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# Effects of SARS-CoV-2 infection during IVF treatment on embryo morphokinetics and pregnancy outcomes after fresh transfer: a prospective cohort study

Jialyu Huang[1†], Yuxin Liu[2†], Leizhen Xia[1†], Haisheng Wu[3], Houyang Chen[1], Zhihui Huang[1], Yajie Liao[2], Xinxia Wan[1], Hong Chen[1], Yan Zhao[1*], Frauke von Versen-Höynck[4*] and Xingwu Wu[1*]

## Abstract

**Objectives** Prior studies have assessed the association between SARS-CoV-2 convalescence and subsequent in vitro fertilization (IVF) treatment, while the outcomes of couples infected during controlled ovarian stimulation (COS) were limited and controversial. The aim of this study was to clarify the effects of SARS-CoV-2 infection during COS on embryo morphokinetics and IVF clinical outcomes with the use of time-lapse monitoring.

**Methods** We conducted a prospective cohort study of 230 couples who underwent IVF cycles between April 2023 and April 2024 in an academic fertility center. Participants were divided into four groups based on the nucleic acid testing for SARS-CoV-2 during COS: both positive ($n=31$), female positive ($n=64$), male positive ($n=20$), and both negative ($n=115$). A time-lapse imaging system was used for embryo culture. Multivariate logistic regression and generalized linear models were performed to control for potential confounders.

**Results** Compared with the both negative group, the both positive group had a significantly lower cleavage rate (97.4 ± 7.7% vs. 93.6 ± 11.5%; $\beta_{adjusted}$ = -0.04, 95% confidence interval [CI]: -0.07– -0.01) and blastocyst formation rate (85.4 ± 18.9% vs. 73.0 ± 29.4%; $\beta_{adjusted}$ = -0.15, 95% CI: -0.28– -0.03). Embryos derived from the both positive group also presented significantly longer time to form 5, 6, 7, and 8 cells (t5-t8), as well as time to start compaction (tSC), time to morulation (tM), time to start blastulation (tSB), time to blastocyst (tB), and time to expanding blastocyst (tEB). No adverse impacts were observed on oocyte- and embryo-related outcomes in female positive or male positive group. The four groups were also comparable in live birth rate and neonatal outcomes after fresh embryo transfer.

†Jialyu Huang, Yuxin Liu and Leizhen Xia contributed equally to this work.

*Correspondence:
Yan Zhao
zhaoyan1967_jx@126.com
Frauke von Versen-Höynck
vonVersen-Hoeynck.Frauke@mh-hannover.de
Xingwu Wu
wuxingwujy@163.com

Full list of author information is available at the end of the article

**Conclusion** SARS-CoV-2 infection in both partners affects morphokinetic parameters of embryo development with decreased cleavage rate and blastocyst formation rate, but does not influence pregnancy and neonatal outcomes after fresh embryo transfer. Our study implies that reproductive physicians should pay attention to infertile couples with SARS-CoV-2 infection during IVF treatment and should provide adequate counseling on their embryo and pregnancy outcomes.

**Keywords** COVID-19, SARS-CoV-2, *In vitro* fertilization, Embryo morphokinetics, Pregnancy

## Introduction

While the World Health Organization declared an end to global emergency, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and coronavirus disease 2019 (COVID-19) continue to take a significant toll on human health. Since December 2022, the control of COVID-19 was relaxed in China, leading to a steep increase of infected couples during in vitro fertilization (IVF) treatment. Because of the co-expression of SARS-CoV-2 entry factors angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) in the testis, ovary, and uterus [1–3], and the associated systemic inflammation and oxidative stress [4], a viral effect on human reproduction has been strongly suspected.

An increasing number of studies have been carried out to clarify the impact. For males, Yang et al. [5] described the damage of COVID-19 to both Sertoli and Leydig cells using pathological examination. Semen quality, including sperm count, concentration, and motility, was also significantly decreased [6]. For females, some studies suggested that SARS-CoV-2 infection could impair ovarian follicles and their function, especially in severe cases [7–9]. Additionally, endometrial gene expression was significantly altered in symptomatic women with COVID-19 [10].

A recent systematic review with meta-analysis concluded that prior SARS-CoV-2 infection had no measurable adverse effects on IVF treatment outcomes [11]. However, the outcomes of couples infected during controlled ovarian stimulation (COS) are largely unknown. One study did not detect SARS-CoV-2 RNA in endometrial tissue, follicular fluid and cumulus cells among 16 infected women, and suggested reassuring results in oocyte fertilization and embryo development [12]. Another study reported that for women infected before oocyte retrieval, the oocyte utilization rate was significantly decreased [13]. More recently, Tian et al. [14] showed a negative effect of acute SARS-CoV-2 infection during COS on embryo and blastocyst quality. Existing studies failed to reach consensus, and all focused on the laboratory outcomes without further examining clinical outcomes.

In this study, we aimed to fill this knowledge gap and evaluated the impact of SARS-CoV-2 infection during IVF treatment on embryo morphokinetics and clinical outcomes in fresh transfer cycles.

## Materials and methods

### Study design and population

This prospective cohort study was conducted at the Center for Reproductive Medicine, Jiangxi Maternal and Child Health Hospital affiliated to Nanchang Medical College between April 2023 and April 2024. The study was approved by the Reproductive Medicine Ethics Committee of the hospital (No. 2023-04), and conducted in accordance with the Declaration of Helsinki. Before inclusion, written informed consents were obtained from each couple.

Infertile couples undergoing their first or second IVF cycle were screened for participation and subjected to reverse transcription-polymerase chain reaction (RT-PCR) assay for SARS-CoV-2 during COS. The exclusion criteria were: (1) age ≥ 45 years old; (2) undergoing oocyte or sperm donation; (3) oocyte vitrification cycles; (4) preimplantation genetic testing cycles; and (5) rescue intracytoplasmic sperm injection (ICSI) cycles. Participants were divided into four groups, including the both positive group if both partners had COVID-19 during COS, the female positive group if only a female partner had COVID-19, the male positive group if only a male partner had COVID-19, and the negative group if both were uninfected with SARS-CoV-2.

### Controlled ovarian stimulation

The depot gonadotropin releasing hormone agonist protocol was used as previously described [15]. In brief, on day 2–3 of the menstrual cycle, 3.75 mg leuprorelin (Lizhu Parma, China) was administered. After 28 days, successful pituitary desensitization was confirmed when endometrial thickness was below 5 mm with serum concentration of follicle-stimulating hormone (FSH) < 5 mIU/mL, luteinizing hormone < 5 mIU/mL, and estradiol < 50 pg/mL. Exogenous recombinant FSH (Gonal-F, Merck Serono, Switzerland) was then given to initiate stimulation. Based on the patient's age, body mass index (BMI), and ovarian reserve, the starting dose could be ranged from 100 IU to 225 IU. According to subsequent follicular response, adjustment was made whenever necessary. Ovulation was triggered with 250 μg recombinant human chorionic gonadotropin (hCG; Ovidrel, Merck

Serono, Switzerland) when mean diameter of ≥1 follicle reached 20 mm or 2 leading follicles were ≥ 18 mm.

#### Embryo culture and transfer

After 36 to 38 h, oocytes were retrieved through transvaginal ultrasound-guided follicular aspiration. Conventional IVF or ICSI was used for insemination based on the semen quality, with fertilization checked 16 to 18 h later. The G1 plus and G2 plus medium (Vitrolife, Sweden) were sequentially used for cleavage-stage and blastocyst-stage culture. Embryos were incubated under mineral oil in a controlled atmosphere (37℃, 5% $O_2$, 6% $CO_2$, 89% $N_2$) using the EmbryoScope Plus time-lapse monitoring system (Vitrolife, Sweden). Evaluation of embryo morphology and kinetics were done manually by two trained and experienced clinical embryologists using the EmbryoViewer software (Vitrolife, Sweden).

Fresh embryo transfer was fully discussed with patients based on latest clinical evidence, and performed only in those with no fever on the day of transfer. After oocyte retrieval, patients were intramuscularly injected with 60 mg progesterone daily (Xianju Pharma, China) to induce endometrial secretory transformation. Three or five days later, cleavage- or blastocyst-stage embryos were transferred using transabdominal ultrasound guidance. For luteal phase support, vaginal progesterone gel (90 mg/d; Crinone, Merck Serono, Switzerland) and oral dydrogesterone (20 mg/d; Duphaston, Abbott Biologicals, USA) were used and continued until 10 weeks' gestation if pregnancy was confirmed.

#### Outcome measures

For embryo morphokinetics, only normally fertilized oocytes were included in the analysis. All annotations were performed in hours post-insemination (HPI). For IVF cycles, the time of co-incubation with spermatozoa was the start time for HPI (t0), while t0 of the ICSI cycles was expressed as the median of the time taken for fertilization of all oocytes in a single cluster. Recorded kinetic parameters included time to pronuclear appearance (tPNa), time to pronuclear fading (tPNf), time to completion of cleavage to 2 to 8 cells (t2 to t8, respectively), time to start compaction (tSC), time to morulation (tM), time to start blastulation (tSB), time to blastocyst (tB), and time to expanding blastocyst (tEB). The time intervals from first cleavage to 3 cells (t3-t2), first cleavage to 4 cells (t4-t2), 3 cells to 4 cells (t4-t3), and 5 cells to 8 cells (t8-t5) were defined as cc2a, cc2b, s2, and s3, respectively.

For laboratory outcomes, the oocyte maturation rate was calculated as the number of metaphase 2 oocytes divided by the number of oocytes in ICSI cycles. The 2PN fertilization rate was calculated as the number of 2PN zygotes divided by the number of oocytes in both IVF and ICSI cycles. The 2PN cleavage rate was calculated as the number of day 3 (D3) embryos produced from 2PN zygotes divided by the total number of 2PN zygotes. The D3 good quality embryo rate was calculated as the number of D3 good-quality embryos with ≥ 7 even blastomeres, ≤ 15% fragmentation, and no multinucleation and vacuoles, divided by the total number of cleavage-stage embryos. The blastocyst formation rate was calculated as the number of blastocysts divided by the number of D3 embryos for extended culture to days 5 and 6. The viable blastocyst rate was calculated as the number of usable blastocysts (> 4CC) divided by the number of formed blastocysts.

For pregnancy outcomes, the definition of biochemical pregnancy was a serum β-hCG level of ≥ 20 mIU/mL at 10–12 days after embryo transfer. The definition of implantation rate was the number of gestational sacs divided by the number of embryos transferred. The definition of clinical pregnancy was the ultrasound discovery of at least one gestational sac with or without a fetal heart beat at 1 month following embryo transfer. The definition of early miscarriage was pregnancy loss at < 12 gestational weeks, while ongoing pregnancy was a viable pregnancy beyond 12 weeks' gestation. Live birth was defined as the birth of an infant exhibiting life signs beyond 24 weeks of gestation.

For singleton livebirths, neonatal outcomes were further collected from couples via telephone surveys by specially trained nurses using standardized questionnaires. The outcomes included mode of delivery, newborn gender, gestational age, birthweight, and birth defect. Preterm birth (< 37 weeks), low birthweight (< 2500 g), and macrosomia (≥ 4000 g) were categorized. Additionally, birthweight Z-score was calculated by adjusting for gender and gestational week based on the growth standard of Chinese singletons [16]. Small-for-gestational age and large-for-gestational age were defined as Z-score < 10th and > 90th percentiles, respectively.

#### Statistical analysis

Continuous variables were summarized as means with standard deviations, and examined for normality via the Shapiro-Wilk test along with visual inspection of histograms and Q-Q plots. Data with normal and skewed distribution were compared by one-way analysis of variance and Kruskal-Wallis test, respectively. Categorical variables were presented as numbers with percentages, and differences among groups were analyzed by $\chi^2$ test or Fisher's exact test as appropriate.

Generalized linear models were applied to assess the independent effect of SARS-CoV-2 infection on embryo morphokinetics and laboratory outcomes. Adjusted covariates included age, BMI, infertility duration, infertility type, basal FSH, anti-Müllerian hormone (AMH), antral follicle count (AFC), cause of infertility, cycle rank,

COVID-19 vaccination status, and fertilization method. For pregnancy outcomes, logistic regression analysis was used. In addition to the aforementioned variables, we also controlled for the endometrial thickness as well as number and stage of embryos transferred. Using the both negative group as reference, adjusted β and odds ratios (aORs) with 95% confidence intervals (CIs) were calculated for the other three categories.

SAS version 9.4 (SAS Institute, USA) was used for all data analyses. All tests were 2-sided and $P<0.05$ was considered as statistically significant.

## Results

A total of 230 eligible couples were enrolled in the current study, including 31 (13.5%) with both partners infected, 64 (27.8%) with only female partners infected, 20 (8.7%) with only male partners infected, and 115 (50.0%) with both partners uninfected. The majority of infected women (93/95 [97.9%]) and men (50/51 [98.0%]) had mild symptoms, and only three were asymptomatic.

The baseline characteristics of included couples are displayed in Table 1. No significant differences were observed in female age and BMI, infertility duration and type, basal FSH, AMH, AFC, cause of infertility, as well as cycle rank. Additionally, the four groups were comparable in terms of vaccination rates among women and men.

The COS and laboratory outcomes grouped by the infection status are detailed in Table 2. The duration of stimulation, dosage of gonadotropin, and hormone levels on trigger day were all similar among groups. Compared with the both negative group, the both positive group had a significantly decreased 2PN cleavage rate (97.4 ± 7.7% vs. 93.6 ± 11.5%; $P=0.014$) and blastocyst formation rate (85.4 ± 18.9% vs. 73.0 ± 29.4%; $P=0.011$) (Fig. 1A). After adjusting for potential confounding factors, the significant negative association remained between SARS-CoV-2 infection in both partners and 2PN cleavage rate ($\beta_{adjusted}$ = -0.04, 95% CI: -0.07– -0.01) and blastocyst formation rate ($\beta_{adjusted}$ = -0.15, 95% CI: -0.28– -0.03) (Fig. 1B). No significant differences were detected on the number of oocytes as well as oocyte maturation rate, 2PN fertilization rate, D3 good quality embryo rate, and viable blastocyst rate. Compared with the both negative group, female positive group or male positive group did not differ significantly in all laboratory parameters before and after adjustment.

Table 3 shows the embryo morphokinetics based on time-lapse imaging. The normally fertilized oocyte numbers of both positive, female positive, male positive, and both negative groups were 249, 507, 154, and 959, respectively. Compared with the both negative group, the both positive group presented significantly longer t5 (48.3 ± 9.1 vs. 50.3 ± 9.4 h; $P=0.005$), t6 (52.6 ± 9.6 vs. 54.9 ± 11.3 h; $P=0.002$), t7 (56.0 ± 10.1 vs. 57.9 ± 10.7 h; $P=0.012$), t8 (60.2 ± 11.7 vs. 62.3 ± 12.2 h; $P=0.025$), tSC (79.0 ± 9.6 vs. 82.5 ± 7.9 h; $P<0.001$), tM (87.5 ± 10.0 vs. 91.3 ± 9.2 h; $P<0.001$), tSB (98.0 ± 9.8 vs. 101.6 ± 10.0 h; $P<0.001$), tB (107.6 ± 10.9 vs. 111.2 ± 11.1 h; $P<0.001$), and tEB (117.2 ± 10.8 vs. 120.5 ± 11.5 h; $P=0.001$) (Fig. 1A). After adjusting for potential confounders,

**Table 1** Baseline characteristics of enrolled couples

| | Both positive (*n* = 31) | Female positive (*n* = 64) | Male positive (*n* = 20) | Both negative (*n* = 115) | *P*-value |
|---|---|---|---|---|---|
| Female age (years) | 31.7 ± 3.9 | 31.1 ± 4.4 | 31.1 ± 3.5 | 30.3 ± 3.6 | 0.347 |
| Female BMI (kg/m2) | 22.8 ± 4.9 | 21.9 ± 3.0 | 22.7 ± 4.0 | 22.0 ± 3.1 | 0.911 |
| Infertility duration (years) | 4.3 ± 3.3 | 3.8 ± 2.8 | 4.0 ± 1.9 | 4.1 ± 2.6 | 0.387 |
| Infertility type, *n* (%) | | | | | 0.544 |
| Primary | 10 (32.3) | 22 (34.4) | 8 (40.0) | 50 (43.5) | |
| Secondary | 21 (67.7) | 42 (65.6) | 12 (60.0) | 65 (56.5) | |
| Basal FSH (mIU/mL) | 6.4 ± 1.9 | 6.5 ± 2.0 | 5.5 ± 2.1 | 5.9 ± 2.0 | 0.104 |
| AMH (ng/mL) | 3.2 ± 1.8 | 3.1 ± 1.5 | 4.1 ± 2.1 | 3.5 ± 1.7 | 0.140 |
| AFC | 13.0 ± 4.4 | 13.1 ± 4.8 | 14.5 ± 4.4 | 13.4 ± 4 | 0.509 |
| Cause of infertility, *n* (%) | | | | | 0.874 |
| Female factor | 20 (64.5) | 37 (57.8) | 14 (70.0) | 66 (57.4) | |
| Male factor | 3 (9.7) | 7 (10.9) | 3 (15.0) | 13 (11.3) | |
| Mixed | 4 (12.9) | 14 (21.9) | 3 (15.0) | 22 (19.1) | |
| Unexplained | 4 (12.9) | 6 (9.4) | 0 (0) | 14 (12.2) | |
| Cycle rank, *n* (%) | | | | | 0.767 |
| 1 | 25 (80.7) | 56 (87.5) | 18 (90.0) | 97 (84.4) | |
| 2 | 6 (19.4) | 8 (12.5) | 2 (10.0) | 18 (15.7) | |
| Female vaccination rate, *n* (%) | 26 (83.9) | 54 (84.4) | 19 (95.0) | 101 (87.8) | 0.615 |
| Male vaccination rate, *n* (%) | 29 (93.6) | 57 (89.1) | 19 (95.0) | 102 (88.7) | 0.872 |

Data are presented as mean ± SD or number (percentage). Abbreviations: AFC, antral follicle count; AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle-stimulating hormone

**Table 2** Controlled ovarian stimulation and laboratory outcomes

| | Both positive (*n* = 31) | Female positive (*n* = 64) | Male positive (*n* = 20) | Both negative (*n* = 115) | *P*-value |
|---|---|---|---|---|---|
| Duration of stimulation (days) | 11.8 ± 2.0 | 11.0 ± 1.9 | 11.5 ± 1.9 | 11.7 ± 2.5 | 0.522 |
| Dosage of gonadotropin (IU) | 2271.4 ± 793.0 | 2024.8 ± 687.5 | 1958.1 ± 710.8 | 2015.6 ± 768.1 | 0.141 |
| Hormone levels on trigger day | | | | | |
| Estradiol (pg/ml) | 2328.4 ± 1182.2 | 2243.2 ± 1167.2 | 2074.2 ± 894.4 | 2352.8 ± 1000.3 | 0.525 |
| Progesterone (ng/ml) | 0.7 ± 0.5 | 0.5 ± 0.4 | 0.4 ± 0.3 | 0.5 ± 0.3 | 0.150 |
| Luteinizing hormone (mIU/mL) | 2.9 ± 6.6 | 2.1 ± 2 | 1.8 ± 0.5 | 1.8 ± 1.4 | 0.175 |
| No. of oocyte retrieved | 13.2 ± 5.1 | 12.6 ± 4.3 | 12.7 ± 5.1 | 13.6 ± 3.7 | 0.373 |
| Fertilization type, *n* (%) | | | | | 0.404 |
| IVF | 21 (67.7) | 46 (71.9) | 17 (85.0) | 90 (78.3) | |
| ICSI | 10 (32.3) | 18 (28.1) | 3 (15.0) | 25 (21.7) | |
| Oocyte maturation rate (%) * | 71.2 ± 28.4 | 74.0 ± 22.1 | 24.2 ± 42.0 | 67.7 ± 27.4 | 0.194 |
| 2PN fertilization rate (%) | 70.5 ± 16.2 | 68.7 ± 18.2 | 65.3 ± 19.0 | 66.1 ± 19.3 | 0.613 |
| 2PN cleavage rate (%) | 93.6 ± 11.5 | 97.0 ± 6.3 | 99.1 ± 2.8 | 97.4 ± 7.7 | 0.113 |
| D3 good quality embryo rate (%) | 24.8 ± 24.1 | 29.7 ± 28.7 | 29.1 ± 24.7 | 31.5 ± 24.5 | 0.569 |
| Blastocyst formation rate (%) | 73.0 ± 29.4 | 80.0 ± 21.3 | 81.8 ± 24.5 | 85.4 ± 18.9 | 0.065 |
| Viable blastocyst rate (%) | 73.8 ± 26.3 | 81.3 ± 23.3 | 88.8 ± 12.3 | 82.4 ± 18.6 | 0.212 |

* Analysis was based on ICSI cycles only. Data are presented as mean ± SD or number (percentage). Abbreviations: IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; 2PN, two pronuclei

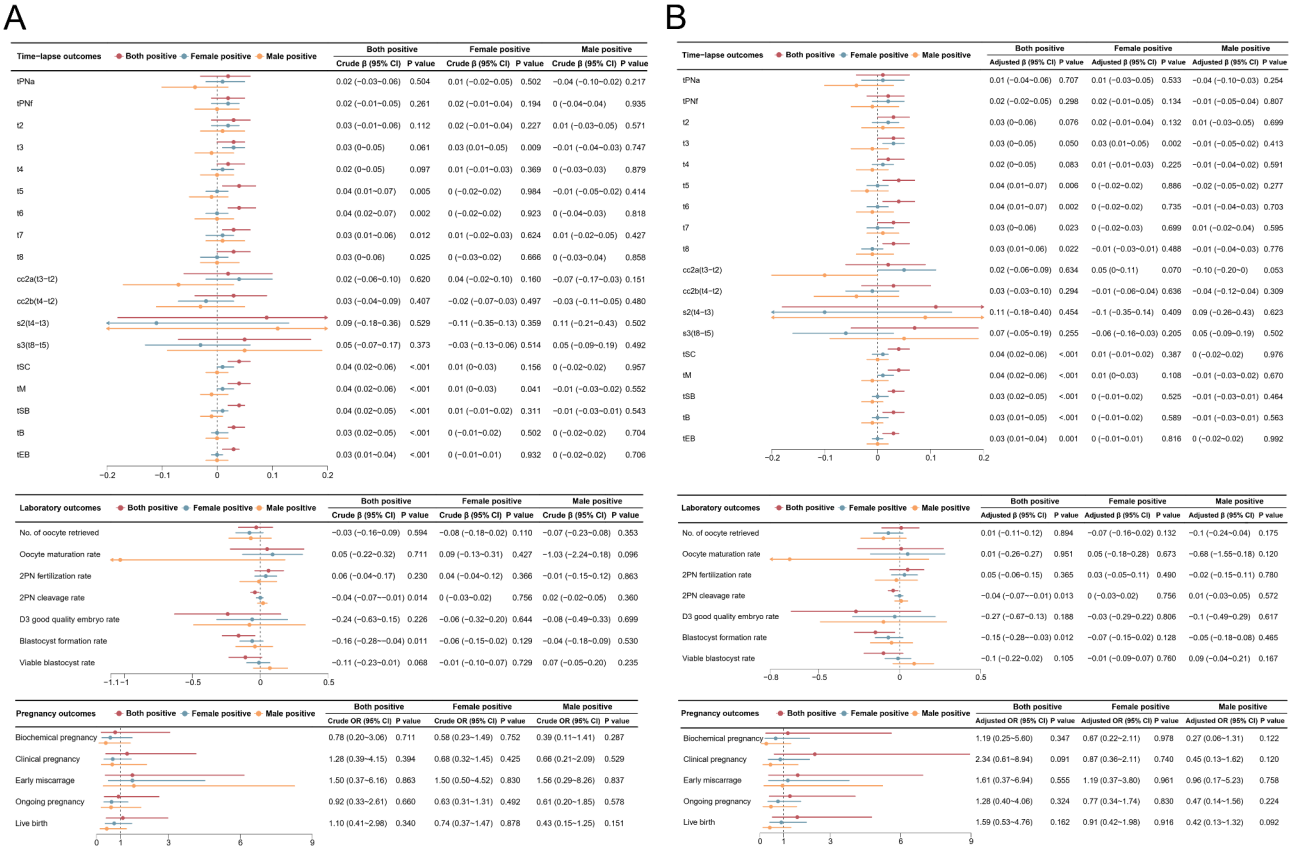


**Fig. 1** Analysis of time-lapse outcomes, laboratory outcomes and pregnancy outcomes in the both positive, female positive and male positive groups using the both negative group as reference. (**A**) Crude analysis. (**B**) Adjusted analysis

**Table 3** Evaluation of embryo morphokinetics by time-lapse imaging

| | Both positive (*n* = 249) | Female positive (*n* = 507) | Male positive (*n* = 154) | Both negative (*n* = 959) | *P*-value |
|---|---|---|---|---|---|
| tPNa | 8.1 ± 3.6 | 8.1 ± 2.2 | 7.7 ± 2.1 | 8.0 ± 2.8 | 0.068 |
| tPNf | 25.2 ± 6.8 | 25.1 ± 5.4 | 24.6 ± 5.1 | 24.7 ± 6.2 | 0.062 |
| t2 | 28.0 ± 7.0 | 27.7 ± 5.7 | 27.6 ± 6.3 | 27.3 ± 6.5 | 0.086 |
| t3 | 37.7 ± 7.5 | 37.8 ± 7.9 | 36.6 ± 6.8 | 36.8 ± 6.3 | 0.026 |
| t4 | 39.7 ± 7.2 | 39.2 ± 7.2 | 38.7 ± 6.5 | 38.8 ± 7.2 | 0.124 |
| t5 | 50.3 ± 9.4 | 48.3 ± 9.8 | 47.7 ± 9.8 | 48.3 ± 9.1 | 0.003 |
| t6 | 54.9 ± 11.3 | 52.7 ± 9.2 | 52.4 ± 10.4 | 52.6 ± 9.6 | 0.026 |
| t7 | 57.9 ± 10.7 | 56.2 ± 9.9 | 56.7 ± 11.9 | 56.0 ± 10.1 | 0.064 |
| t8 | 62.3 ± 12.2 | 60.0 ± 10.7 | 60.4 ± 13.1 | 60.2 ± 11.7 | 0.068 |
| cc2a (t3-t2) | 9.9 ± 5.1 | 10.1 ± 6.7 | 9.0 ± 4.9 | 9.7 ± 4.4 | 0.082 |
| cc2b (t4-t2) | 12.1 ± 6.0 | 11.6 ± 5.6 | 11.4 ± 4.7 | 11.8 ± 5.1 | 0.052 |
| s2 (t4-t3) | 2.3 ± 4.7 | 1.9 ± 4.2 | 2.3 ± 4.2 | 2.1 ± 4.1 | < 0.001 |
| s3 (t8-t5) | 13.1 ± 10.7 | 12.0 ± 9.2 | 13.0 ± 11.3 | 12.4 ± 10.1 | 0.869 |
| tSC | 82.5 ± 7.9 | 79.9 ± 9.8 | 79.1 ± 10.2 | 79.0 ± 9.6 | < 0.001 |
| tM | 91.3 ± 9.2 | 88.8 ± 10.6 | 86.9 ± 10.4 | 87.5 ± 10.0 | < 0.001 |
| tSB | 101.6 ± 10.0 | 98.7 ± 9.4 | 97.4 ± 9.1 | 98.0 ± 9.8 | < 0.001 |
| tB | 111.2 ± 11.1 | 108.0 ± 10.7 | 107.1 ± 10.4 | 107.6 ± 10.9 | 0.002 |
| tEB | 120.5 ± 11.5 | 117.1 ± 10.4 | 117.6 ± 10.6 | 117.2 ± 10.8 | 0.014 |

Data are presented as mean ± SD (hours). Abbreviations: tPNa, time to pronuclear appearance; tPNf, time to pronuclear fading; t2, time to two cells; t3, time to three cells; t4, time to four cells; t5, time to five cells; t6, time to six cells; t7, time to seven cells; t8, time to eight cells; tSC, time to start compaction; tM, time to morulation; tSB, time to start blastulation; tB, time to blastocyst; tEB, time to expanding blastocyst

**Table 4** Clinical outcomes of fresh embryo transfer

| | Both positive (*n* = 21) | Female positive (*n* = 49) | Male positive (*n* = 16) | Both negative (*n* = 104) | *P*-value |
|---|---|---|---|---|---|
| Stage of embryos transferred, *n* (%) | | | | | 0.470 |
| Cleavage | 5 (23.8) | 6 (12.2) | 3 (18.8) | 13 (12.5) | |
| Blastocyst | 16 (76.2) | 43 (87.8) | 13 (81.3) | 91 (87.5) | |
| No. of embryos transferred, *n* (%) | | | | | 0.581 |
| Single | 16 (76.2) | 43 (87.8) | 13 (81.3) | 89 (85.6) | |
| Double | 5 (23.8) | 6 (12.2) | 3 (18.8) | 15 (14.4) | |
| Endometrial thickness on trigger day (mm) | 11.8 ± 3.4 | 10.4 ± 3.0 | 11.8 ± 3.2 | 11.1 ± 2.6 | 0.146 |
| Biochemical pregnancy rate, *n* (%) | 18 (85.7) | 40 (81.6) | 12 (75.0) | 92 (88.5) | 0.387 |
| Implantation rate, *n/N* (%) | 20/26 (76.9) | 36/55 (65.5) | 11/19 (57.9) | 82/119 (68.9) | 0.561 |
| Clinical pregnancy rate, *n* (%) | 17 (81.0) | 34 (69.4) | 11 (68.8) | 80 (76.9) | 0.628 |
| Early miscarriage rate, *n/N* (%) | 2/17 (11.8) | 3/34 (8.8) | 1/11 (9.1) | 4/80 (5.0) | 0.484 |
| Ongoing pregnancy rate, *n* (%) | 15 (71.4) | 31 (63.3) | 10 (62.5) | 76 (73.1) | 0.587 |
| Live birth rate, *n* (%) | 14 (66.7) | 28 (57.1) | 7 (43.8) | 67 (64.4) | 0.376 |

Data are presented as mean ± SD or number (percentage)

SARS-CoV-2 infection in both partners still had a significant impact on t5 ($\beta_{adjusted}$ = 0.04, 95% CI: 0.01–0.07), t6 ($\beta_{adjusted}$ = 0.04, 95% CI: 0.01–0.07), t7 ($\beta_{adjusted}$ = 0.03, 95% CI: 0.00–0.06), t8 ($\beta_{adjusted}$ = 0.03, 95% CI: 0.01–0.06), tSC ($\beta_{adjusted}$ = 0.04, 95% CI: 0.02–0.06), tM ($\beta_{adjusted}$ = 0.04, 95% CI: 0.02–0.06), tSB ($\beta_{adjusted}$ = 0.03, 95% CI: 0.02–0.05), tB ($\beta_{adjusted}$ = 0.03, 95% CI: 0.01–0.05), and tEB ($\beta_{adjusted}$ = 0.03, 95% CI: 0.01–0.04) (Fig. 1B). For the female positive group, a significant difference was observed only in t3 compared with the both negative group. No discernible differences were observed for the male positive group in both crude and adjusted analyses.

A total of 190 patients underwent fresh embryo transfer and were included in the pregnancy outcome analysis (Table 4). There were no significant differences in the developmental stage of embryos, number of embryos transferred, and endometrial thickness on trigger day between the four groups. The live birth rate *per* cycle was 64.4%, 57.1% (aOR = 0.91, 95% CI: 0.42–1.98), 43.8% (aOR = 0.42, 95% CI: 0.13–1.32), and 66.7% (aOR = 1.59, 95% CI: 0.53–4.76) for the both negative, female positive, male positive, and both positive groups, respectively (Fig. 1B). Likewise, the biochemical pregnancy rate, embryo implantation rate, clinical pregnancy rate, early

miscarriage rate and ongoing pregnancy rate were all comparable among the four groups.

The outcomes of 108 singleton livebirths are further summarized in Table S1. No significant differences were observed in the mode of delivery, newborn gender, gestational age, birthweight and its Z-score. The four groups were also comparable in the proportions of preterm birth, low birthweight, macrosomia, small-for-gestational age, large-for-gestational age as well as birth defect.

## Discussion

We described the impact of SARS-CoV-2 infection during COS on IVF treatment outcomes with the use of time-lapse monitoring. The results of our study demonstrated that SARS-CoV-2 infection in both partners adversely affected morphokinetic parameters of embryo development with decreased 2PN cleavage rate and blastocyst formation rate, but did not influence clinical outcomes after fresh embryo transfer. Moreover, SARS-CoV-2 infection in only males or females had no adverse impact on either laboratory or pregnancy outcomes.

Prior to our study, Boudry et al. [12] found that the fertilization rate and the rate of excellent and good quality embryos were within normal limits in a case series of 16 asymptomatic or mildly infected women. Controversially, another study reported that SARS-CoV-2 infection before oocyte retrieval could lead to a diminished oocyte utilization rate, defined as the number of viable embryos divided by the number of oocytes retrieved [13]. After stratification by sex, the male positive group showed a descending trend in D3 good quality embryo rate, while the female positive group demonstrated an unexpectedly higher number of 2PN zygotes [13]. Moreover, a recent study showed a significant decrease in top-quality embryo rate, blastocyst formation rate, viable blastocyst rate, and top-quality blastocyst rate in SARS-CoV-2 infected couples during COS compared with the uninfected group [14]. Impaired oocyte- and embryo-related outcomes were similarly observed in the female positive group and the male positive group [14]. In the present study, we found a significantly lower 2PN cleavage rate and blastocyst formation rate in the both positive group, which corresponded well with the longer time of t5 to t8, tSC, tM, tSB, tB, and tEB observed in time-lapse imaging. Nonetheless, there were no significant differences in the female positive or male positive group, indicating that SARS-CoV-2 infection in both partners may have a jointly negative effect on embryo development.

Using the time-lapse monitoring system, a retrospective cohort by Braga et al. [17] has assessed the impact of previous SARS-CoV-2 infection on embryo morphokinetics in subsequent IVF cycles. The study found that the times to pronuclei appearance and fading, time to form 2 to 5 cells, time to blastulation, and time to complete t2-tPNf and t4-t3 synchronous divisions were significantly increased in embryos derived from infected patients. However, the results may be biased due to the lack of knowledge about male infection. In our prospective cohort, the kinetic parameters were more obviously influenced after the second cleavage (4-cell stage) and during the blastocyst stage in the both positive group. This finding suggested that SARS-CoV-2 infection during IVF may affect embryo morphokinetics in a different manner than treatments started after infection.

While more studies are needed for elucidation, most findings to date have suggested a detrimental effect of COVID-19 on human embryo development. However, the exact mechanisms still remain unclear. Based on published single cell RNA sequencing datasets, Weatherbee et al. [18] showed expression of ACE2 and TMPRSS2 in the trophectoderm of the blastocyst as well as syncytiotrophoblast and hypoblast of the implantation stages, which develop into tissues that interact with the maternal blood supply for nutrient exchange. While the presence of SARS-CoV-2 RNA has not been reported in embryos, an in vitro study by Montano et al. [19] demonstrated that both trophectoderm and inner cell mass of human blastocysts could be infected by live SARS-CoV-2. Infected embryos also displayed various degrees of cytopathic effects, ranging from focal cell degradation to total collapse and death. In addition to direct viral invasion, COVID-19 could also induce aberrant oxidative stress and systemic inflammatory response [4], thus affecting early embryo development indirectly [20].

Several studies have investigated the pregnancy outcomes of recovered patients, and found no adverse effects in fresh embryo transfer cycles [21–25]. Nonetheless, no evidence was available for those infected during IVF treatment. A transcriptomic study found that in endometrial tissue of infected women, 235 genes were differentially expressed and functionally enriched in regulating cytokine inflammation and immune responses to viruses [10], which may lead to compromised endometrial receptivity for embryo implantation. Considering the potential negative effects, the experts group in China recommended cancellation of fresh embryo transfer when patients were infected during COS [26]. In this study, we found that SARS-CoV-2 infection had no negative effect on ongoing pregnancy rate if infected women presented no high fever. This finding may provide guidance for infertile couples who would like to conceive at the soonest.

There are a few limitations that need to be acknowledged. Firstly, the observational design of the study exposes it to potential selection bias and confounding risks. For example, the effect of viral loads was not analyzed, and we did not detect SARS-CoV-2 RNA and antibody in semen, follicular fluid or granulosa

cells. Secondly, SARS-CoV-2 Omicron variant was predominant during the study timeframe. Most participants were reinfected and had mild symptoms. In this regard, our finding should not be extrapolated directly to more severely infected populations, which may have worse morphokinetic outcomes. Finally, the sample size remains limited and only fresh transfer outcomes were followed up. Therefore, larger prospective cohorts are needed for the assessment of cumulative live birth outcomes.

## Conclusion

In conclusion, our prospective cohort study demonstrated that SARS-CoV-2 infection in both partners had an adverse influence on 2PN cleavage rate, blastocyst formation rate, and embryo morphokinetics. However, the pregnancy and neonatal outcomes after fresh embryo transfer were not significantly affected. The findings should be helpful to understand the effects of COVID-19 on human embryo development and provide an important basis for counseling with infected couples during IVF treatment. Further studies with larger sample size are warranted to prolong the follow-up period and validate our conclusion.

### Abbreviations

| | |
|---|---|
| ACE2 | Angiotensin-converting enzyme 2 (ACE2) |
| AFC | Antral follicle count |
| AMH | Anti-müllerian hormone |
| aOR | Adjusted odds ratio |
| BMI | Body mass index |
| CI | Confidence interval |
| COS | Controlled ovarian stimulation |
| COVID-19 | Coronavirus disease 2019 |
| FSH | Follicle-stimulating hormone |
| hCG | Human chorionic gonadotropin |
| HPI | Hours post-insemination |
| ICSI | Intracytoplasmic sperm injection |
| IVF | In vitro fertilization |
| RT-PCR | Reverse transcription-polymerase chain reaction |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus 2 |
| TMPRSS2 | Transmembrane protease serine 2 |
| tPNa | Time to pronuclear appearance |
| tPNf | Time to pronuclear fading |
| tSC | Time to start compaction |
| tM | Time to morulation |
| tSB | Time to start blastulation |
| tB | Time to blastocyst |
| tEB | Time to expanding blastocyst |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-025-01355-6 .

Supplementary Material 1

### Author contributions

Conception and design of the work: X.W.W. and J.L.H. Data acquisition: Y.X.L., H.Y.C., Z.H.H., Y.J.L., X.X.W., H.C. and Y.Z. Statistical analysis: L.Z.X., J.L.H. and H.S.W. Manuscript drafting: J.L.H. and Y.X.L. Manuscript revision: F.V.V.H. Project supervision: Y.Z. and X.W.W. All authors reviewed the results and approved the final version of the manuscript.

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### Data availability

The datasets generated during and/or analyzed during the current study are not publicly available but may be made available upon reasonable request to the corresponding author.

## Declarations

### Ethics approval and consent to participate

The study was approved by the Reproductive Medicine Ethics Committee of Jiangxi Maternal and Child Health Hospital (No. 2023-04), and conducted in accordance with the Declaration of Helsinki. All patients signed informed contents for participation.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

### Author details

[1]Center for Reproductive Medicine, Jiangxi Key Laboratory of Reproductive Health, Jiangxi Branch of National Clinical Research Center for Obstetrics and Gynecology, Jiangxi Maternal and Child Health Hospital, Nanchang Medical College, 318 Bayi Avenue, Donghu District, Nanchang 330006, China
[2]Department of Clinical Medicine, School of Queen Mary, Nanchang University, Nanchang, China
[3]School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China
[4]Department of Obstetrics and Gynecology and Gynecology Research Unit, Hannover Medical School, Carl- Neuberg-Strasse 1, D-30625 Hannover, Germany

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