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•LETTER TO THE EDITOR•

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The effect of SARS-CoV-2 infection on human embryo early development: a multicenter prospective cohort study

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Dear Editor,

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has swept the globe for 3 years (Zhou et al., 2020). With the nationwide relaxation of controls on the coronavirus disease 2019 (COVID-19) epidemic since December 2022 in China, fertility and *in vitro* fertilization (IVF) centers are receiving increasing numbers of infected patients. However, there is still a lack of high-quality evidence on the effects of the virus on human oocytes and early-stage embryos (Ata et al., 2022). Given the co-expression of SARS-CoV-2-associated angiotensin-converting enzyme 2 (ACE2) and the cellular transmembrane serine protease 2 (TMPRSS2) in gametes and fertilized eggs as well as in blastocyst trophectoderm cells, it is reasonable to suspect that infection may affect oocyte and early-stage embryo quality (Rajput et al., 2021). Thus, at some centers, the decision has been made to cancel cycles or simply freeze oocytes for infected patients due to safety concerns (Boudry et al., 2022; Esposito et al., 2020). Herein we established a prospective cohort study to assess the impact of COVID-19 on oocyte quality and embryo development.

Valid data from a total of 906 couples were obtained from three reproductive centers in Shandong province and Shanghai municipality from December 1, 2022, to January 11, 2023. Women over the age of 42 or those who had chosen thaw-frozen oocytes or donated oocytes were excluded from the study. All enrolled patients completed a COVID-19 infection questionnaire. COVID-19 infection was diagnosed by nucleic acid test or antigen test of SARS-CoV-2. Couples were divided into COVID-19 and non-COVID-19 groups depending on whether one member of the couples had been infected with SARS-CoV-2 before oocyte retrieval. Based on the time interval from the infection of women to oocyte retrieval, the COVID-19 group was further subdivided into ≤7 days group, 7–14 days group and >14 days group. The

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specific flow chart was shown in Figure S1 in Supporting Information. Important laboratory indicators were assessed to evaluate the quality of oocytes and embryos. All statistical analyses were performed using SPSS 26.0.

Among the 906 couples, 206 self-reported SARS-CoV-2 infection before oocyte retrieval, including 93 women in the ≤7 days group, 38 in the 7–14 days group, and 52 in the >14 days group, with the rest of 700 unaffected. Baseline characteristics were similar between the two groups, with the exceptions of body mass index (BMI), vaccination status and ovarian stimulation protocols (Table 1). The median age of women in the COVID-19 group was 33.0 (IQR 30.8–36.0) years, compared with 33.0 (IQR 30.0–36.0) years in the non-COVID-19 group. More women had applied an agonist protocol for ovarian stimulation in the COVID-19 group (45.6% *vs*. 34.7%). In addition, COVID-19 group had a higher vaccination rate than non-COVID-19 group (73.8% *vs*. 60.4% in female; 81.6% *vs*. 68.4% in male). There were no significant differences in oocyte-related outcomes, including the number of oocytes retrieved [9.0 (IQR 5.8–15.0) *vs*. 8.5 (IQR 4.0–14.0)], oocyte maturation rate [90.0% (IQR 78.3%–100.0%) *vs*. 87.5% (IQR 75.0%–100.0%)], and normal fertilization rate [75.0% (IQR 53.3%–85.7%) *vs*. 70.0% (IQR 50.0%–86.2%)], between the COVID-19 and non-COVID-19 groups, except the number of bipronuclear (2PN) zygotes [6.0 (IQR 3.0–10.0) *vs*. 5.0 (IQR 2.0–8.0], P=0.021) (Table 1). Embryo development outcomes also did not differ between the two groups regarding the number of good-quality embryos on day 3 [3.0 (IQR 2.0–6.0) *vs*. 3.0 (IQR 1.0–6.0)], rate of good-quality embryos on day 3 [66.7% (IQR 42.9%–85.7%) *vs*. 69.2% (IQR 50.0%–100.0%)], and oocyte utilization rate [40.0% (IQR 25.0%–53.6%) *vs*. 35.0% (IQR 20.0%–53.8%)]. To eliminate possible confounding effects, we performed adjustments for BMI, vaccination rate and ovarian stimulation protocols in linear regression model 1, and for these variables plus female age and anti-Müllerian hormone (AMH) in model 2. The adjusted results still suggested that infection status almost had no influence on oocyte and embryo outcomes (Table S1 in Supporting Information).

Subgroup analysis showed ≤7 days group, >14 days group and non-COVID-19 group did not differ in oocyte and embryo laboratory outcomes with each other. However, for women who were infected 7–14 days before oocyte retrieval, more 2PN zygotes (8.5 (IQR 4.0–11.0) *vs*. 5.0 (IQR 2.0–8.0)) were obtained, along with a higher oocyte utilization rate (45.3% (IQR 34.6%–60.0%) *vs*. 35.0% (IQR 20.0%–53.8%)), than the non-COVID-19 group (Table 1). Subsequent multivariable linear regression analysis (model 3) further supplemented the results of this subgroup analysis (Table S1 in Supporting Information). Similarly, no differences in embryo outcomes between the ≤7 days group and the non-COVID-19 group were identified in regression model 3 except oocyte utilization rate [β1: −6.343 (95%CI: −12.617, −0.070), P=0.047]. In addition, after adjusting confounding effects, the occurrence of SARS-CoV-2 infection 7–14 days before oocyte retrieval was found to increase the number of oocytes obtained by 2.267 (95%CI: 0.507, 4.027) (P=0.012, in model 3), the number of 2PN zygotes by 1.646 (95%CI: 0.351, 2.941) (P=0.013, in model 3), and the number of good-quality embryos by 1.298 (95%CI: 0.222, 2.375) (P=0.018, in model 3). Analogous regression results to the 7–14 days group were also found in the >14 days group. But there was no statistical significance in the number of oocytes retrieved in >14 days group compared to non-COVID-19 group [1.535 (95%CI: −0.043, 3.113), P=0.057, model 3] (Table S1 in Supporting Information).

The separate effects of male or female infection on embryo quality were further analyzed. When comparing 23 infected men whose wives had not been infected with the non-COVID-19 group, baseline characteristics and laboratory outcomes were similar. Notably, although there was no statistical difference in embryo outcomes, male-only-infection group showed a descending trend in rate of good-quality embryos (60.0% (IQR 27.3%–75.0%) *vs*. 69.2% (IQR 50.0%–100.0%), P=0.112) compared with the non-COVID-19 group. In 41 couples, only the wives had been infected before oocyte retrieval, the number of 2PN was more than that in the non-COVID-19 group (mean 8.0, median 5.0 (IQR 4.0–14.0) *vs*. mean 6.0, median 5.0 (IQR 2.0–8.0), P=0.034) (Table S2 in Supporting Information).

To the best of our knowledge, this is the largest prospective study to investigate the associations between SARS-CoV-2 infection and early-stage embryo quality in assisted reproductive technology (ART). Our research revealed that infection before oocyte retrieval did not have clear negative effects on oocyte and embryo outcomes, including the number of oocytes retrieved, oocyte maturation rate, normal fertilization rate, or rate of good-quality embryos. A study by Youngster et al. (2022) also showed similar results with our conclusion that COVID-19 does not appear to be a risk factor in human early development. But linear regression results in this study indicated that infection within 7 days before oocyte retrieval diminished oocyte utilization rate. And other outcome indicators also showed a descending trend in the ≤7 days group. This seemed to suggest that infection within 7 days may still have a negative impact on outcomes. We were also surprised to find that those women with a time interval of more than 7 days appeared to have better outcomes. Given the limitations of the sample size, this finding still warrants further discussion and mechanism research. From the perspective of male infection, a retrospective study reported that IVF outcomes were not related to male COVID-19 infection (Wang et al., 2022). In our study, a reduction in good-quality embryos rate in men only infected population was identified despite there was no statistical significance. Due to small sample size

Table 1 Baseline characteristics and embryo outcomes in COVID-19 and non-COVID-19 group[a)]

Characteristics	COVID-19 versus non-COVID-19			Subgroup analysis				
	COVID-19 (*n*=206)	Non-COVID-19 (*n*=700)	*P* value	≤7 days (*n*=93)	7–14 days (*n*=38)	>14 days (*n*=52)	Non-COVID-19 (*n*=700)	*P* value
Female age (year)	33.0 (30.75–36.0)	33.0 (30.0–36.0)	0.820	34.0 (31.0–36.0)	32.0 (30.0–34.3)	33.0 (31.0–36.0)	33.0 (30.0–36.0)	0.465
BMI, (kg m^{-2})	22.5 (20.6–25.1)	21.8 (20.0–24.2)	**0.019***	22.3 (20.4–24.8)	23.0 (21.0–25.9)	22.2 (21.1–25.0)	21.8 (20.0–24.2)	0.105
AMH, (ng mL^{-1})	2.5 (1.4–4.3)	2.8 (1.6–4.6)	0.336	2.5 (1.4–4.5)	3.0 (1.8–4.4)	2.5 (1.2–4.8)	2.8 (1.6–4.6)	0.886
Causes of infertility, *n* (%)			0.381					0.648
Tubal factors	100 (49.5%)	333 (48.8%)		44 (49.4%)	22 (57.9%)	26 (50.0%)	333 (48.8%)	
Male factor	29 (14.4%)	125 (18.3%)		12 (13.5%)	6 (15.8%)	6 (11.5%)	125 (18.3%)	
Others	73 (36.1%)	224 (32.8%)		33 (37.1%)	10 (26.3%)	20 (38.5%)	224 (32.8%)	
Female vaccination status								
Vaccination rate, *n* (%)	152 (73.8%)	423 (60.4%)	**<0.001***	69 (74.2%) [a]	24 (63.2%)[ab]	43 (82.7%) [ab]	423 (60.4%)[b]	**0.001***
TI, (d)	380.0 (314.0–495.4)	400.5 (297.0–509.3)	0.902	384.0 (322.0–489.0)	381.0 (311.5–497.8)	416.0 (303.0–524.0)	400.5 (297.0–509.3)	0.881
Male vaccination status								
Vaccination rate, *n* (%)	168 (81.6%)	479 (68.4%)	**<0.001***	70 (75.3%) [a]	34 (89.5%)[ab]	46 (88.5%) [b]	479 (68.4%) [b]	**0.001***
TI, (d)	378.0 (309.0–502.0)	410.0 (319.0–523.0)	0.230	382.5 (321.0–502.0)	378.0 (319.0–499.0)	358.5 (293.0–522.3)	410.0 (319.0–523.0)	0.542
Ovarian stimulation protocols, *n* (%)			**0.017***					**0.020***
Agonist protocol	93 (45.6%)	233 (34.7%)		39 (42.9%)	24 (63.2%)	21 (40.4%)	233 (34.7%)	
Antagonist protocol	85 (41.7%)	326 (48.5%)		37 (40.7%)	12 (31.6%)	23 (44.2%)	326 (48.5%)	
Others	26 (12.7%)	113 (16.8%)		15 (16.5%)	2 (5.3%)	8 (15.4%)	113 (16.8%)	
Fertilization type, *n* (%)			0.696					0.306
IVF	78 (44.1%)	309 (45.7%)		32 (44.4%)	21 (56.8%)	19 (36.5%)	309 (45.7%)	
ICSI	99 (55.9%)	367 (54.3%)		40 (55.6%)	16 (43.2%)	33 (63.5%)	367 (54.3%)	
No. of oocytes retrieved	9.0 (5.8–15.0)	8.5 (4.0–14.0)	0.157	8.0 (5.0–13.0)	12.0 (5.8–18.0)	10.0 (6.0–15.0)	8.5 (4.0–14.0)	0.057
Oocyte maturation rate (ICSI only), (%)	90.0 (78.3–100.0)	87.5 (75.0–100.0)	0.555	87.5 (71.4–100.0)	93.3 (84.4–100.0)	87.0 (71.4–100.0)	87.5 (75.0–100.0)	0.784
No. of 2PN zygotes	6.0 (3.0–10.0)	5.0 (2.0–8.0)	**0.021***	5.0 (3.0–7.25)[ab]	8.5 (4.0–11.0)[a]	7.0 (3.8–10.0)[ab]	5.0 (2.0–8.0)[b]	**0.004***
Normal fertilization rate, (%)	75.0 (53.3–85.7)	70.0 (50.0–86.2)	0.610	71.8 (50.0–85.7)	70.7 (58.4–83.3)	75.0 (62.7–86.9)	70.0 (50.0–86.2)	0.439
No. of good-quality embryos (D3)	3.0 (2.0–6.0)	3.0 (1.0–6.0)	0.188	3.0 (1.0–5.0)[a]	5.0 (2.0–8.75)[a]	4.0 (2.0–8.0)[a]	3.0 (1.0–6.0)[a]	**0.014***
Rate of good-quality embryos (D3), (%)	66.7 (42.9–85.7)	69.2 (50.0–100.0)	0.216	60.0 (33.3–80.0)	78.9 (47.5–100.0)	71.4 (47.2–88.2)	69.2 (50.0–100.0)	0.273
Oocyte utilization rate, (%)	40.0 (25.0–53.6)	35.0 (20.0–53.8)	0.378	33.3 (16.7–48.4)[a]	45.3 (34.6–60.0)[b]	47.2 (30.4–60.5)[ab]	35.0 (20.0–53.8)[a]	**0.006***

a) BMI, Body mass index; AMH, anti-Müllerian hormone; TI, time interval between last dose of vaccination and the day of female oocyte retrieval; ICSI, intracytoplasmic sperm injection; MII, metaphase II; D3, Day3 after fertilization. Oocyte utilization rate=(embryos transferred+embryos frozen)/oocyte retrieved. Values are presented as median (IQR) or *n* (%); *, $P<0.05$; a, b, same subscript letter denotes proportions or values do not differ significantly from each other at the 0.05 level.

of these studies, the role of male infection in ART still needs to be further explored. The available evidence suggests that viral RNA is not present in follicular fluid and oocytes (Boudry et al., 2022), and our results tentatively revealed that couples with COVID-19 seemed to have comparable oocyte quality and embryo development to those without COVID-19. However, caution should be taken when attempting to draw any definitive conclusions about the impact of viruses in ART, since several infection-related factors may affect final reproductive outcomes, including endocrine levels, endometrial receptivity, and anxiety during and after IVF treatment (Asghar et al., 2021; Henarejos-Castillo et al., 2020; Kothandaraman et al., 2021). Owing to the limitation of this study having a short follow-up, the effects of COVID-19 on pregnancy and live birth are not clear yet. We will further trace long-term pregnancy outcomes as well as the health of offspring in this prospective cohort study. Overall, our study suggests that COVID-19 does not have a clear negative effect

on oocyte quality or embryo development, while assuaging current clinical concerns.

Compliance and ethics *This study was approved by the Institutional Review Ethics Board of Center for Reproductive Medicine, Shandong University (IRB 2022/Ethical Review #134). The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

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