Ovarian granulosa cells from women with PCOS express low levels of SARS-CoV-2 receptors and co-factors

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Abstract

Purpose Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is global pandemic with more than 5 million deaths so far. Female reproductive tract organs express coronavirus-associated receptors and factors (SCARFs), suggesting they may be susceptible to SARS-CoV-2 infection; however, the susceptibility of ovary/follicle/oocyte to the same is still elusive. Co-morbidities like obesity, type-2 diabetes mellitus, cardiovascular disease, etc. increase the risk of SARS-CoV-2 infection. These features are common in women with polycystic ovary syndrome (PCOS), warranting further scope to study SCARFs expression in ovary of these women.

Materials and methods SCARFs expression in ovary and ovarian tissues of women with PCOS and healthy women was explored by analyzing publically available microarray datasets. Transcript expressions of SCARFs were investigated in mural and cumulus granulosa cells (MGCs and CGCs) from control and PCOS women undergoing in vitro fertilization (IVF).

Results Microarray data revealed that ovary expresses all genes necessary for SARS-CoV-2 infection. PCOS women mostly showed down-regulated/unchanged levels of SCARFs. MGCs and CGCs from PCOS women showed lower expression of receptors *ACE2*, *BSG* and *DPP4* and protease *CTSB* than in controls. MGCs showed lower expression of protease *CTSL* in PCOS than in controls. Expression of *TMPRSS2* was not detected in both cell types.

Conclusion Human ovarian follicle may be susceptible to SARS-CoV-2 infection. Lower expression of SCARFs in PCOS indicates that the risk of SARS-CoV-2 infection to the ovary may be lesser in these women than controls. This knowledge may help in safe practices at IVF settings in the current pandemic.

Keywords SARS-CoV-2 · COVID-19 · Ovarian granulosa cells · Oocyte · PCOS · IVF

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the infection coronavirus disease 2019 (COVID-19) which is currently a public health emergency

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worldwide. The disease in symptomic patients shows a broad spectrum of clinical manifestations ranging from fever, cough, tiredness, joint/muscle pain, shortness of breath, headache, hemoptysis, diarrhea, dyspnea, etc. [1]. Since the first case in December 2019, SARS-CoV-2 has infected more than 250 million individuals as of November 11, 2021, leading to more than 5 million deaths (WHO, 2021). Besides the challenge of vaccinating the population, the emerging mutations in SARS-CoV-2 genome may enable it with increased infectivity and virulence, immune evasion and impedance against diagnostics and therapy, hence raising the concerns of scientific and medical community to a greater extent [2].

The recent outbreak of COVID-19 is much more contagious than SARS-CoV. Information about molecular determinants of the coronavirus helps us to understand the mechanism of infection which involves both viral and host cellular machinery. There are around 28 SARS-CoV-2 and coronavirus-associated receptors and factors (SCARFs), out

of which we are hereby discussing that are in the interest of this study [3]. The transmembrane glycoprotein, spike protein (S protein) which is involved in binding and infection to host cell is responsible for variation in coronavirus and host tropism [4]. S protein binds to the host cells by the canonical receptor, angiotensin-converting enzyme II (ACE2). The virus may also bind to Basigin (BSG) (non-canonical receptor) [3]. Dipeptidyl peptidase 4 (DPP4) and C-type lectin domain family four member M (CLEC4M) are other molecules proposed to be used by virus for entry into the host cell [5, 6]. The receptor attachment is followed by priming of S protein by host proteases to facilitate viral entry. Transmembrane protease, serine 2 (TMPRSS2) mediates entry of the virus into the host cells by cleaving the S protein followed by membrane fusion. In the absence of TMPRSS2, the virus can use cathepsin B/L (CTSB and CTSL), FURIN etc. as alternate proteases [3]. Infection of gametes by SARS-CoV-2 is a matter of concern as it may affect the embryo and fetus, may affect pregnancy and can have long-term health consequences [7, 8]. Although pulmonary damage has been the most prevalent cause of death due to COVID-19, there is growing evidence for the emerging multi-organ infectious nature of SARS-CoV-2 [3]. Theoretically, SARS-CoV-2 may infect any cell or tissue type that co-expresses receptor and proteases, i.e., ACE2 and TMPRSS2 [9]. There are a few studies focused on expression of SCARFs in reproductive organs. Zhou et al. performed in situ protein-proofed single-cell RNA profiling of several human tissues which indicated that ovary can be a likely target for SARS-CoV-2 infection [10]. ACE2 is expressed in female reproductive organs like ovary, uterus, vagina and placenta [11–15]. In addition, expression of other receptors and proteases (BSG, CTSL, FURIN) has been reported in male and female reproductive tissue by several studies using single-cell RNA sequencing approach and by analyzing publically available microarray datasets [3, 16] but reports on TMPRSS2 expression in ovary are inconsistent. However, alternative proteases still remain in contention to facilitate the SARS-CoV-2 infection at ovary.

The susceptibility of the human oocyte/follicle to SARS-CoV-2 infection is not widely studied and the results are variable. Transcriptomic and proteomic data analysis showed that SCARFs are expressed in human ovarian tissue. Also, they have reported that oocytes of non-human primate showed ACE2 expression along with the expression of co-receptor BSG and alternative protease CTSL [9]. However, receptor DPP4 and alternative proteases CTSB and FURIN have not been explored by them. In a study by Hikmet et al. the expression of ACE2 mRNA and protein was found to be low in female reproductive organs [17]. Two other studies also showed expression of both *ACE2* and *BSG* receptors in human oocytes and preimplantation embryos (blastocyst) [7, 18]. In the

follicle, oocyte is surrounded by companion somatic cells i.e. cumulus granulosa cells (CGCs) whereas the mural granulosa cells (MGCs) line the follicular wall around the antrum. While MGCs are involved in more of steroidal functions and providing mechanical support to oocyte, the CGCs are in immediate contact with oocyte and act as metabolic drivers of the oocyte [19]. As MGCs and CGCs are in close vicinity of oocyte, if they are susceptible to infection by SARS-CoV-2, there are high chances that the oocyte may also get infected and in turn it may reflect in the embryo as well. Hence, it is important to know about SCARFs expression in these cells which would indicate the susceptibility of oocyte and in turn the embryo towards SARS-CoV-2 infection. SCARFs expression in granulosa cells has not been studied much apart from a few reports [9, 20]; hence, there is a need to examine SCARFs expression in them. Therefore, we investigated the expression of receptor and proteases (which facilitate the entry of SARS-CoV-2 inside the cells) in granulosa cells.

Our other interest is to investigate the expression of SCARFs in granulosa cells of women with polycystic ovary syndrome (PCOS), which is the major endocrinopathy in women of reproductive age. The characteristic features of this disorder are hyper-androgenemia, obesity and increased risk for development of type-2 diabetes mellitus (T2D), cardiovascular disease (CVD), which increases the susceptibility to SARS-CoV-2 infection and may even worsen clinical outcomes of COVID-19 [21]. Hyperandrogenism in these women may lead to elevated ACE expression which can augment viral entry [22]. Recently, Subramanian et al. using a regression model predicted that the women with PCOS are at increased COVID-19 risk as compared to controls based on the hazard scores of features like BMI, impaired glucose regulation, androgen excess, anovulation, vitamin D deficiency, hypertension, etc. [23]. Moreover, there are evidences showing that the receptors for SARS-CoV-2 (ACE2, TMPRSS2) can be regulated by androgens, which are elevated in PCOS [24]. BSG too may be regulated by estrogen and progesterone [25]. Hence, one can expect altered SCARFs expression in PCOS which may lead to severe ramifications of COVID-19 due to hormonal imbalance and other clinical features in these women. However, the possible influence or chances of infection to follicles/oocyte which may affect the fertility/pregnancy and its outcome due to COVID-19 in PCOS women are still not clear.

Hence, we first explored the expression of SCARFs in ovarian tissue of healthy women and women with PCOS by analyzing publically available microarray datasets. Also, we investigated transcript expression of SCARFs in MGCs and CGCs of women with PCOS and control women which have not been studied as much till date.

Materials and methods

Microarray data analysis

The Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database was searched for gene expression datasets on PCOS compared to controls (Table 1). Seven PCOS datasets which compared gene expression patterns of ovarian cells/tissues in control women and women with PCOS were selected and downloaded from the GEO database [26]. Each of the datasets was processed independently to identify differentially expressed genes. The microarray data analysis was carried using R 4.0.2. The raw CEL files for the Affymetrix arrays were downloaded and normalized using the robust multi-array average method (RMA) to allow for background correction, normalization and summarization. In case of the Agilent arrays, the pre-processed quantile normalized series matrix file were used. After preprocessing, the unmapped probes were removed. Duplicate gene entries were collapsed using the collapse Row function in R. The limma package in R was used to identify differentially expressed genes [27]. Multiple testing correction was performed using the Benjamini and Hochberg method. Genes with P value < 0.05 and fold change > 1.5 were considered to be differentially expressed.

Study subjects and sample collection

This study was carried out at ICMR-National Institute for Research in Reproductive Health (ICMR-NIRRH), India after ethical approval. We recruited women with PCOS ($n = 14$) as per the Rotterdam consensus criteria [28]. Regularly menstruating women having no reproductive complications and undergoing IVF due to male factor infertility or oocyte donors were recruited as controls ($n = 15$). All participants were undergoing controlled ovarian hyperstimulation using a GnRH agonist protocol for IVF at P. D. Hinduja National Hospital and Medical Research Centre, Mumbai. They were enrolled in the study after obtaining written consent. On the day of ovum pick-up (d-OPU), blood was collected from all participants for carrying out biochemical and hormonal assays. On the same day, macroscopically clear follicular fluid was collected, processed as described previously [29]. Serum and follicular fluid collected on d-OPU were assayed for estradiol (E_2), progesterone (P_4), total testosterone (TT) and SHBG by electro-chemiluminescence technology using Roche e411 automated analyzer (Roche, Basel, Switzerland). Baseline levels for LH, FSH, prolactin and TSH estimated between days 3 and 7 of menstrual cycle were obtained from clinical records. TT and SHBG values were used to calculate androgen excess indices (http://www.issam.ch/freetesto.htm).

Isolation of granulosa cells

Immediately after oocyte collection, CGCs were separated manually from the cumulus oocyte complex suspended in aspirated follicular fluid. From the IVF center, CGCs were transported in ovum buffer to the lab for further processing. CGCs were then washed with PBS and cell lysis buffer was added to extract RNA. MGCs were collected and separated from red blood cells by centrifugation through a Ficoll gradient (HiMedia, India) at 600g for 20 min. MGCs were carefully removed from the middle layer of Ficoll gradient and washed with phosphate-buffered saline. The enriched CGCs and MGCs were used for quantitative gene expression analysis.

Table 1 Gene expression datasets included in the study

Dataset	Sample used for analysis	Platform	Array
GSE114419	Controls (3) PCOS (3)	GPL17586	Affymetrix Human Transcriptome Array 2.0
GSE137684	Controls (4) Normo-androgenic PCOS (3)	GPL17077	Agilent-039494 SurePrint G3 Human GE v2 8×60K Microarray 039,381
GSE106724	Controls (4) Normo-androgenic PCOS (4)	GPL21096	Agilent-062918 Human lncRNA array V4.0
GSE102293	Controls (4) PCOS (2)	GPL570	Affymetrix Human Genome U133 Plus 2.0 Array
GSE34526	Controls (3) PCOS (7)	GPL570	Affymetrix Human Genome U133 Plus 2.0 Array
GSE5850	Controls (6) PCOS (6)	GPL570	Affymetrix Human Genome U133 Plus 2.0 Array
GSE1615	Controls (4) PCOS (5)	GPL96	Affymetrix Human Genome U133A Array

List of microarray datasets downloaded from GEO. The studies were carried out in women with PCOS and controls were used for the analysis using R package

Real-time PCR

RNA was extracted from cells using Qiagen miRNA easy kit (Qiagen, Hilden, Germany) and quantified by Nanodrop Synergy HT (Biotek, Germany). The cDNA was synthesized by high-capacity cDNA reverse transcription kit (Applied Biosystems, CA, USA). The expression of genes was investigated using the Takyon SYBR mastermix (Eurogentec, Europe) and appropriate primers (Supplemental Table 1) using cDNA samples. These included receptors *ACE2, BSG, CLEC4M, DPP4* and proteases *TMPRSS2, CTSB, CTSL* and *FURIN*. The mRNA abundance was normalized to the expression of housekeeping gene 18S rRNA, and the gene expression levels were represented as fold change values by the Δ threshold cycle (Ct) $2^{-\Delta\Delta Ct}$ method.

Results

Clinical characteristics of the study participants

There was no significant difference between age and BMI between control and PCOS group (Table 2). On the d-OPU, P4 levels in follicular fluid and SHBG levels both in follicular fluid and serum of women with PCOS were significantly lower than controls. Basal LH and LH:FSH ratio in serum and total, bioavailable and free testosterone as well as free androgen index were significantly higher in follicular fluid and serum collected on d-OPU in PCOS group as compared to control group.

Table 2 Demographic and clinical characteristics of the study participants undergoing controlled ovarian hyper-stimulation during IVF

	Control ($n=15$), median (IQR)	PCOS ($n=14$), median (IQR)	P value
Age, years	28 (22–31)	28.50 (25.75–31)	0.430
BMI (kg/m^2)	20.50 (18.40–25.40)	24.50 (21.58–28.26)	0.077
Basal LH levels (µU/mL)	3.6 (2.8–6.13)	7.57 (4.9–8.995)	0.0007**
Basal FSH levels (µU/mL)	5 (4.48–7.09)	5.65 (4.11–6.14)	0.810
LH:FSH	0.656 (0.54–0.9)	1.37 (1.04–1.65)	0.0006**
Prolactin (ng/mL)	14.2 (6.37–16.98)	12.3 (10.18–14.56)	0.743
TSH (mIU/mL)	1.87 (1.6–3.08)	2.08 (1.29–3.20)	0.777
E2 (ng/mL) before hCG administration	1.68 (1.5–2.24)	1.79 (1.22–2.4)	0.948
E2 (ng/mL) on hCG administration day	2.49 (2.1–3.8)	2.54 (2.25–4.19)	0.694
[a]E_2 (ng/mL) Serum	2.14 (2–2.8)	0.997 (0.078–2.61)	0.067
[a]E_2 (ng/mL) FF	840 (435–1042)	912.6 (608.9–1260)	0.420
[a]P_4 (ng/mL) Serum	2.6 (1–5)	0.73 (0.188–2.98)	0.073
[a]P_4 (µg/mL) FF	23.02 (15–30)	15.48 (7.03–21.06)	0.036*
[a]TT (ng/dL) Serum	111 (90–128)	161.5 (123.7–192.5)	0.019*
[a]TT (ng/dL) FF	372.6 (260–600)	617.5 (400.3–787.4)	0.025*
[a]SHBG (nmol/L) Serum	125 (80–234)	89.85 (47.25–108.4)	0.045*
[a]SHBG (nmol/L) FF	153.4 (133.2–175)	95.55 (78.25–149.9)	0.034*
[a]Free T (pmol/L) Serum	28.10 (19.5–34)	52.87 (36.37–61.75)	0.001**
[a]Free T (pmol/L) FF	87.09 (58–127)	183 (142.8–224.5)	0.0005**
[a]Bio T (nmol/L) Serum	0.66 (0.46–0.79)	1.24 (0.85–1.45)	0.001**
[a]Bio T (nmol/L) FF	2.09 (1.35–2.98)	4.3 (3.34–4.93)	0.0005**
[a]FAI Serum	3.28 (2.26–4.25)	6.24 (4.27–8.88)	0.001**
[a]FAI FF	7.66 (6.44–13.15)	19.50 (16.29–24.41)	0.0004**

Data are represented as the median (inter-quartile range) for demographic, hormonal, and biochemical profiles compared between control and women with PCOS. Statistical comparison was performed using the Mann–Whitney U test. P values < 0.05 are considered significant for all statistical tests

IVF in vitro fertilization, *BMI* body mass index, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *TSH* thyroid stimulating hormone, *FF* follicular fluid, E_2 estradiol, P_4 progesterone, *TT* total testosterone, S*HBG* sex hormone-binding globulin, *Free T* free testosterone, *Bio T* bioavailable testosterone, *FAI* free androgen index

[*]$P < 0.05$, [**]$P < 0.01$

[a]Parameters were measured in serum and follicular fluids obtained on the day of ovum pick-up

Microarray data analysis

We carried out microarray data analysis using gene expression datasets on studies involving PCOS and control women (Fig. 1). Interestingly, all SCARFs which we have focused on in the present study are expressed in the ovary of healthy women. Between controls and PCOS, majority of the COVID-related host genes did not show any differential expression except few. Analysis showed *ACE2* was down-regulated in granulosa cells of women with PCOS compared to controls [30]. Another receptor, *BSG* showed lower expression in theca cells, but in granulosa cells, its expression was increased in PCOS women than in controls [31, 32]. DPP4 was found to be decreased in granulosa cells of women with PCOS [32]. Further, *FURIN* was observed to be down-regulated in oocyte and up-regulated in granulosa cells of women with PCOS [30, 33]. *CTSB* expression was observed to be up-regulated in both oocyte and granulosa cells of women with PCOS [32, 33]. *CTSL* expression was not consistent in granulosa cells of PCOS across different microarray studies (GSE106724), [32, 34]. The expression of *CLEC4M* and *TMPRSS2* was comparable across all
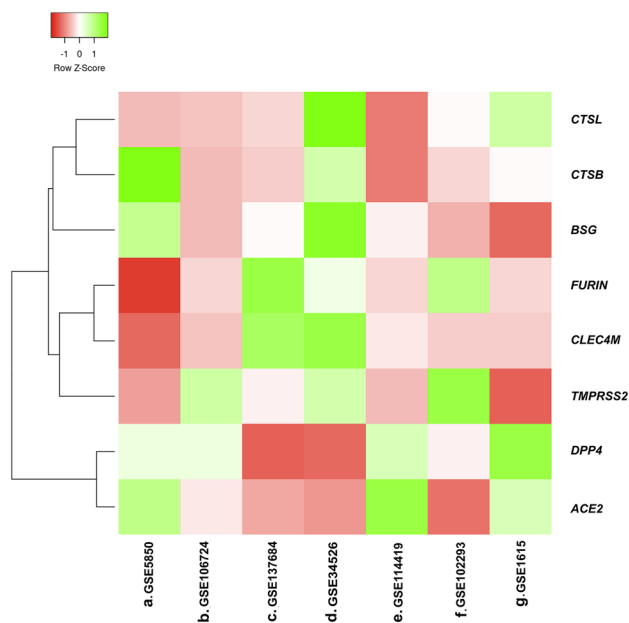
analyzed studies. However, the microarray data for these genes are not validated by gene expression analysis.

Transcript levels of SCARFs in MGCs and CGCs

We measured the transcript levels of several SARS-CoV-2 receptors (*ACE2*, *BSG*, *CLEC4M*, *DPP4*) and proteases (*TMPRSS2*, *CTSL*, *CTSB*, *FURIN)* and found all of them are expressed in ovarian granulosa cells (both MGC and CGCs) except *TMPRSS2* (Fig. 2). In women with PCOS, mostly gene expression pattern of SCARFs in MGCs and CGCs accorded with each other. The expression of receptors *ACE2, BSG* and co-receptor *DPP4* was significantly lower, but *CLEC4M* transcript level was comparable in women with PCOS and controls in both MGCs and CGCs. In case of proteases, transcript levels of both *CTSB* and *CTSL* were significantly lower in MGC, whereas only *CTSL* transcript level was decreased in CGCs of women with PCOS compared to controls. Expression of *FURIN* was comparable between control and women with PCOS in both MGC and CGC.

Discussion

COVID-19, one of the most threatening zoonotic outbreaks seen so far, affects respiratory tract majorly but it may infect other organs too [3]. Studies suggest that there could be possibility of infection to the ovary and ovarian cells/tissues that may hamper fertilization and pregnancy outcomes [7, 35, 36]. Taking this into account, we have analyzed publically available microarray datasets for expression of SCARFs in ovarian tissue of women with PCOS and compared it to controls. We measured the expression level of SCARFs transcript in CGCs and MGCs as these cells play pivotal role in oocyte development, maturation, its fertilization potential which would influence embryo quality [19].

The analysis of microarray data revealed that the healthy ovary expresses all genes necessary for the SARS-CoV-2 infection which indicates that the ovary might be susceptible to infection by SARS-CoV-2. The comparative microarray analysis between controls and PCOS showed lower or comparable receptor expression and either similar or higher protease (CTSB) expression across all analyzed studies and the results are not consistent. Interestingly, in women with PCOS, all the receptors were down-regulated in ovary, hinting towards the reduced risk of ovarian infection in them.

Apart from high-throughput microarray studies, reports on the expression of SCARFs in MGCs and CGCs are rare [9, 20]. Therefore, we investigated the expression of SCARFs in granulosa cells of PCOS and controls which may shed light on susceptibility of the ovary/ovarian follicle to SARS-CoV-2 infection. The *ACE2* transcript expression was
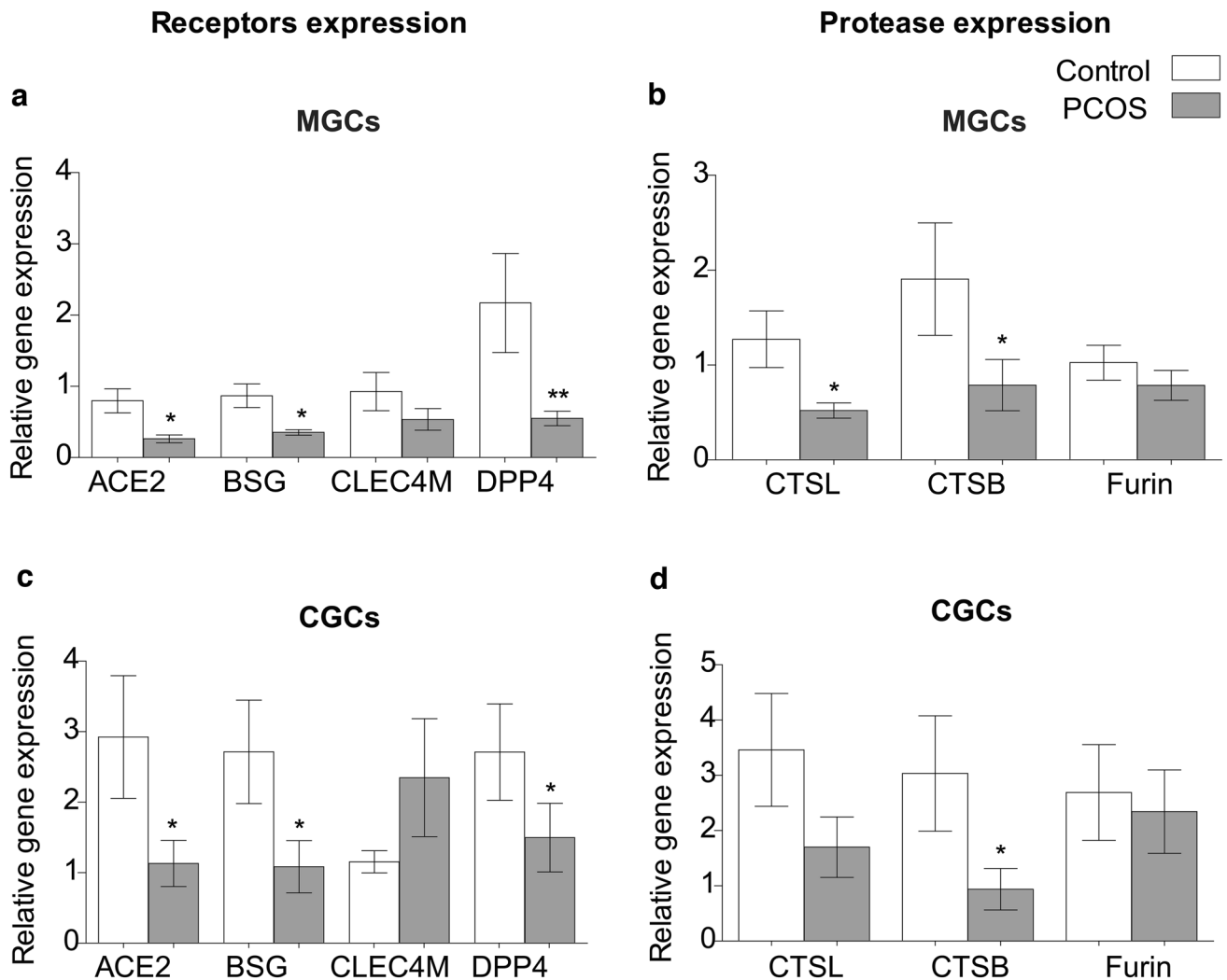

Fig. 1 Heat map showing the expression of genes for SARS-CoV-2 receptors and proteases in ovary and ovarian tissues from women with PCOS compared to controls. Differential expression (Log2fold change values) of genes for SARS-CoV-2 receptors and spike protein processing enzymes in ovary obtained by independently reanalyzing the microarray gene expression datasets obtained from the GEO database. Microarray analysis was carried out in following samples **a** oocyte, (**b–f**) gonadotropin-stimulated mural granulosa cells. *ACE2* angiotensin-converting enzyme II, *BSG* Basigin, *DPP4* Dipeptidyl peptidase 4, *CLEC4M* C-type lectin domain family 4 member M, *TMPRSS2* Transmembrane protease, serine 2*; CTSB* cathepsin B*, CTSL* cathepsin L

Receptors expression

Protease expression

Fig. 2 Relative expression of SCARFs in granulosa cells. Relative genes expression levels of SARS-CoV-2 receptors (*ACE2, BSG, CLEC4M, DPP4*) and spike protein processing enzymes (*CTSB, CTSL and FURIN*) in MGCs (**a**, **b**) and CGCs (**c**, **d**) compared between PCOS and control groups. Fold change was evaluated using the $2^{-\Delta\Delta Ct}$ method. Expression was normalized to the 18S rRNA gene as an endogenous control and granulosa cells calibrator sample. Bar graphs represent "mean ± SEM" and *$P < 0.05$ considered significant. Data are analyzed using the Mann–Whitney *U* test. *MGCs* mural granulosa cells, *CGCs* cumulus granulosa cells, *ACE2* angiotensin-converting enzyme II, *BSG* Basigin, *DPP4* Dipeptidyl peptidase 4, *CLEC4M* C-type lectin domain family 4 member M, *TMPRSS2* Transmembrane protease, serine 2; *CTSB* cathepsin B, *CTSL* cathepsin L

downregulated in both CGCs and MGCs from women with PCOS. This observation corroborated with our microarray gene expression analysis, reported plasma levels of ACE2 in PCOS women and expression level in oocytes of rat model of PCOS [30, 37, 38]. BSG is known to be regulated by estrogen and progesterone and is important for fertilization and implantation [25]. Its presence is reported in oocyte, granulosa cells (follicles of all stages), ovarian surface epithelium, and corpora lutea [12] [18]. *BSG* was found to be down-regulated in theca cells and up-regulated in granulosa cells of PCOS women in microarray analysis [31, 32]. On the contrary, we found its significantly lower expression in MGCs and CGCs of women with PCOS. Our data on

receptor expression in both types of granulosa cells further indicate that oocyte can be infected by SARS-CoV-2; however, women with PCOS may be at lower risk of infection due to lower expression of major receptors of SARS-CoV-2.

Expression of co-receptor *DPP4* was low in both MGCs and CGCs of PCOS women supporting the microarray data analysis [32]. The DHT/insulin-induced rat model of PCOS showed lower expression of *DPP4* mRNA and protein in ovarian tissue than that of controls which accorded with our results [39, 40]. However, higher or comparable levels of DPP4 protein were found in serum and plasma of PCOS women respectively [41, 42]. Higher DPP4 mRNA levels were also observed in KGN granulosa cells after androgen

treatment. Further, CLEC4M- C-type lectin receptor expression was found in the ovary and MGCs by single-cell RNA sequencing and flow cytometry [14]. Even we observed its presence in the granulosa cells and expression levels were comparable between PCOS and controls. Overall, the expression of SARS-CoV-2 receptors was low in granulosa cells of PCOS women.

The SARS-CoV-2 hijacks transmembrane proteases for priming of its S protein out of which TMPRSS2 is the primary protease [43]. The expression of TMPRSS2 was not different in the ovary between women with PCOS and controls across microarray datasets. However, we could not detect its expression in the granulosa cells. In the same line, few other studies reported extremely low or no expression of TMPRSS2 in the ovary and ovarian tissues [9, 10, 16]. The available data regarding TMPRSS2 expression in ovary are therefore inconsistent and warrant further research on the same and alternative proteases.

We investigated the expression of alternative proteases cathepsins (CTSB/L) and FURIN as they also can prime the S protein [3, 21, 44]. CTSL is known to be involved in expansion of cumulus–oocyte complex at the time of ovulation [45]. Microarray data analysis revealed variable expression of *CTSL* in granulosa cells of women with PCOS (GSE106724), [32]. We found down-regulated *CTSL* expression in MGCs of women with PCOS contrasting to the report by Oksjoki et al. in the ovarian tissue [46]. *CTSB* expression was reported to be higher in oocytes and granulosa cells of women with PCOS [32, 33]. However, we found a significantly lower expression *of CTSB* in MGCs and CGCs from PCOS women which accorded with the data obtained from mouse model of PCOS. [47]. *FURIN* is reported to be expressed in granulosa cells and may be playing role in apoptosis and proliferation of these cells [48, 49]. Microarray data showed that *FURIN* was down-regulated in oocyte, whereas another study reported its higher expression in granulosa cells of women with PCOS [30, 33]. We found comparable expression of *FURIN* in granulosa cells of control and PCOS women. FURIN can cleave S protein at multiple sites and it is hypothesized that because of this, SARS-CoV-2 may possess higher membrane fusion capacity than other coronaviruses which encourages further research on this molecule [50].

Our study shows SCARFs are expressed in the follicular compartment of ovary; however, low expression of receptors may reduce the susceptibility of ovary to infection. We did not find TMPRSS2 expression, but other proteases are present. The current data on association between PCOS and COVID-19 are limited. Though studies indicate that women with PCOS may be at higher risk of COVID-19 due to excess androgen, other co-morbidities, lower vitamin D; and interplay between these elements and cytokine levels in women with PCOS [22, 51, 52]. However, these studies

feature systemic SARS-CoV-2 infection of women with PCOS. To our surprise, our data show down-regulation of few SCARFs in MGCs and CGCs from women with PCOS, indicating risk of COVID infection at the ovarian level is less in PCOS women compared to healthy controls. Our findings need to be established with higher number of samples. Moreover, the expression of SCARFs in granulosa cells may vary in different phases of menstrual cycle as some of them are regulated by sex hormones [25, 53, 54]. Despite these limiting factors, we believe that delineating SCARFs expression in various cell types and physiological conditions holds prime importance as they are putative candidates for developing diagnostic, therapeutic interventions and to better understand the pathogenesis and prognosis of COVID-19. There are past evidences showing that viruses like Epstein–Barr virus, hepatitis virus can infect ovary and replicate in ovum, hence leading to vertical transmission and may be responsible for either infertility, oocyte apoptosis, ovarian failure, or may even cause chronic inflammation and cause ovarian cancer too [55–57].

Further studies are warranted in animals and in infected women to understand effect of SARS-CoV-2 infection to the ovary, oocyte and its plausible influence on fertilization. Additionally, this information of expression of SCARFs in ovary may prove to be crucial to prevent the possible spread of transmission of infection in IVF procedures as oocyte/embryo may be exposed to infection from potential sources (other than plausible maternal transmission), such as semen from infected males, liquid nitrogen spills, infected healthcare workers, etc. Appropriate SOPs can be laid down in ART procedure to reduce the risk of oocyte and embryo with SARS-CoV-2 infection. It is thus crucial to know about expression of receptors and molecules that can effectuate SARS-CoV-2 infection in follicular cells. Emerging data from ongoing animal model studies may provide more insights about the effects of SARS-Co-2 infection on ovary and events proceeding and following fertilization.

Data availability Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest None of the authors has any conflict of interest to declare.

Ethical approval This study was approved by Ethics committee of ICMR-National Institute for Research in Reproductive Health (ICMR-NIRRH).

Consent to participate Informed written consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

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