Yang et al. Journal of Ovarian Research (2024) 17:231
https://doi.org/10.1186/s13048-024-01553-7

Journal of Ovarian Research

RESEARCH Open Access

Characterization of the gut microbiota in patients with SARS-CoV-2 infection during controlled ovarian stimulation

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Abstract

Background The Coronavirus disease 2019 (COVID-19) pandemic has emerged as a global health crisis, with clinical manifestations including those suggesting injury to various organs such as the ovaries, which implies that it extends beyond respiratory infections. Changes in gut microbiota may exhibit correlations with the mechanisms and stages of severity in COVID-19, as well as a link with sex hormones, embryo development, and pregnancy. Controlled ovarian stimulation (COS) is used to induce the development of multiple high-quality follicles during in vitro fertilization (IVF). Our research aimed to investigate whether patients infected with COVID-19 have altered gut microbiota compositions that would affect the outcomes of COS.

Methods Twenty-one healthy females and seventeen patients with COVID-19 were enrolled. Samples were sequenced for gut microbiota identification through 16 S rRNA V3-V4 region, including species annotation, community diversity, and community functions.

Results No significant differences were found between the groups in terms of in IVF cycle outcomes and laboratory parameters. Patients with COVID-19 and healthy women showed no significant difference in the total number of available blastocyst embryos. Furthermore, the gut microbiota alpha diversity index in the COVID-19 group were markedly reduced compared to those of healthy females. Comparing the COVID-19 group to the controls, the gut microbiota dysbiosis decreased levels of *Ruminococcus*, and *Agathobater*, and elevated levels of *Achromobacter* and *Raistonia*. Finally, we identified a series of microbial functional characteristics, including membrane transport and carbohydrate metabolism, that exhibited significant disparities between the two groups.

Conclusions Patients in the COVID-19 group exhibited significant disparities in the gut microbiota composition compared to the healthy women during COS. However, the IVF outcomes did not show any significant differences between the two groups. Collectively, our speculation suggests that SARS-COV-2 infection may alter the gut microbiota without impacting IVF outcomes.

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Keywords COVID-19, Gut microbiota, Controlled ovarian stimulation, SARS-CoV-2, In vitro fertilization

Background

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) appears to be the pathogen causing the global outbreak of coronavirus disease 2019 (COVID-19). During the fifth year of the pandemic, more than 770 million people were confirmed with the disease. Although the prevalence of COVID-19 has decreased, approximately 200,000 new cases are reported monthly [1]. The presence of SARS-CoV-2 is expected to be long-lasting. The clinical manifestations of COVID-19 are diverse, the two most common presentations are dry cough and fever [2]. However, the clinical manifestations are not limited to respiratory infections but also extend to kidney, testicular, hepatic, and ovarian injuries [3–7].

The pathogenesis of COVID-19 involves SARS-CoV-2 entering host cells via attaching to the receptor angiotensin-converting enzyme 2 (ACE2) [8]. ACE2 is highly expressed in the small intestine in addition to the nasal mucosa and alveoli [9, 10]. The intestinal inflammation and the composition of gut microbiome can be regulated by ACE2 receptors [11]. Previous research has indicated that the gut microbiota compositions of patients with COVID-19 differs from that of healthy individuals, which are linked to the severity of disease [12, 13]. Overall, gut microbial alterations may be correlated with the underlying mechanisms and severity stages of COVID-19.

Gut microbiota, considered as the second genome, is linked to sex hormones, embryo development, and pregnancy [14–16]. The reabsorption of free estrogens may be facilitated by the estrobolome, which refers to the gene profile responsible for metabolizing estrogens in the gut microbiota [17]. Additionally, the gut microbiota is altered in a rodent model of ovariectomy [14]. Therefore, the gut microbiota can interact with estrogens. Controlled ovarian stimulation (COS) is used to induce the development of multiple high-quality follicles during in vitro fertilization (IVF). COS involves the application of exogenous gonadotropins and supraphysiological elevated estradiol levels [18]. However, how COS impacts embryonic development remains unclear. Therefore, our objective was to investigate whether individuals infected with SARS-COV-2 exhibit alterations in gut microbiota composition that may impact the outcomes of COS.

Materials and methods

Human subjects and ethics approval

This study was conducted at the Reproductive Medicine Center of The First Affiliated Hospital of Anhui Medical University (Hefei, China) and approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (PJ 2023-01-64). All participants supplied written informed consent and were recruited between December 3, 2022, and May 30, 2023. Nasopharyngeal swabs were collected from all participants to confirm SARS-COV-2 infection using reverse transcriptase polymerase chain reaction (RT-PCR) testing before COS initiation. RT-PCR testing was conducted every three days until the end of COS. The participants were categorized into two groups, control and COVID-19, based on RT-PCR test results and clinical symptoms. The COVID-19 group included 17 patients who tested positive for RT-PCR test, exhibiting either asymptomatic or just mild symptoms. The control group consisted of age-matched individuals without COVID-19 who underwent COS throughout the same period.

Based on patient records and embryology reports, the following clinical parameters related to treatment outcomes were collected: age, partner's age, body mass index (BMI) (weight[kg]/height[m]^2), infertility duration, basal follicle-stimulating hormone (FSH) concentration, basal follicular number, sperm concentration, progressive motility (PR), deformity rate of sperm. The serum hormone levels were measured on cycle day five (T1), day eight (T2), and the trigger day (T3) during COS. Outcome assessments include the number of retrieved oocytes, fertilization rate, mature oocyte (MII stage) rate, and number of available blastocysts for transfer.

Gut microbial sequencing

Fresh stool samples were collected from all participants for gut microbiota testing. The feces samples were processed using the cetyltrimethylammonium bromide (CTAB) method to extract the DNA. The 16 S rRNA genes were amplified using V3-V4 primers. PCR products were denatured, extended, and purified using a Qiagen Gel Extraction Kit (Qiagen, Germany). After quality evaluation using a Qubit@2.0 Fluorometer (Thermo Scientific), the library was sequenced on an Illumina platform (Illumina NovaSeq 6000 System).

Bioinformatics analysis

To remove low-quality sequences, the raw data were filtered using Fastp software (Version 0.23.1). Alpha diversity analysis and specie annotation were carried out using QIIME (Version 1.9.1). Unweighted UniFrac distance and principal coordinate analysis (PCoA) were performed to visualize sample clustering according to the specie composition profiles.The various gut microbiota or biomarkers were analyzed using t-test and linear discriminant analysis effect size (LEfSe). Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

Table 1 Demographic and clinical characteristics of the COVID-19 group and the control group

Group	Control (*N* = 21)	COVID-19 (*N* = 17)	*P* value
Patient age (y)	32.00 ± 2.41	30.82 ± 5.73	0.437[a]
Partners age (y)	32.14 ± 3.53	33.12 ± 5.28	0.501[a]
BMI (kg/m^2)	21.93 ± 2.77	24.08 ± 4.69	0.087[a]
Infertile duration (y)	3.43 ± 1.60	3.76 ± 3.25	0.700[a]
Basal FSH (mIU/L)	7.06 ± 1.33	7.35 ± 2.44	0.664[a]
Basal follicular number			
Right	7.48 ± 2.32	7.35 ± 3.52	0.902[a]
Left	6.76 ± 2.57	6.71 ± 2.66	0.948[a]
Sperm concentration (×10^6/mL)	50.13 ± 58.05	63.92 ± 62.96	0.493[a]
PR(%)	32.48 ± 18.20	30.96 ± 26.81	0.843[a]
Sperm abnormality rate (%)	89.77 ± 23.23	89.51 ± 24.84	0.976[a]

COVID-19, coronavirus disease 2019; BMI, body mass index; FSH, follicular stimulating hormone; PR, progressive motility

[a] independent sample t-tests

(PICRUSt2) software (version 2.1.2-b) was used to perform functional annotation.

Statistical analysis

The normally distributed data were expressed as mean ± standard deviation, while the non-normally distributed data were expressed as medians (interquartile range, IQRs). Differences between the two groups were assessed using independent sample t-tests or Mann-Whitney U tests. Categorical data were analyzed by chi-square test. Statistical significance was defined when $P<0.05$.

Results

The clinical characteristics of patients with COVID-19 and control group

The demographic of the 17 female patients infected with COVID-19 and the 21 controls during COS are presented in Table 1. The ages of patients, partners, and BMI values were similar between the COVID-19 group and the controls. No differences were shown between the two groups in basal FSH levels, basal follicular number, infertility duration, sperm concentration, sperm motility, and sperm deformity rate.

Analysis of cycle characteristics and results in COS for the COVID-19 group and the control group

The cycle characteristics and hormones levels during COS in 21controls and 17 patients affected COVID-19 are shown in Table 2. No significant differences in cycle protocols, gonadotropin dosage and duration of COS in two groups. The control group has elevated levels of luteinizing hormone (LH) concentration than the COVID-19 group, while there were no significant differences in the serum levels of progestin (P) and estradiol (E2) on day five of COS. No significant differences were observed in hormones including P, E2, and LH between the two groups on day eight and the trigger day of COS (Table 2). Table 3 displayed the treatment outcomes of patients in the COVID-19 group and the controls. The patients affected by COVID-19 and the controls had similar fertilization methods and mean number of retrieved oocytes. Meanwhile, parameters including fertilization rate and number of blastocysts available for transfer are also similar in both groups.

Table 2 Cycle characteristics and hormone levels for the COVID-19 and the control groups during COS

Group	Control (*N* = 21)	COVID-19 (*N* = 17)	*P* value
Protocol			0.064[b]
Antagonist	16	8	
Long luteal	5	9	
Total dosage of gonadotropin (IU)	2700.0 (2400 to 4350)	2825.0 (2375 to 3900)	0.705 [c]
Duration of COS (d)	10.5 (10 to 11)	11.5 (11 to 12)	0.075[c]
T1			
P	2.12 (0.75 to 3.07)	2.08 (1.54 to 3.05)	0.758[c]
E2	671.14 (104.35 to 3672.88)	574.93 (344.17 to 1438.63)	0.988[c]
LH	4.20 ± 1.80	1.78 ± 1.86	0.000[a]
T2			
P	2.52 ± 1.55	2.82 ± 1.43	0.523[a]
E2	4928.50 ± 3354.94	3567.29 ± 2299.30	0.173 [a]
LH	2.17 ± 1.74	1.91 ± 1.47	0.356[a]
T3			
P	3.51 ± 2.34	3.91 ± 1.04	0.489[a]
E2	8992.32 ± 5869.93	10996.26 ± 5687.55	0.296[a]
LH	1.75 (1.04 to 2.24)	1.41 (0.84 to 2.64)	0.177[c]

P, progestin; E2, estradiol; LH, luteinizing hormone. T1, the serum hormone levels on day 5; T2, the serum hormone levels on day 8; T3, the serum hormone levels on the trigger day. [a] independent sample t-tests; [b] chi-square test; [c] Mann-Whitney U tests

Table 3 The IVF outcomes of the COVID-19 group and the control group

Group	Control (*N* = 21)	COVID-19 (*N* = 17)	*P* value
Fertilization method			0.224[b]
IVF	14	8	
ICSI	7	9	
Oocytes retrieved	13.95 ± 6.32	14.12 ± 5.51	0.932[a]
MII/oocytes (%)	88.74	90.83	0.637[b]
Fertilization rate(%)	58.36	57.5	0.934[b]
Total available blastocyst embryos	5.33 ± 2.87	6.29 ± 2.97	0.319[a]

COVID-19, coronavirus disease 2019; IVF, in vitro fertilization; MII, metaphase II; ICSI, intracytoplasmic sperm injection. [a] independent sample t-tests; [b] chi-square test;

Altered gut microbiota diversities in the COVID-19 group during COS

We detected the α and β diversities of the microbial communities in both groups. In the rarefaction curve, the number of observed operational taxonomic units (OTUs) in the COVID-19 group was decreased compared to the control group (Fig. 1A). The alpha diversity (chao1, observed_species, shannon, and simpson indices) significantly decreased compared to non-infected females (Fig. 1B). The two groups shared 1398 out of 3687 OTUs, with 992 OTUs exclusively found in the COVID-19 group (Fig. 1C). Additionally, the β diversity of microbial communities assessed by PCoA, is depicted in Fig. 1D. Samples from the control and COVID-19 groups could not be completely discriminated.

Gut microbiome dysbiosis in the COVID-19 group

We further identified the specific composition of gut microbiota in both groups. The gut microbiota in both groups primarily consist of Firmicutes, Bacteroidota, Proteobacteria, and Actiobacteria (Fig. 2A). Compared to healthy women, the abundance of Proteobacteria was found to be significantly higher, while Acidobacteriota showed a notable decrease in the COVID-19 group (Fig. 2B). The top five genus composition at the control group and COVID-19 group were *Bacteroides*, *Prevotella_9*, *Romboutsia*, and *Achromobacter*, accounting for 34.75% and 37.09%, respectively (Fig. 2C). Additionally, the COVID-19 group had decreased levels of *Lachnospira*, *Ruminococcus*, *Agathobater*, *[Eubacterium]_eligens_group*, and *Ligilactobacillus*, however, increased levels of *Achromobacter*, *Pedobacter*, and *Ralstonia* compared to healthy female (Fig. 2D). Next, we analyzed the potential biomarker gut bacterial genera in two groups using LEfSe. We found two genera (*Ruminococcus* and *Agathobacter*) in the control group, while three genera (*Pedobacter*, *Ralstonia*, and *Achromobacter)* in the COVID-19 group (Fig. 2E and F).

PICRUSt analysis

Functional characteristics of the gut microbial communities were investigated utilizing PICRUSt analysis. We identified some different microbial functional characteristics between the two groups, which including membrane transport, carbohydrate metabolism, xenobiotic biodegradation and metabolism, replication and repair, and genetic information processing (Fig. 3A). Additionally, the majority of genes were associated with membrane transport and upregulated in the COVID-19 group (Fig. 3B).

Discussion

Our study compared the outcomes of COS and gut microbiota in SARS-COV-2-infected patients and healthy women. The serum LH level was lower in the COVID-19 group compared to the control group during the COS on day five. The two groups did not show any significant differences in terms of other outcomes of COS, such as the number of retrieved oocytes and available blastocysts. Moreover, the composition of the gut microbiota was different from that of the controls. This study represents the first research focusing on the gut microbiome of female individuals affected by COVID-19 during COS.

The microbiota composition in numerous diseases was altered. In previous studies, gut microbiota diversity has been found to differ significantly between patients affected by COVID-19 and unaffected controls [19–21]. The majority of research participants were predominantly male. Our research showed that COVID-19 patients had significantly lower α diversity during COS to those without the infection. Additionally, a different gut microbial composition between the two groups has been found, which revealed a higher abundance of *Ralstonia* and *Achoromobacter* in patients with COVID-19. *Ralstonia* and *Achoromobacter* were both related to respiratory diseases [22, 23]. *Achoromobacter* belongs to the family *Alcaligenaceae*, appears to correlate with disease severity. Nasopharynx microbiota analysis revealed a positive correlation between increasing abundance of *Achromobacter* and severity of conditions in patients infected with SARS-COV-2 [24]. *Achromobacter* is the predominant bacterial genus in patients with mild disease, based on the blood samples collected [25]. In contrast, butyrate-related gut microbiota, such as *Ruminococcus* and *Agathobacter*, were more abundant in healthy controls. Butyrate, a key short-chain fatty acid (SCFA), could regulate the intestinal barrier function and immune system [26]. In addition, it is negatively associated with respiratory viral infections (e.g., COVID-19) and drug resistance [27, 28]. Altogether, these findings suggested a correlation between COVID-19 and an imbalance in the gut microbiota.

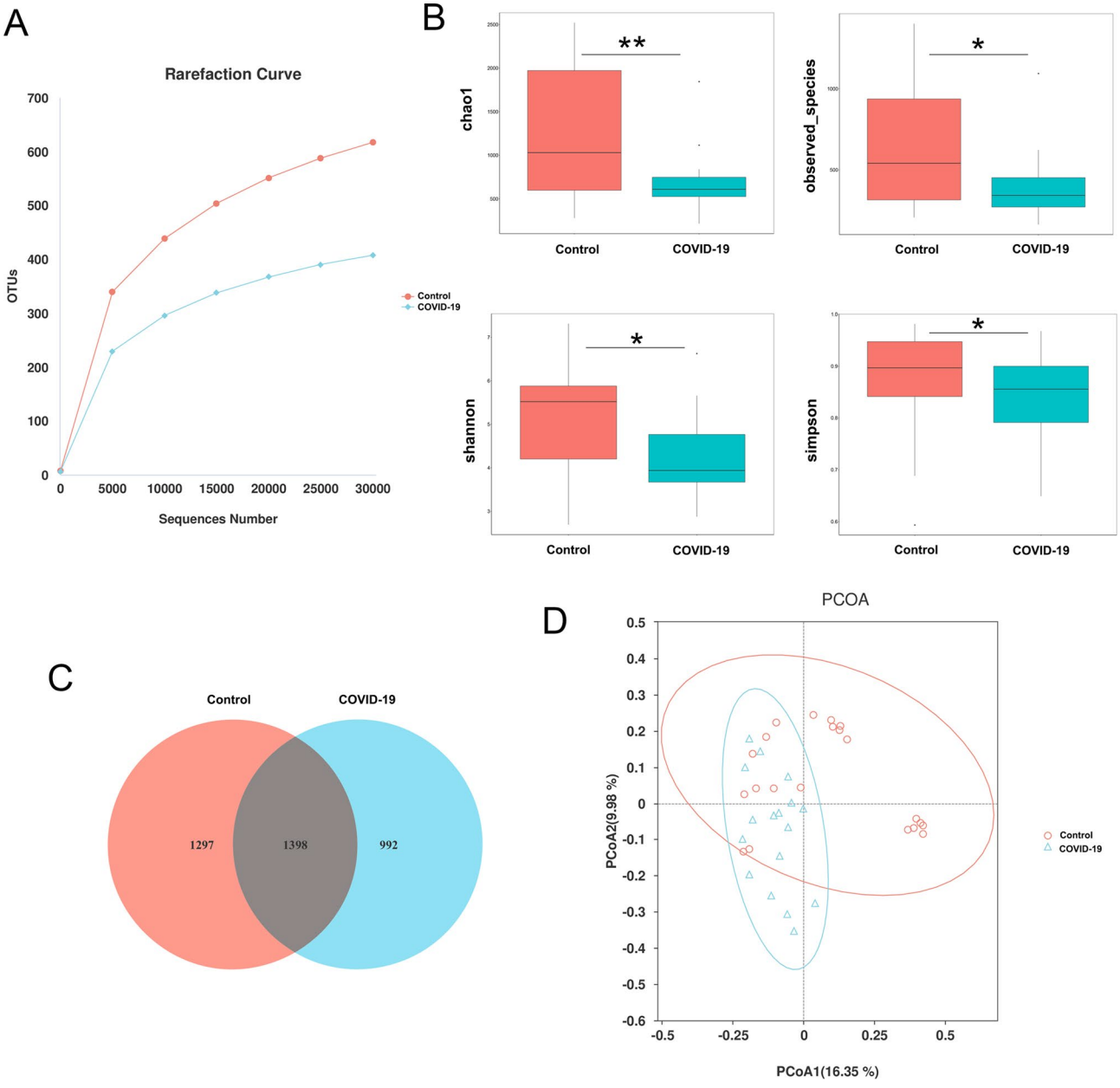

Fig. 1 Gut microbiota diversities in patients infected with SARS-CoV-2 and controls during COS. (**A**) The rarefaction curve of observed OTUs number. (**B**) The boxplots of alpha diversity based on chao1, observed_species, shannon, and simpson indices. (**C**) Venn diagram displaying the common and distinct OTUs between control and COVID-19 group. (**D**) Unweighted UniFrac metric was used to perform PCoA analysis on gut microbial communities

However, association between SARS-COV-2 and antiretroviral treatment (ART) outcomes remains inconclusive. Previous studies have suggested negative correlation between positive SARS-COV-2 test and ART, specifically pertaining to the quantity of retrieved oocytes and quality of embryos [29, 30]. The ACE2 receptor expressed in ovarian cells may enhance the susceptibility of these cells to SARS-COV-2 infection, potentially affecting female fertility [7]. Meanwhile, ACE2 acts as a partner for amino-acid transporter B^0AT1, which can regulate the compositions and the functions of gut microbiota and have a significantly important impact on immune responses [31]. ACE2 may also influence the composition of gut microbiota by regulating the level of tryptophan in the small intestine [11]. However, several studies indicated no differences in laboratory outcomes, including the rate of mature oocytes and blastocyst formation, between patients infected with SARA-COV-2 and controls [32, 33]. In our study, patients with COVID-19 had lower LH level on day five during COS. We also found that an infection with SARS-CoV-2 does not have a detrimental impact on the success of ART, including

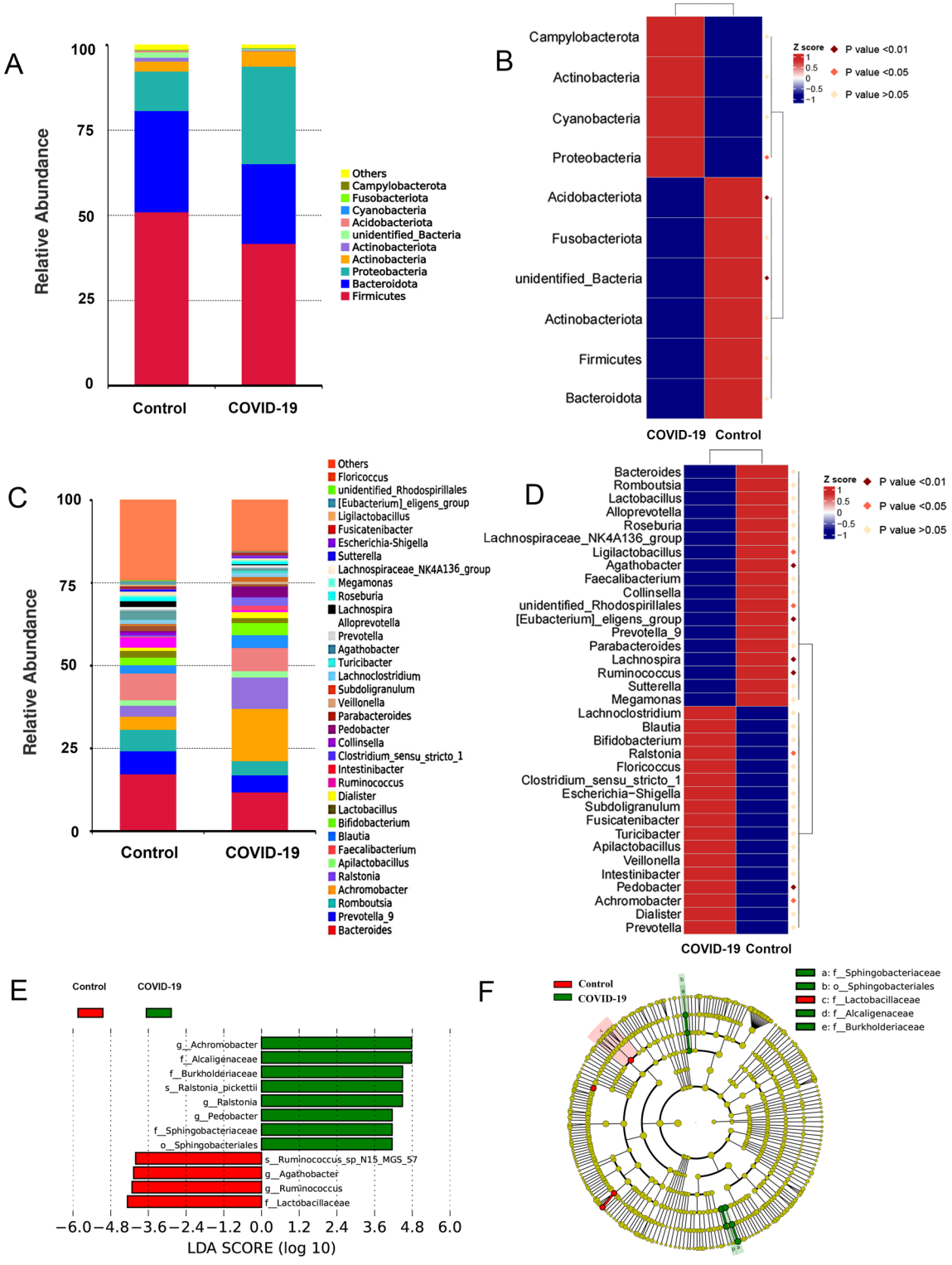

Fig. 2 Patients with COVID-19 have distinct gut microbiome dysbiosis. (**A**) A phylum-level histogram of the gut microbiota between the control and COVID-19 groups. (**B**) Heatmap of the abundance between two groups at phylum-level. (**C**) A genus-level bar plot of gut microbiota between control and COVID-19 groups. (**D**) Heatmap of the abundance between two groups at genus-level. (**E**) LEfSe analysis of significantly different gut microbiota between two groups (LDA > 3). (**F**) Cladogram analysis of significantly different classification units between control and COVID-19 groups. COVID-19, coronavirus disease 2019; LDA, Linear Discriminant Analysis

Fig. 3 Pathway features related to gut microbial communities based on PICRUSt analysis. (**A**) PICRUSt analysis of functional pathways in gut microbiota between the two groups. (**B**) KEGG pathway annotation. The data are statistically significant ($P < 0.05$). PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; KEGG, Kyoto Encyclopedia of Genes and Genome

oocyte retrieval and embryo development. In addition, alterations in the gut microbiota are associated with infertility [34]. The abundance of anti-inflammatory gut microbiota, including *Ruminococcus*, *Lachnospiraceae*, and *Agathobacter* [35–37], was markedly lower in the COVID-19 patients than in the control group. Inflammatory conditions affect oocyte quality and maturity [38]. Interestingly, our study found no significant correlation between gut microbial composition and ART laboratory outcomes in the COVID-19 group. However, a rodent study revealed a positive correlation between *Ruminococcus* and LH level [39]. Collectively, our findings suggested that alterations in the gut microbiota do not impact ART laboratory results but may affect sex-hormone levels in patients with COVID-19 during COS.

Finally, the functional profiles of the gut microbial communities were investigated using PICRUSt analysis. Our study indicated that the majority of the genes annotated in the KEGG database were associated with membrane transport and were upregulated in the COVID-19 group. ACE2 acts as a membrane protein widely expressed in the small intestine, which can bind to SARS-CoV-2 spike glycoproteins and exhibits significantly enriched expression [10, 40]. Moreover, several studies have indicated that SARS-COV-2 infection is correlated with transmembrane transport [41]. Alterations in the gut microbiota composition were found to be associated with SARS-COV-2 infection.

Our study is the first to investigate the correlation between gut microbiota and COVID-19 in female patients during COS. In addition, we provide valuable insights into the development of microbiota-based interventions for COVID-19 and the impact of SARS-COV-2 on ART outcomes. However, there still exist several limitations in the study. Due to the strict exclusion criteria, our small sample size may not accurately reflect the overall features and could be biased. The association between viral load of SARS-COV-2 and gut microbiota dysbiosis remains unclear as we did not measure viral load to stratify groups based to COVID-19 severity. Due to including asymptomatic patients and those with mild COVID-19 symptoms, the relationship between severe symptoms of patients and gut microbiota is also unclear.

Conclusions

The COVID-19 group exhibited a significant disparity in the composition of the gut microbiota compared to the healthy female controls during COS. Compared to non-COVID-19 patients, patients infected with SARS-COV-2 show increased levels of *Ralstonia* and *Achoromobacte* but decreased levels of *Ruminococcus* and *Agathobacter*. However, there were no significant differences in the IVF outcomes between the two groups. Collectively, our findings suggest that SARS-COV-2 may induce alterations in the gut microbiota without impacting IVF outcomes. These findings need to be corroborated in larger cohort. Particularly, prospective studies are required to identify mechanisms.

Abbreviations

COVID-19	Coronavirus disease 2019
COS	Controlled Ovarian Stimulation
IVF	In vitro fertilization
SARS-CoV-2	Severe acute Respiratory Syndrome Coronavirus 2
ACE2	Angiotensin-converting enzyme 2
BMI	Body Mass Index
FSH	Follicle-stimulating hormone
PCoA	Principal Coordinate Analysis
LEfSe	Linear discriminant analysis effect size
PICRUSt2	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
LH	Luteinizing Hormone
E2	Estradiol
P	Progestin
OTUs	Operational Taxonomic Units
SCFA	Short-chain Fatty Acid
ART	Antiretroviral treatment
LDA	Linear Discriminant Analysis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-024-01553-7.

Supplementary Material 1

Acknowledgements

We appreciate the patients who took part in this study and the professionals from the First Affiliated Hospital of Anhui Medical University for their cooperation.

Author contributions

Conceptualization, Y.X.C.and B.S; Methodology, L.M.W. and G.J.L.; Formal analysis and investigation, T.J.Y. and H.Y.Y.; Writing–original draft, T.J.Y. and H.Y.Y.; Visualization, G.J.L. and L.M.W.; Writing- review and editing, Y.X.C. and B.S.; Funding acquisition, G.J.L. and B.S.; Supervision, Y.X.C.and B.S.

Funding

This work was funded by the National Natural Science Foundation of China (82201777), Project of Anhui Institute of Translational Medicine (2021zhyx-C36) and Foundation of Education Department of Anhui Province for Outstanding Young Teachers (gxgwfx2022009).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The studies were approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (PJ2023-01-64), and in accordance with the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Clinical trial number

Not applicable.

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Received: 17 April 2024 / Accepted: 5 November 2024
Published online: 20 November 2024

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