Journal of Ovarian Research

RESEARCH

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Asymptomatic or mild COVID-19 infection in women prior to oocyte retrieval has no impact on embryo laboratory outcomes: a retrospective study

Yanhong Wu[1], Shenghao Wu[1], Weijue Su[1], Junzhao Zhao[1*] and Liangliang Ma[2*]

Abstract

Background Few previous studies have addressed the impact of COVID-19 infection status on assisted reproductive technology outcomes. The purpose of this study was to assess whether COVID-19 infection affects ovulation induction outcomes and the laboratory outcomes of women undergoing assisted reproductive technology treatment.

Methods In total, 363 patients were divided into three groups: the COVID-19 infection group (group A, $n=49$), the COVID-19 recovery group (group B, $n=119$) and the COVID-19 non-infection group (group C, $n=195$). Intergroup comparisons of baseline characteristics, stimulation characteristics and laboratory outcomes were performed.

Results The Gn dosage in group A was significantly higher than those in groups B and C. The duration of Gn treatment was longer in group A than in group B. In group B, the number of high-quality blastocysts was lower than that in group C. The rates of blastocyst formation (42.56%) and high-quality blastocyst formation (12.05%) in group B were significantly lower than those in group A (51.51%; $P=0.003$, 16.58%; $P=0.026$) and C (48.20%; $P=0.005$, 16.49%; $P=0.002$). The high-quality blastocyst rate in group C (34.20%) was the highest and was different from that in group B (28.33%). The main risk factor for high-quality blastocyst formation according to multivariate logistic regression analysis was recovery from COVID-19 (0.599, 95% CI: 0.360–0.996; $P=0.048$).

Conclusion Asymptomatic or mild COVID-19 infection prior to oocyte retrieval may not has a significant negative effect on ovulation induction outcomes or laboratory outcomes, although the number of Gn days and dose of Gn may increase. In addition, we should pay attention to infertile women recovering from COVID-19 infection and be aware of the significant reduction in the number of high-quality blastocysts in this population.

Keywords Assisted reproductive technology, COVID-19, High-quality blastocyst, Oocyte retrieval, Ovulation induction

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Background

The new coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus (SARS-CoV)-2, has become the most concerning public health event worldwide [1]. In the last three years, the Chinese government has legally classified COVID-19 as an infectious category B disease and managed it as a category A infectious disease in terms of prevention and control, and the effectiveness of this approach in controlling the outbreak has been remarkable. However, after the Chinese government issued a notice on further optimizing the implementation of prevention and control measures for COVID-19 in December 2022, the number of pneumonia infections continued to increase, with the peak number of confirmed cases reaching nearly 7 million that day, and as of May 3, 2023, the WHO announced that the outbreak had caused nearly 100 million infections and more than 120,000 deaths in China [2]. Although the epidemic has since stabilized in China, it is not known whether there will be another outbreak of mass infections in the future, so the situation remains critical.

Angiotensin-converting enzyme 2 (ACE2) is a recognized site of entry of COVID-19 into target cells [3] and is widely expressed in the vascular endothelium, respiratory epithelium, and alveolar mononuclear cells, with ACE2 as the main receptor. When the virus replicates in the lower respiratory tract, it attacks a wide range of ACE2 target organs, such as the heart, gastrointestinal tract, kidney, and reproductive organs [4, 5]. Some studies have shown that COVID-19 does not significantly affect female ovarian follicular function [6]. However, Jing Y et al. have argued against it [7]. There is still controversy as to whether COVID-19 causes reproductive system damage in women. Most previous studies involved patients who had recovered from COVID-19 infection and those without COVID-19 infection, yet there are few studies examining patients in a COVID-19 infection state. In this study, we retrospectively analyzed the clinical data of 363 patients who underwent oocyte retrieval at our center from December 2022 to February 2023, among whom 49 patients were still infected with COVID-19 on the day of oocyte retrieval. An analysis of the impact of COVID-19 infection on the outcome of in vitro fertilization (IVF) in females can provide a reference for practitioners of reproductive medicine addressing patients with COVID-19 infection.

Materials & methods

Patients

A retrospective analysis of women who underwent ovum pick-up (OPU) at the infertility center of the Second Hospital of Wenzhou Medical University from December 2022 to February 2023 was conducted. The inclusion criteria were as follows: (1) age ≤ 42 years; (2) no more than 4 cycles of OPU; (3) the early follicular-phase long-acting gonadotropin-releasing hormone agonist (GnRH-a) long (EFLL) protocol for controlled ovarian hyperstimulation (COH); and (4) an interval between recovery and oocyte retrieval of no more than 2 months after COVID-19 infection. The exclusion criteria were as follows: (1) malignancy or other systemic chronic diseases, such as those of the autoimmune system or hematological system; (2) a history of hereditary disease or chromosomal abnormality in one of the partners; (3) OPU cancellation regardless of the reason (e.g., fever); or (4) severe or critical COVID-19 infection.

In total, 363 eligible patients were enrolled in the study and divided into three groups on the basis of the results of a novel coronavirus ribonucleic acid (RNA) in a nasopharyngeal swab obtained 48 h prior to oocyte retrieval. Those with a positive result were allocated to the COVID-19 infection group (group A, $n = 49$), and those with two consecutive negative novel coronavirus nucleic acid tests (at least 24 h between sampling) were allocated to one of two groups on the basis of the serology of the novel coronavirus antibodies: the COVID-19 recovery group (group B, $n = 119$), if there was a previous history of neocoronavirus infection and immunoglobulin M (IgM) negativity and immunoglobulin G (IgG) positivity, or the COVID-19 non-infection group (group C, $n = 195$), if both were negative (Fig. 1).

Detection of novel coronavirus antibodies in serum and detection of novel coronavirus RNA in nasopharyngeal swabs

Patients who visited our center were routinely tested for novel coronavirus antibodies (IgG and IgM) twice at the start of COH and 48 h before oocyte retrieval and for novel coronavirus RNA in throat swabs. Antibody detection was performed with a Zhuhai Livzon Co., Ltd. novel coronavirus IgG and IgM kit (colloidal gold method), RNA detection was performed via real-time fluorescence reverse transcription–polymerase chain reaction, and the detection reagent used was a nucleic acid detection kit (20203400749) from Guangzhou Daan Company.

EFLL protocol

A single full-dose injection of 3.75 mg GnRH-a (Decapeptyl, Ferring GmbH, Germany) was administered between days 3 and 5 of the menstrual cycle. After 33–36 days, when the pituitary gland was desensitized (follicle-stimulating hormone (FSH) < 5 mIU/ml, luteinizing hormone (LH) < 5 mlU/ml, estradiol (E2) < 50 pg/ml, no follicles > 10 mm in diameter on ultrasound, endometrium < 5 mm), ovarian stimulation was commenced by means of treatment with gonadotropins (Gn) selected according to the patient's age, anti-Müllerian hormone (AMH), body mass index (BMI), antral follicle count

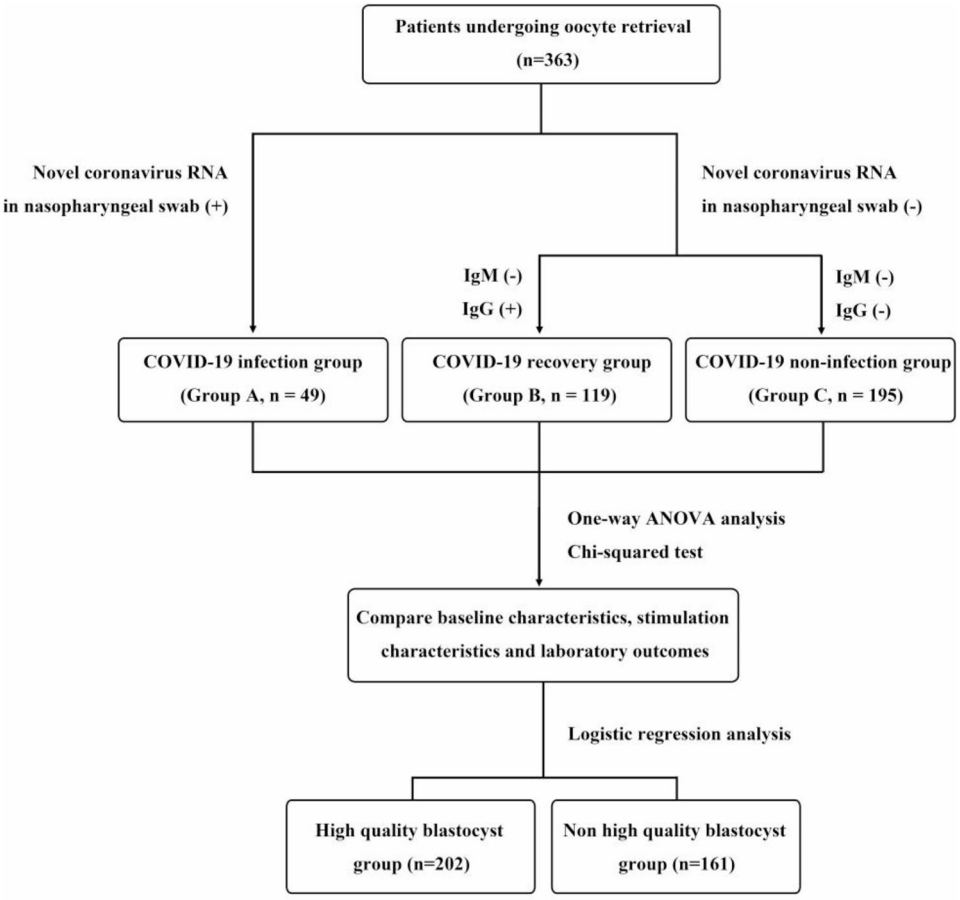

Fig. 1 Flow chart. A total of 363 eligible patients underwent oocyte retrieval at the infertility center of the Second Hospital of Wenzhou Medical University from December 2022 to February 2023. The results revealed novel coronavirus RNA in nasopharyngeal swabs 48 h prior to oocyte retrieval, with a positive result in the COVID-19 infection group (group A, $n=49$). If two consecutive novel coronavirus nucleic acid tests were negative (at least 24 h between sampling), then the remaining patients were divided into two groups on the basis of the serology of the novel coronavirus antibodies: the COVID-19 recovery group (group B, $n=119$), if there was a previous history of COVID-19 infection and IgM negativity and IgG positivity; and the COVID-19 non-infection group (group C, $n=195$), if both were negative. Statistical analysis was used for comparisons of the patients' baseline characteristics, stimulation characteristics and laboratory outcomes. All patients were divided into a high-quality blastocyst group ($n=202$) and a non-high-quality blastocyst group ($n=161$) according to whether they had high-quality blastocysts. Multivariate logistic regression analysis was performed to explore whether COVID-19 infection affects the formation of high-quality blastocysts

(AFC), and basal FSH. Monitoring was started on days 5–6 of Gn stimulation, and the Gn type and dose were adjusted according to observations on ultrasound during monitoring and serum sex hormone levels. Ovulation was triggered with 4000-10,000 IU human chorionic gonadotropin (hCG, Livzon Pharmaceuticals, Zhuhai, Guangzhou, China) or 250 μg of human chorionic gonadotropin (Merck Serono, Italy) when 2–3 follicles had reached ≥ 18 mm in diameter. If the ultrasound examination indicated that the endometrial thickness was above 5 mm after downregulation, a urine pregnancy test was performed to rule out pregnancy before the use of Gn to trigger ovulation (Fig. 2).

Oocyte retrieval and embryo culture

At 36 to 38 h after the trigger, the oocytes were retrieved transvaginally under vaginal ultrasound guidance. After oocyte retrieval, the number of oocytes was recorded microscopically by a laboratory embryologist, and fertilization was performed by means of conventional IVF or intracytoplasmic sperm injection (ICSI) methods. 2PN oocytes at 16–18 h after IVF/ICSI indicated normal fertilization. The development of cleavage-stage embryos was observed on the third day after oocyte retrieval, and blastocysts were observed on the fifth and/or sixth day.

Freeze-thawing of blastocysts

In accordance with the Gardner blastocyst grading method [8], the dilatation and hatching status of the blastocysts were graded within the range of 1–6, the inner cell mass (ICM) and trophoblastic ectodermal (TE) cells were graded as A, B, or C. High-quality blastocysts were defined as those categorized as stage 3 or above with ICM and TE cells graded as B or above [9]. The vitrification

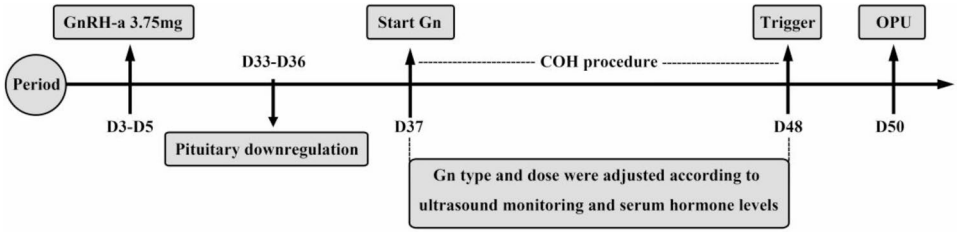

Fig. 2 Early Follicular-phase Long-acting GnRH-a Long protocol. A single full-dose injection of 3.75 mg GnRH-a was administered on days 3–5 of the menstrual cycle. After 33–36 days, when the pituitary downregulation was determined, Gn was then individually selected to initiate ovulation stimulation. The Gn type and dose were adjusted according to ultrasound monitoring and serum sex hormone levels. The trigger was administered when there were 2–3 follicles ≥ 18 mm or more in diameter. At 36 to 38 h after the trigger, OPU was performed under transvaginal ultrasound guidance. (GnRH-a, gonadotropin-releasing hormone agonist; Gn, gonadotropins; OPU, ovum pick-up; COH, controlled ovarian hyperstimulation)

freezing solution (Kitazato, Shizuoka, Japan), including No. 1 equilibration solution (ES) and No. 2 vitrification solution (VS), was prewarmed at room temperature. In accordance with the instructions of the freezing kit, the blastocysts were frozen in ES, and the timer was started. After 5 - 8 min the blastomeres of the embryos were observed, and the embryos were transferred into the VS (approximately 0.2 ml). The embryos in the VS were washed several times and placed near the black mark at the front end of the freezing carrier rod within 1 min, and the freezing carrier rod was quickly loaded with the embryos and immediately placed into liquid nitrogen, with an outer sleeve in the liquid nitrogen.

Assessment of laboratory outcomes

According to the expert consensus on the quality control of key indicators of the embryo laboratory proposed by the Reproductive Medicine Branch of the Chinese Medical Association [10], the rate of cleavage = the number of cleavage-stage embryos/the number of normal fertilized oocytes ×100%; the rate of blastocyst formation = the total number of blastocysts/the number of normal fertilized oocytes ×100%; the rate of high-quality blastocyst formation = the number of high-quality blastocysts/the number of normal fertilized oocytes ×100%; and the high-quality blastocyst rate = the number of high-quality blastocysts/the total number of blastocysts ×100%.

Statistical methods

All statistical analyses were performed with SPSS software (version 26.0; IBM, Chicago). Continuous variables that fit a normal distribution were presented as the means and standard deviations or medians, and comparisons were made via one-way ANOVA. Measured variables that did not follow a normal distribution were expressed as medians and interquartile ranges (IQRs), and differences were analyzed by means of the Kruskal–Wallis H test. The Bonferroni method was used for pairwise comparisons in multiple-group comparisons. Enumeration variables were compared via Pearson's chi-square test or Fisher's exact test. Multivariate logistic regression analysis was used to analyze the main factors affecting the formation of high-quality blastocysts, and the odds ratios (ORs) and 95% confidence intervals (CIs) of the independent variables were calculated. $P<0.05$ indicated a statistically significant difference.

Results

Comparison of baseline characteristics

There were no significant differences in the age of either partner, infertility duration, female BMI, AMH, infertility type, infertility causes, fertilization method, OPU cycle or basic hormone levels (all $P>0.05$; Table 1).

Comparison of stimulation characteristics and laboratory outcomes

There were no significant differences in trigger day hormones, the number of oocytes retrieved, the number of mature oocytes, the number of normal fertilized oocytes, the number of cleavage-stage embryos, the number of blastocysts or the rate of cleavages among the three groups (all $P>0.05$). The dosage of Gn in group A (2969.94 ± 1112.23 IU) was significantly higher than that in group B (2385.77 ± 884.67 IU; $P<0.001$) and group C (2478.32 ± 879.68 IU; $P=0.001$). The duration of Gn treatment was significantly longer in group A (12.22 ± 2.31 days) than in group B (11.35 ± 2.48 days; $P=0.022$). In group B, the number of high-quality blastocysts (0 [0, 2]) was significantly lower than that in group C (0 [1, 2]; $P=0.013$). The rates of blastocyst formation (42.56%) and high-quality blastocyst formation (12.05%) in group B were significantly lower than those in groups A (51.51%; $P=0.003$, 16.58%; $P=0.026$) and C (48.20%; $P=0.005$, 16.49%; $P=0.002$). The percentage of high-quality blastocysts in group C (34.20%) was the highest, and the rate significantly differed from that in group B (28.33%; $P=0.039$; Table 2).

Factors affecting the formation of high-quality blastocysts

The 363 patients included in this study were divided into a high-quality blastocyst group ($n=202$) or a non-high-quality blastocyst group ($n=161$) on the basis of

Table 1 Comparison of baseline characteristics

	Group A (*n* = 49)	Group B (*n* = 119)	Group C (*n* = 195)	*P* value
Female age, mean(SD) (year)	33.23 ± 5.05	32.11 ± 4.20	33.04 ± 4.77	0.151
Male age, mean(SD) (year)	34.92 ± 5.21	34.34 ± 5.48	34.68 ± 5.02	0.766
Infertility duration, mean(SD)(year)	3.51 ± 2.65	3.51 ± 2.49	3.40 ± 2.63	0.919
Female BMI, mean(SD)(kg/m2)	22.76 ± 3.44	22.16 ± 2.76	22.10 ± 3.57	0.452
AMH, mean(SD)(ng/ml)	3.26 ± 2.70	3.46 ± 2.91	3.26 ± 2.35	0.800
Infertility type				
Primary infertility%(n)	34.69(17/49)	42.86(51/119)	39.49(77/195)	0.606
Secondary infertility%(n)	65.31(32/49)	57.14(68/119)	60.51(118/195)	0.606
Infertile causes				
Female factors%(n)[#]	57.14(28/49)	57.14(68/119)	61.54(120/195)	0.696
Male factor%(n)	10.20(5/49)	15.97(19/119)	13.33(26/195)	0.595
Both factors%(n)^	14.29(7/49)	9.24(11/119)	8.21(16/195)	0.397
Unexplained factor%(n)	18.37(9/49)	17.65(21/119)	16.92(33/195)	0.962
Fertilization method				
IVF%(n)	87.76(43/49)	79.83(95/119)	78.97(154/195)	0.373
ICSI%(n)	12.24(6/49)	20.17(24/119)	21.03(41/195)	0.373
OPU cycle				
First cycle%(n)	75.51(37/49)	80.67(96/119)	85.12(166/195)	0.244
Second cycle%(n)	18.37(9/49)	15.97(19/119)	8.72(17/195)	0.066
Third cycle%(n)	4.08(2/49)	2.52(3/119)	4.10(8/195)	0.725
Fourth cycle%(n)	2.04(1/49)	0.84(1/119)	2.06(4/195)	0.728
Basic hormones				
LH, mean(SD)(IU/L)	4.29 ± 2.24	4.50 ± 2.25	4.94 ± 3.11	0.205
FSH, mean(SD)(IU/L)	7.79 ± 2.66	7.01 ± 2.24	7.43 ± 2.30	0.108
E2, mean(SD)(pg/ml)	46.60 ± 19.69	43.35 ± 17.04	44.71 ± 17.74	0.548
PRL, median(IQR)(ng/ml)[U]	11.74(7.51,15.67)	11.50(9.70,13.94)	10.96(8.72,13.25)	0.081
P, median(IQR))(ng/ml)[U]	0.48(0.35,0.61)	0.48(0.40,0.58)	0.48(0.42,0.61)	0.194

[#]Female factors mainly included polycystic ovary syndrome, endometriosis, tubal obstruction

^Both factors were defined as more than one reason causing infertility

[U]Kruskal-Wallis H test/groups individually tested by Mann–Whitney U-test

[*]$P < 0.05$ was statistical signifificance. "a" represents *P* value less than 0.05 between groups A and B, "b" represents *P* value less than 0.05 between groups A and C, "c" represents *P* value less than 0.05 between groups B and C

SD, Standard deviation; IQR, Inter Quartile Range; BMI, Body mass index; AMH, Anti-mullerian hormone; IVF, In vitro fertilization; ICSI, Intracytoplasmic sperm injection; OPU, Ovum pick up; LH, Luteinizing hormone; FSH, Follicle-stimulating hormone; E2, Estradiol; PRL, Prolactin; P, Progesterone

the presence or absence of high-quality blastocysts. In the univariate analysis, the main factors associated with high-quality blastocysts were male age, OPU cycle, AMH, the number of oocytes retrieved, Gn dosage, COVID-19 infection at the time of OPU and the E2 level of the trigger day.

Multivariate logistic regression analysis excluded male age, OPU cycle, AMH, Gn dosage and the E2 level on the trigger day, as their *P* values were all greater than 0.05. The main risk factor for high-quality blastocyst formation in the multivariate logistic regression analysis was recovery from COVID-19 (0.599, 95% CI: 0.360–0.996; $P = 0.048$). The number of retrieved oocytes ranged from 10 to 14 (3.345, 95% CI: 1.832–6.107, $P < 0.001$), and the number of retrieved oocytes equal to or greater than 15 (3.820, 95% CI: 1.925–7.581, $P < 0.001$) was found to be a protective factor for high-quality blastocyst formation (Table 3).

Discussion

This study provides a clinical reference for physicians in reproductive medicine to continue oocyte retrieval surgery when treating patients with COVID-19 infection. The results of the present study suggest that asymptomatic or mild COVID-19 infection in women prior to oocyte retrieval may not has a significant negative impact on ovulation induction outcomes or embryo laboratory results. It is not necessary to cancel the oocyte retrieval procedure due to infection with COVID-19, but it is worth noting that the number of stimulation days and dose of Gn may increase. To our knowledge, this is the first study to investigate whether preretrieval COVID-19 infection has an impact on ovulation induction outcomes and laboratory embryo outcomes in women.

At the beginning of the outbreak, the American Society for Reproductive Medicine (ASRM) and the European Society for Human Reproduction and

Table 2 Comparison of stimulation characteristics and laboratory outcomes

	Group A (*n* = 49)	Group B (*n* = 119)	Group C (*n* = 195)	*P* value
Trigger day hormones				
LH, median(IQR)(IU/L)[U]	0.59(0.41,0.92)	0.60(0.33,0.97)	0.59(0.42,0.91)	0.985
E2, mean(SD)(pg/ml)	2101.70 ± 1257.04	2165.16 ± 1286.97	2172.15 ± 1384.97	0.946
P, mean(SD)(ng/ml)	0.69 ± 0.34	0.70 ± 0.43	0.71 ± 0.40	0.965
Gn dosage, mean(SD)(IU)	2969.94 ± 1112.23[a, b]	2385.77 ± 884.67	2478.32 ± 879.68	0.001*
Duration of Gn treatment, mean(SD)(day)	12.22 ± 2.31[a]	11.35 ± 2.48	11.58 ± 2.05	0.073
No. of oocytes retrieved	11.33 ± 6.42	11.55 ± 6.38	12.43 ± 6.72	0.388
No. of mature oocytes	10.14 ± 5.85	9.71 ± 5.96	10.52 ± 5.66	0.477
No. of normal fertilized oocytes	8.22 ± 5.25	8.02 ± 5.33	8.55 ± 4.87	0.653
No. of cleavage embryos	7.92 ± 5.11	7.72 ± 5.16	8.31 ± 4.75	0.576
No. of blastocysts	4.22 ± 3.72	3.41 ± 3.01	4.12 ± 3.04	0.112
No. of high quality blastocysts[U]	1.0(0, 2.0)	0(0, 2.0)[c]	0(1, 2.0)	0.078
Rate of cleavage%(n)	97.49(388/398)	96.33(919/954)	97.18(1621/1668)	0.381
Rate of blastocyst formation%(n)	51.51(205/398)[a]	42.56(406/954)[c]	48.20(804/1668)	0.003*
Rate of high quality blastocyst formation%(n)	16.58(66/398)[a]	12.05(115/954)[c]	16.49(275/1668)	0.006*
High quality blastocyst rate%(n)	32.20(66/205)	28.33(115/406)[c]	34.20(275/804)	0.118

[U]Kruskal-Wallis H test/groups individually tested by Mann–Whitney U-test

*$P < 0.05$ was statistical signifificance. "a" represents *P* value less than 0.05 between groups A and B, "b" represents *P* value less than 0.05 between groups A and C, "c" represents *P* value less than 0.05 between groups B and C

SD, Standard deviation; IQR, Inter Quartile Range; LH, Luteinizing hormone; E2, Estradiol; P, Progesterone; Gn, gonadotropins;

Embryology (ESHRE) issued guidelines recommending that all assisted reproductive treatment (ART) be postponed as much as possible except in emergencies. With the effective control of the epidemic in some countries and regions, these medical societies recommend that reproductive health care be fully restarted on the premise of ensuring the safety of medical staff [11]. However, it is not clear whether previous infection of the parent with the novel coronavirus infection has a significant negative effect on gametes or embryos. In particular, if an infertile woman faces many obstacles in her attempt to complete the process of COH, before oocyte retrieval reveals COVID-19 infection, should she proceed with oocyte retrieval surgery, or cancel the surgery and negate all her efforts those of the physicians? This is a difficult decision for both doctors and patients.

The ovarian reserve and ovarian response are common indicators of ovarian function, among which AMH is considered the most sensitive marker of the ovarian reserve [12]. In one study the serum AMH levels in women infected with COVID-19 were reported to be significantly decreased, and the testosterone/prolactin (T/PRL) ratio was found to be significantly increased [13], indicating that the ovarian reserve function of these patients was significantly reduced; however, the validity of this conclusion remains to be confirmed, as the findings may have been influenced by differences in sample collection times between the infection and control groups. Other studies have shown that there are no significant difference in sex hormones, including FSH, LH and AMH, between patients with COVID-19 infection and patients in the control group [14, 15]. This finding is consistent with the results of this study; compared with those in the non-infection group, there were no significant differences in basal hormone levels, AMH levels, AFC or trigger day hormone levels in either the infection group or the recovery group, suggesting that COVID-19 infection may has a limited impact on the ovarian reserve of female patients.

In this study, we found, for the first time, that the Gn dose and duration of Gn treatment were significantly higher in the infection group than in the other two groups. At present, there have been no studies confirming that infection with COVID-19 increases the number of days and dose of Gn stimulation. A 2024 study revealed that the COVID-19 infection group had lower Gn doses and shorter Gn durations than did non-infection group. This may be because the study included a greater proportion of patients receiving antagonist therapy, favoring short-term ovulation induction regimens during pandemics to minimize hospital visits, reduce the risk of nosocomial infections, and improve treatment efficiency [16]. The ovulation induction protocols of the subjects included in this study were all EFLL protocol, thereby excluding the influence of the ovulation induction protocol on Gn use. The reason that COVID-19 infection increased the number of days and dose of Gn stimulation has not been clarified. The study sample size is limited, so this gap in knowledge will need further clarification in future studies. Additionally, in some studies, the effects of the COVID-19 vaccine were evaluated only in terms of female reproductive function, but their conclusions

Table 3 Factors affecting the formation of high quality blastocysts

Factors	Univariable		Multivariable	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Infertility duration (year)				
< 3	Ref			
≥ 3	0.949(0.627–1.436)	0.804		
BMI(kg/m^2)				
< 24	Ref			
≥ 24	0.762(0.485–1.198)	0.239		
Female age(year)				
< 35	Ref			
35 ≤ age ≤ 42	0.683(0.436–1.068)	0.095		
Male age(year)				
< 35	Ref			
≥ 35	0.534(0.351–0.812)	0.003*	0.723(0.447–1.169)	0.186
Infertility type				
Primary infertility	Ref			
Secondary infertiliy	1.082(0.709–1.65)	0.716		
OPU cycle				
First cycle	Ref			
Second cycle	0.511(0.271–0.964)	0.038*	0.575(0.284–1.163)	0.124
Third cycle	0.437(0.140–1.367)	0.155	0.488(0.133–1.796)	0.281
Fourth cycle	0.349(0.063–1.938)	0.229	0.733(0.124–4.340)	0.732
AMH(ng/ml)				
< 2	Ref			
2–5	1.952(1.222–3.117)	0.005*	1.104(0.593–2.056)	0.755
> 5	3.148(1.672–5.927)	< 0.001*	1.543(0.660–3.608)	0.317
No. of oocytes retrieved				
< 10	Ref			
10–14	3.444(2.013–5.893)	< 0.001*	3.345(1.832–6.107)	< 0.001*
≥ 15	5.437(3.182–9.290)	< 0.001*	3.820(1.925–7.581)	< 0.001*
Duration of Gn treatment(day)				
< 10	Ref			
10–14	1.463(0.812–2.637)	0.206		
≥ 15	0.765(0.319–1.837)	0.549		
Gn dosage(IU)				
< 1500	Ref			
1500–3000	0.984(0.506–1.912)	0.961	1.382(0.634–3.014)	0.416
> 3000	0.430(0.211–0.875)	0.020*	0.755(0.312–1.823)	0.531
Fertilization method				
IVF	Ref			
ICSI	0.995(0.592–1.673)	0.986		
PCOS				
No	Ref			
Yes	1.035(0.530–2.023)	0.919		
Endometriosis				
No	Ref			
Yes	1.488(0.538–4.114)	0.444		
DOR				
No	Ref			
Yes	0.323(0.168–0.621)	0.001*	1.178(0.496–2.794)	0.711
COVID-19 infection at the time of OPU				
No	Ref			
Yes	0.889(0.471–1.676)	0.716	1.255(0.611–2.578)	0.537
Recovery	0.613(0.387–0.971)	0.037*	0.599(0.360–0.996)	0.048*

Table 3 (continued)

Factors	Univariable		Multivariable	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Male factor				
No	Ref			
Yes	0.699(0.428–1.140)	0.151		
Trigger day E2(pg/ml)				
< 2500	Ref			
2500 ≤ E2 < 4000	2.123(1.278–3.526)	0.004*	1.016(0.546–1.889)	0.960
≥ 4000	2.972(1.382–6.393)	0.005*	1.089(0.447–2.655)	0.852
Trigger day P(ng/ml)				
< 1.5	Ref			
≥ 1.5	2.743(0.742–10.139)	0.130		

*$P < 0.05$ was statistical significance

BMI, Body mass index; AMH, Anti-mullerian hormone; IVF, In vitro fertilization; ICSI, Intracytoplasmic sperm injection; OPU, Ovum pick up; PCOS, Polycystic Ovary Syndrome; DOR, Diminished ovarian reserve; E2, Estradiol; P, Progesterone

were controversial. Specifically, Aránzazu Bosch et al. reported that oocyte donors who were vaccinated with the COVID-19 mRNA vaccine required longer ovarian stimulation times and higher Gn dosages than did those who were not vaccinated [17]. However, Antonio Requena et al. reported that there was no significant difference in the number of days or dose of Gn, regardless of the vaccine administered, and that it had no measurable harmful effects on reproductive outcomes [18]. Surprisingly, in this study, despite the use of more Gn stimulation, there was no significant increase in the number of oocytes recovered in the infection group. In addition, COVID-19 infection had no significant effect on subsequent oocyte maturation, embryo fertilization, embryo cleavage or blastocyst formation.

In a retrospective study from Wuhan, the authors reported that although the blastocyst formation rate in the case group decreased slightly, the final laboratory and clinical outcomes did not significantly differ from those in the non-infection group [15]. The results of this study revealed that the number of blastocysts, rate of blastocyst formation, rate of high-quality blastocyst formation and number of high-quality blastocysts in the recovery group were significantly lower than those in the non-infection group. Even the blastocyst formation rate and high-quality blastocyst formation rate were also significantly lower in the recovery group than in the infection group; however, there was no significant difference between the infection group and non-infection group ($P > 0.05$). This suggests that when infertile women are unfortunately infected with asymptomatic or mildly symptomatic COVID-19 prior to oocyte retrieval, there may not be a significant negative impact on embryo laboratory results, which is consistent with the results of a multicenter prospective cohort study led by Shandong University [19]. Therefore, there is no need to cancel oocyte retrieval due to COVID-19 infection, but infertile women who recover from COVID-19 infection need to be aware of the impact of infection on blastocyst formation, especially with regard to the significant reduction in the number of high-quality blastocysts. Multivariate regression analysis also revealed a 0.599-fold increase in the number of high-quality blastocysts obtained from women who had recovered from COVID-19 infection compared to uninfected women, again confirming the disadvantage of COVID-19 infection to the subsequent formation of high-quality blastocysts. The reason may be that the microenvironment of oocyte development after COVID-19 infection is abnormal, which has a long-term negative effect. If ART is used before the recovery period, embryo quality may be affected, especially as the number of high-quality blastocysts decreases. In addition, COVID-19 can also cause a decline in male semen quality, as well as embryo quality.

Unfortunately, data on the minimum time interval required between recovery from COVID-19 infection and ART treatment are not available at this stage [20]. Some authors recommend a 3-month interval between recovery from COVID-19 infection and ART treatment, primarily to allow men to enter a new spermatogenic cycle [21]. The shortest interval between recovery from COVID-19 infection and ART treatment among the subjects included in the Wuhan retrospective study was 4 months [15], whereas the interval between recovery from COVID-19 infection and ART treatment among the subjects in the recovery group included in this study was no more than 2 months. At this stage, there is no consensus on the appropriate interval between recovery from COVID-19 infection and initiation of fertility treatment, so further research is needed and is the focus of our team's future research.

This study has three major limitations. First, this was a single-center retrospective study with a limited sample size, and we were unable to classify subgroups to study the effects of different characteristics (e.g., severity, complications, recovery time), and a multicenter study with a larger sample size is needed to support the conclusions of this study to eliminate the limitations of sample size and study design. Second, only the effects of COVID-19

infection on ovarian function and laboratory outcomes in women were studied, and the effects of COVID-19 infection on pregnancy outcomes, especially the live birth rate, were not evaluated. The impact of COVID-19 infection on the clinical outcomes of ART and offspring should be further studied via follow-up of infected patients. Finally, most of the patients included were asymptomatic or had mild disease, so the conclusions of this study may not be applicable to all patients with COVID-19 infection, especially those with severe disease.

Conclusion

The results of this retrospective study suggest that asymptomatic or mild COVID-19 infection in women prior to oocyte retrieval may not has a significant negative effect on ovulation induction outcomes or embryo laboratory outcomes. There is no need to cancel the oocyte retrieval procedure due to infection with COVID-19, but notably, the number of Gn days and dose of Gn may be increased. In addition, the focus should be on infertile women recovering from COVID-19 infection, and we must be aware of the potential subsequent long-term effects of COVID-19 infection on embryogenesis, especially as evidenced by the significant reduction in the number of high-quality blastocysts. Unfortunately, there is no consensus on the minimum interval required between recovery from COVID-19 infection and ART treatment.

Acknowledgements
The authors wish to express their thanks for the financial support of Wenzhou City Key Innovation Team of Reproductive Genetics Grant.

Author contributions
YW and LM contributed to the conception and design of this study. SW and WS acquired and interpreted the data. YW wrote the first draft of the manuscript. JZ approved the final manuscript. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

Funding
This work was supported by the Wenzhou City Key Innovation Team of Reproductive Genetics Grant, Zhejiang, China (grant number: C20170007).

Data availability
The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee (Institutional Review Board) of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University and informed written consent was obtained from all participants (Reference: 2023-K-88-01).

Competing interests
The authors declare no competing interests.

Received: 2 November 2023 / Accepted: 15 January 2025
Published online: 03 February 2025

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