor alpha-induced protein 6 (TNFAIP6), epiregulin (EREG), and betacellulin (BTC), follicle rupture defects, reduced ovulatory capacity, and, ultimately, infertility (Buensuceso *et al.* 2016). Third, *IGF2* activates signaling pathways involved in embryo implantation and embryo-endometrium crosstalk during the implantation process (Zhou *et al.* 2021) and also plays a key role in follicular growth, oocyte and embryo development, fetal growth, and the regulation of fetoplacental development (Wang *et al.* 2006, Harris *et al.* 2011, Liu *et al.* 2019, Muhammad *et al.* 2020). Fourth, not only *H19* is expressed in embryogenesis but it was also observed during studies of *H19* knockout mice that the ovaries of *H19* knockout mice exhibit accelerated follicular recruitment and atresia, produce less anti-Mullerian hormone (AMH), and experience a more rapid decline in fertility in comparison to wild-type counterparts (Qin *et al.* 2019, Zhu *et al.* 2020).

Analysis of the secretion-related gene *MON1A*, the angiogenesis-related gene *CYR61*, and the hemostasis-related gene *TFPI2* revealed no significant association between anxiety symptoms and infertility treatment outcomes. The overall lowest value of *CYR61* expression in not conceived women with anxiety could reflect the negative effect of anxiety on this gene expression and, also, the negative effect of reduced *CYR61* expression on endometrial decidualization and blastocyst implantation through disturbances in some processes. The protein encoded by the *CYR61* gene is a member of the CCN family and thus participates in many important processes, including cell proliferation, migration, adhesion, and extracellular matrix synthesis (Yang *et al.* 2018). At the same time, it is also associated with the angiogenesis process. For example, it is known that a mutation in the *CYR61* could lead to impaired angiogenesis in placenta and concomitantly impaired fertility (Mo *et al.* 2002). In *in vivo* study, high expression of CYR61 protein has also been found to support pregnancy outcomes in endometrial and myometrial regeneration and induction of angiogenesis (Li *et al.* 2019). Moreover, the results of *TFPI2* expression, when a lower value was observed in not conceived women who expressed anxiety compared to conceived women who did not express anxiety, could be explained by a couple of reasons. First, TFPI2 protein has been found to significantly inhibit the activity of plasmin, a hydrolase class enzyme found in blood plasma (Kobayashi *et al.* 2023). Plasmin is involved in fibrin degradation, clot lysis, and inactivation of blood coagulation factors such as V, VIII, IX, and X (Kobayashi *et al.* 2023). Thus, TFPI2 contributes to the stabilization of hemostasis during pregnancy and childbirth by inhibiting bleeding and reducing the risk of maternal and fetal complications. Second, it is known that the decreased value of the TFPI2 protein may lead to disturbances in the processes of intercellular signal transmission and intercellular interactions (Bashiri *et al.* 2014). However, further research is needed to establish consistent links between *TFPI2* expression, women's psychological health, and pregnancy success. In summary, the results of this study highlight the need for further experimental research with larger sample sizes to better understand the relationship between stress or anxiety and gene expression patterns involved in reproductive processes. Moreover, with the extensive growth of the genetic and epigenetic studies, it is believed that deeper clarification of relations between the reproductive health, psychological well-being, and genetic mechanisms will come in the wider field exploring the genome with the goal of applying this knowledge in the daily clinical practice.

Despite the interesting observations reported in this study, several limitations should be acknowledged. Most importantly, the relatively small sample size, particularly in the subgroup of women with anxiety who did not conceive, limits the statistical power of the analysis and the generalizability of the findings. Therefore, the results should be interpreted cautiously and considered exploratory. Further studies involving larger cohorts are required to confirm the potential association between anxiety symptoms, gene expression patterns, and reproductive outcomes.

Conclusion

This study explored potential links between anxiety symptoms, infertility, and gene expression patterns in endometrial stromal cells of women undergoing ART. The findings suggest that anxiety symptoms may be associated with differences in gene expression related to processes important for endometrial function and embryo implantation. However, due to the relatively small sample size, these results should be interpreted cautiously and considered exploratory.

The results highlight the potential importance of psychological well-being in fertility treatment and support the need for appropriate psychological assessment and counseling for individuals undergoing ART. This perspective is also reflected in recent European fertility treatment policy recommendations,

which recognize psychological support as an important component of comprehensive fertility care (Fincham *et al.* 2022). Further research involving larger cohorts is required to confirm these findings.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/RAF-25-0165.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the work reported.

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Author contribution statement

RB, BVM, and ES designed the study and selected the participants for the study. RB and BVM performed clinical interventional procedures. GS and EV isolated and cultivated EnSCs from collected endometrium samples, performed gene expression analysis by RT-qPCR, and statistically analyzed and interpreted the obtained results. GS, EV, and ES performed visualization of the manuscript. ES performed a psychological assessment of anxiety, depression, and quality of life of women undergoing ART due to couple infertility, using the HADS, as well as analyzed and interpreted the results of descriptive data and psychological assessment. RB collected and systematized descriptive data, summarized results of the descriptive data, psychological assessment, outcomes of performed ART procedures, and gene expression, and wrote the original draft. EK and DR validated data. DR and RN conceptualized the study. BVM, EK, DR, and RN performed review and editing of the manuscript. RN supervised the study and writing processes. All authors read and approved the final manuscript.

Data availability

The data and materials used and analyzed within this article are available from the corresponding author upon reasonable request.

Ethical approval and consent to participate

This study was approved by the Ethics Committee of Biomedical Research of Vilnius Region, No. 158200-18/7-1049-550. All participants received written and oral information and signed informed consent before the study.

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