# Undetectable viral RNA in follicular fluid, cumulus cells, and endometrial tissue samples in SARS-CoV-2–positive women

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**Objective:** To study the presence of viral RNA in the follicular fluid, cumulus cells, and endometrial tissue samples in SARS-CoV-2–positive women undergoing assisted reproductive technology (ART).
**Design:** Prospective, single-center, observational study.
**Setting:** Tertiary hospital.
**Patient(s):** A total of 16 patients undergoing transvaginal oocyte retrieval who had a positive SARS-CoV-2 RNA test <48 hours before the procedure. All patients underwent the retrieval between September 2020 and June 2021 and used in vitro fertilization or intracytoplasmic sperm injection. All embryos were vitrified to avoid conception during SARS-CoV-2 infection.
**Intervention(s):** Follicular fluid aspirated during oocyte retrieval, cumulus cells, and endometrial samples were analyzed for SARS-CoV-2 RNA using the RealStar SARS-CoV-2 RT-PCR-Kit1.0.
**Main Outcome Measure(s):** The primary outcome parameter was the detection of viral RNA in the follicular fluid, cumulus cells, and endometrial cells. Fertilization rate, embryo developmental potential, and clinical outcome after frozen embryo transfer were secondary outcome parameters.
**Result(s):** Samples from 16 patients were analyzed. Cycle threshold values of <40 were considered positive. All samples were negative for SARS-CoV-2 viral RNA. No inflammatory lesions of the endometrium were identified histologically. Fertilization rate, embryo development, and clinical outcomes after embryo transfer were reassuring.
**Conclusion(s):** In women infected with SARS-CoV-2 who underwent ART, viral RNA was undetectable in the follicular fluid, cumulus cells, and endometrium. Caution is warranted in view of the small sample size, and the risk of SARS-CoV-2 affecting the embryo via ART cannot be ruled out. Adequate counseling of women and couples undergoing ART is crucial in parallel with further research on the effect of exposure of the early human embryo to SARS-CoV-2.
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**El resumen está disponible en Español al final del artículo.**

**Key Words:** SARS-CoV-2, follicular fluid, cumulus cells, endometrium, human embryo



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The outbreak of the respiratory disease COVID-19, caused by the novel SARS-CoV-2, has resulted in millions of infected patients and deaths worldwide since the end of 2019 (1, 2). COVID-19 can be complicated by pneumonia, respiratory failure, systemic inflammation, and coagulopathy and has a steep age risk gradient of severe morbidity and mortality (1, 3). Because of alarming levels of its spread and severity, social and traveling restrictions were implemented in most countries, and several national authorities recommended the cessation of fertility treatments to prevent overburdening health care systems.

As in many countries, in Belgium too, assisted reproductive technology (ART) cycles were abruptly discontinued, except for oncofertility patients. As the number of infections and hospitalizations declined, ART cycles were relaunched, and fertility clinics took precautionary measures to ensure patient care on the basis of safety guidelines provided by national and international societies, while mitigating the risk of SARS-CoV-2 passage in the in vitro fertilization (IVF) laboratory (4). Recommendations were updated continuously as evidence regarding patient and staff safety accumulated.

Real-time polymerase chain reaction (RT-PCR) testing on a nasopharyngeal swab to diagnose SARS-CoV-2 infection has been a real game changer in this pandemic and was instrumental in restarting routine fertility care (3). Our center adopted a policy of cycle cancelation when women had a positive RT-PCR test during ovarian stimulation. In case of a positive RT-PCR test <48 hours before oocyte retrieval in an asymptomatic patient, couples were given the option to proceed with the oocyte retrieval as planned or to cancel the cycle. In view of the paucity of data regarding possible periimplantation transmission of SARS-CoV-2 to the conceptus, we adhered to a strict freeze-all approach to avoid conception in SARS-CoV-2–positive patients (5–7). Moreover, published data illustrate an increased risk of pregnancy loss and congenital abnormalities in women who had hyperthermia during pregnancy (8, 9).

The primary aim of this study was to identify SARS-CoV-2 RNA in the follicular fluid, cumulus cells, and endometrial samples in women who had tested positive for SARS-CoV-2 RNA <48 hours before oocyte retrieval. The secondary outcomes included the fertilization rate, embryo developmental potential, and clinical outcome after frozen embryo transfer (FET).

## MATERIALS AND METHODS

### Study Design

This was a single-center, prospective cohort study of consecutive cases of SARS-CoV-2–infected women with a positive RT-PCR test after nasopharyngeal swab screening <48 hours before oocyte retrieval. All women who were scheduled for ovarian stimulation for ART between September 1, 2020, and June 1, 2021, were asked to complete a COVID-19 triage questionnaire before the start of ovarian stimulation. All patients from the SARS-CoV-2 endemic geographical areas that had an increased risk of infection based on the questionnaire underwent nasopharyngeal swab screening. The SARS-CoV-2–positive patients were not allowed to start ovarian stimulation. All patients who had symptoms suggesting SARS-CoV-2 infection during ovarian stimulation or who were at high risk of SARS-CoV-2 infection after direct contact with infected people also underwent nasopharyngeal swab screening. Ovarian stimulation was discontinued when the test result was positive. During the months corresponding to the peak of the COVID-19 pandemic, routine nasopharyngeal RT-PCR testing of all patients, including asymptomatic ones who were scheduled for an ART cycle, was performed <48 hours before oocyte retrieval; in case of a positive RT-PCR test on the day of ovulation triggering or on the following day, the patient or couple was counseled about the unknown effect of viral infection on the outcome of ART treatment and the unknown risk of vertical transmission. If the patient or couple decided not to cancel the oocyte retrieval but to proceed, the procedure was performed as planned, considering all necessary protective safety measures according to the best practice guidelines, and a freeze-all approach was adopted.

Asymptomatic patients or patients with very mild symptoms who had tested positive for SARS-CoV-2 <48 hours before oocyte retrieval were asked to participate in this study if they decided not to cancel the ART cycle. Ethics approval was obtained from the institutional review board at our hospital (IRB No. B1432020000145), and the study was registered in clinicaltrials.gov (identifier: NCT04425317). The patients had a signed informed consent on the day of oocyte retrieval. Between September 1, 2020, and June 1, 2021, a total of 3,554 patients underwent oocyte retrieval in our center, of whom 0.53% (19/3,554) tested positive for SARS-CoV-2 <48 hours before oocyte retrieval. Of those, 1 patient was an anonymous oocyte donor and, therefore, was not considered eligible for inclusion and another had a negative RT-PCR confirmation test. A third patient refused to participate in the trial. Ultimately, 16 patients were considered for the final analysis.

The study participants underwent ovarian stimulation for ART either in a fixed gonadotropin-releasing hormone antagonist or long gonadotropin-releasing hormone agonist protocol or in a modified natural cycle. Final oocyte maturation was induced with 5,000 IU of human chorionic gonadotropin (Pregnyl; Organon, Oss, The Netherlands) or with 0.2 mg of triptorelin acetate (Gonapeptyl, Ferring). Oocyte retrieval was performed under vaginal ultrasound–guided aspiration 34–36 hours after the above trigger. All procedures were performed in a dedicated operating room, considering all necessary protective safety measures to avoid infection of staff.

When IVF or intracytoplasmic sperm injection (ICSI) was performed with autologous sperm, a SARS-CoV-2 RT-PCR test was performed in the partner, using a nasopharyngeal swab at our hospital or at a distant COVID-19 test site.

### Follicular Fluid

After each oocyte retrieval procedure, all tubes containing follicular fluid and cumulus-oocyte complexes (COCs) were transported to our specific laboratory room designed to accommodate IVF and ICSI procedures of patients with a viral infection, using a secured shipping container at 37°C. After

COC isolation, follicular fluid from the first punctured follicle from each ovary was discarded to minimize the risk of contamination with vaginal mucus and cells, unless only 1 follicle had been punctured. The remaining follicular fluid was pooled and, a sample of 500 μL was sent to the microbiology laboratory for SARS-CoV-2 RNA testing using RT-PCR test.

## Cumulus Cells

All COCs were collected and rinsed in Quinn's Advantage Medium with Hepes (Sage In-Vitro Fertilization, Inc.; CooperSurgical) and transferred to 6% $CO_2$/5% $O_2$ at 37°C in Origio Sequential Fert medium (CooperSurgical) until oocytes were denuded in group in a 100 μL *ICSI*Cumulase drop (CooperSurgical) covered with Ovoil (Vitrolife, Sweden) before their insemination with partner or donor sperm using ICSI. After oocyte denudation, cumulus cells were transferred to RNAse-free Eppendorf tubes, snap-frozen in liquid nitrogen, and stored at −80°C for SARS-CoV-2 RT-PCR testing at a later stage. In case of insemination by IVF, COCs were denuded the day after insemination, but no cumulus cells were collected for the analysis.

## Embryo Culture and Vitrification

The oocytes were fertilized by ICSI using ejaculated sperm (except 1 cycle where IVF was performed), and the resulting embryos were cultured in individual 25 μL culture media droplets covered with oil until day 3 (Origio Sequential Cleav; CooperSurgical) or day 5 (Origio Sequential Blast; CooperSurgical) after fertilization. All embryos with excellent or good quality were vitrified (10). Vitrification was performed using closed CBS-VIT high-security straws (CryoBioSystem, L'Aigle, France) in combination with dimethyl sulfoxide, ethylene glycol, and sucrose as cryoprotectants (Irvine Scientific Freeze kit; Irvine Scientific, Newtown Mount Kennedy, Ireland), according to the method described previously by Van Landuyt et al. (11). In cycles with preimplantation genetic testing, a trophectoderm biopsy was performed before cryopreservation.

## Endometrial Samples

On the day of oocyte retrieval, all patients underwent endometrial biopsy using a Pipelle de Cornier. Each sample specimen was portioned equally for histopathology and virology testing: 1 portion was immersed in RNAlater stabilization reagent (Qiagen) for RNA extraction followed by RT-PCR test, whereas the other portion was immersed in formalin for histopathology. The specimens for histopathology were cut into 5-μm thin sections and mounted on precoated slides after formalin fixation and paraffin embedding. Hematoxylin-eosin staining was performed using a Tissue-Tek Prisma Plus Automated Slide Stainer. The slides were scanned using a 3D Histech slide scanner (3DHISTECH Ltd., Budapest, Hungary) and evaluated using the Pathomation pma.studio digital microscopy software (Sakura Finetek Europe B.V., Alphen aan den Rijn, The Netherlands; Pathomation, Berchem, Belgium).

## Molecular Detection of SARS-CoV-2 in the Follicular Fluid, Cumulus Cells, and Endometrial Samples

Nasopharyngeal swab samples for SARS-CoV-2 were analyzed at our hospital (n = 11) or at a distant COVID-19 test site (n = 5, protocol not available). RNA extraction from 11 nasopharyngeal swabs, 16 follicular fluid aspirates, and 15 cumulus cell samples was performed using the NucliSens easyMAG system (BioMérieux, Marcy l'Etoile, France), according to the manufacturers' instructions. To validate the RNA extraction method, 2 samples of cumulus cells were portioned for RNA extraction using 1 of 2 additional methods. In brief, RNA extraction from 1 sample portion was performed using PicoPure RNA isolation kit (kit0204; Arcturus), whereas RNA extraction from a further sample portion was performed using Trizol; 750 μL of Trizol (15596026; Thermo Fisher), and 200 μL of chloroform (1024451000; Merck) were added to the sample. The extract obtained was clarified by centrifugation for 15 minutes at 4°C at 12,000 rpm. The aqueous phase was kept, and 450 μL of isopropanol (59304; Sigma) and 1 μL of glycogen (10901393001; Sigma) were added. The sample was stored for 1 hour at 4°C and subsequently centrifuged as described above. The pellet was analyzed using RT-PCR.

In addition, 14 endometrial samples (sample sizes from 2 patients were too small for analysis) were treated with Trizol as described above. In analogy with the analysis of cumulus cell samples, 1 endometrial sample was portioned for RNA extraction using RNAeasy Minikit (74104; Qiagen), according to the manufacturers' instructions. Molecular detection using RT-PCR test was performed using the RealStar SARS-CoV-2 RT-PCR Kit 1.0 for qualitative detection and differentiation of lineage B betacoronavirus and SARS-CoV-2–specific RNA (Altona Diagnostics, Hamburg, Germany). All probes were labeled differently. The probe specific for lineage B betacoronavirus (target E gene) RNA was labeled with the fluorophore carboxyfluorescein (FAM, Altona Diagnostics, Hamburg, Germany), whereas that specific for SARS-CoV-2 (target S gene) RNA was labeled with the fluorophore Cy5. The probe specific for internal control was labeled with the fluorophore 5'-dichloro-dimethoxy-fluorescein (JOE, Altona Diagnostics, Hamburg, Germany). The RT-PCR kit catalyzes reverse transcription of the target and internal control RNA to cDNA; results in PCR amplification of the target and internal control cDNA; and allows simultaneous detection of PCR amplicons.

The PCR cycle threshold ($C_t$) value is an indicator of the number of viral copies, with lower $C_t$ values corresponding to a higher concentration of viral genetic material, although $C_t$ values are not a standardized measure for the quantification of viral load. A $C_t$ value of <40 was considered a SARS-CoV-2 RNA–positive result (12).

## Embryological Data and Clinical Outcome After FET

The fertilization rate was calculated as the number of fertilized oocytes per number of metaphase II oocytes in ICSI cases

and per number of COC in 1 case where IVF was used for insemination. The embryos were scored as described by De Munck et al. (13). On day 3, high-quality cleavage stage embryos (grade 1 embryo quality [EQ]) had at least 7 blastomeres of equal or marginally different sizes and $\leq$10% fragmentation; good-quality embryos (grade 2, EQ2) had at least 6 blastomeres and/or $\leq$20% fragmentation and/or blastomeres of marginally different sizes. On day 5, the top-quality blastocysts (grade 1, EQ1) were full or expanded with trophectoderm type A and inner cell mass types A or B; early or expanded blastocysts with a trophectoderm type B and inner cell mass types A or B were considered blastocysts of good quality (grade 2, EQ2).

All FET procedures were performed in a natural or artificial cycle. Cycle monitoring, endometrial preparation, and vitrified-warmed embryo transfer were conducted as described previously (14, 15). Clinical pregnancy was defined by the presence of an intrauterine gestational sac, as visualized by transvaginal ultrasound examination (16). Ongoing pregnancy was defined as a positive heartbeat at or beyond 12 weeks of gestation.

## RESULTS

### Primary Outcome

**Viral RNA in follicular fluid, cumulus cells and endometrial samples.** From September 2020 to June 2021, 16 SARS-CoV-2–positive women were included in the study, with nasopharyngeal swab PCR cycle $C_t$ values between 20.2 (high viral load) and 38.5 (low viral load). Of those, 14 underwent IVF or ICSI with partner sperm and 2 with donor sperm. Nine of the 14 male partners tested positive for SARS-CoV-2. Patient characteristics are presented in Table 1. All follicular fluid aspirates and cumulus cell samples had undetectable levels of viral RNA after 40 amplification cycles with PCR, independent of the nasopharyngeal swab PCR $C_t$ value.

To assess the endometrial histology, hematoxylin and eosin staining was performed for the endometrial samples from 15 patients. The endometrial architecture and cell composition was normal in all samples. None of the samples showed any histopathologic changes. No acute or chronic inflammatory infiltrates were detected. Fourteen endometrial samples were analyzed using RT-PCR and showed no detectable viral RNA for SARS-CoV-2, irrespective of the extraction method used.

### Secondary Outcomes

**Fertilization rate, embryo developmental potential and clinical outcome.** Embryology data are presented in Table 2. The fertilization rate (82%; range 0%–100%) was within normal limits and was comparable with uninfected patients in our clinic, as was the rate of excellent and good embryo quality on day 3 after ICSI (68.2%; range 25%–100%) and on day 5 (41.8%; range 20.0%–71.5%). Embryo development after IVF (1 cycle only) also was normal.

In total, 34 embryos were vitrified in 12 cycles, 15 embryos on day 3 and 19 embryos on day 5 or day 6 (including 8 blastocysts after trophectoderm biopsy for preimplantation genetic testing). Four patients had no embryos available for vitrification because of insufficient embryo quality on day 5 and/or day 6 or, in 1 single case, because of absence of fertilization.

After recovery from COVID-19, 8 patients had a FET; 7 patients had 1 FET cycle. Five patients had single-embryo transfers, 1 had a double-embryo transfer, and 1 cycle was canceled because of poor embryo quality after warming. One patient had 4 FET cycles (all single-embryo transfers). From the 12 embryos warmed, 11 were transferred, and 6 embryos resulted in singleton pregnancies. Of those, 2 pregnancies resulted in spontaneous miscarriage, whereas 3 other patients delivered a healthy infant. One pregnancy is ongoing (Supplemental Table 1, available online).

**Comparison with the preceding or subsequent ART cycle.** In an attempt to evaluate the impact of SARS-CoV-2 infection on preimplantation embryo development, we present descriptive embryology data of the ART cycle during SARS-CoV-2 infection with those in the previous and/or subsequent cycle in the same patient (Tables 3 and 4). Ten patients had at least 1 previous IVF/ICSI cycle in our center, not more than 4 years apart and under similar culture conditions (Table 3). Nine patients started a new IVF/ICSI cycle within the next 4 months after SARS-CoV-2 infection (Table 4). Because not all patients had a previous or subsequent ART cycle, comparative analysis was not possible.

## DISCUSSION

To our knowledge, this is the first study investigating the presence or absence of SARS-CoV-2 RNA not only in follicular fluid but also in cumulus cells and endometrium in a cohort of SARS-CoV-2–positive patients. The viral RNA was undetectable in all samples of all patients. Moreover, our findings suggest normal preimplantation embryo development during SARS-CoV-2 infection and normal clinical outcome after FET.

Because this was the outbreak of a new virus, knowledge of the pathophysiology of SARS-CoV-2 and the potential impact on human gametes, preimplantation embryos, reproductive tissues, and early pregnancy was scarce at the start of the COVID-19 pandemic. Significant advances have been made in the understanding of the molecular machinery facilitating viral entry into host cells. The SARS-CoV-2 enters the host cells mainly through the binding of the spike protein to the angiotensin converting enzyme 2 (ACE2) receptor, and the virus interacts with the transmembrane serine protease 2 (TMPRSS2) on host cells for spike-protein priming (17, 18). The coexpression of ACE2 and TMPRSS2 proteins in human metaphase II oocytes, zygotes, and blastocysts marks a theoretical opportunity for invasion by SARS-CoV-2 (19, 20). A second ACE2-independent mechanism has been described recently, with CD147, also known as Basigin, with cellular receptor and cathepsin L as the protease (21, 22). The presence of CD147 protein also has been shown on human oocytes and preimplantation and peri-implantation embryos (19). Montano et al. (23) have studied the exposure of blastocysts to reporter virions pseudotyped with the SARS-CoV-2 spike protein and demonstrated that human

## TABLE 1

**Patient characteristics and sample analysis from the ART cycle during SARS-CoV-2 infection.**

| | Patient characteristics | | | | | | | PCR test | | | | Hematoxylin and eosin staining |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | Age (y) | BMI (kg/m$^2$) | Treatment protocol | Indication | Symptoms | Laboratory | Cycle threshold value (envelope gene; spike gene) | PCR partner | Follicular fluid | Cumulus cells | Endometrium | Endometrium |
| 1 | 38 | 22.8 | Antagonist | Premature ovarian insufficiency | Mild | External | Unknown | Negative | Negative | Negative | Negative | Normal |
| 2 | 37 | 30.3 | Antagonist | Endometriosis | Mild | External | 18; 17 | Positive | Negative | Negative | Negative | Normal |
| 3 | 38 | 20.1 | Agonist | Male factor | Mild | Internal | 25; 21 | Negative | Negative | Negative | Negative | Normal |
| 4 | 34 | 19.5 | Antagonist | Polycystic ovary syndrome | No | External | 26; 26 | Negative | Negative | Negative | Negative | Normal |
| 5 | 37 | 20.8 | Antagonist | Tubal factor | No | External | 21; 21 | Negative | Negative | Negative | NP | NP |
| 6 | 34 | 35.4 | Agonist | Tubal factor | No | Internal | 20; 19 | N/A[a] | Negative | Negative | Negative | Normal |
| 7 | 39 | 22.0 | Antagonist | Preimplantation genetic testing | No | Internal | 29; 28 | Positive | Negative | Negative | Negative | Normal |
| 8 | 40 | 28.7 | Antagonist | Tubal factor | Mild | Internal | 33; 33 | Positive | Negative | Negative | Negative | Normal |
| 9 | 35 | 19.9 | Agonist | Preimplantation genetic testing | No | Internal | 39; 37 | Negative | Negative | Negative | NP | Normal |
| 10 | 40 | 24.7 | Antagonist | Tubal factor | No | Internal | 23; 22 | Positive | Negative | Negative | Negative | Normal |
| 11 | 33 | 24.2 | Modified natural cycle | Premature ovarian insufficiency | No | Internal | 26; 25 | Positive | Negative | Negative | Negative | Normal |
| 12 | 37 | 40.6 | Antagonist | Male factor | No | Internal | 31; 30 | Positive | Negative | Negative | Negative | Normal |
| 13 | 31 | 23.6 | Antagonist | Idiopathic | Mild | Internal | 22; 21 | Positive | Negative | Negative | Negative | Normal |
| 14 | 28 | 27.4 | Antagonist | Nonobstructive azoospermia | No | External | 35; NP | N/A[a] | Negative | Negative | Negative | Normal |
| 15 | 38 | 23.9 | Agonist | Male factor | Mild | Internal | 21; 19 | Positive | Negative | Negative | Negative | Normal |
| 16 | 31 | 16.4 | Antagonist | Polycystic ovary syndrome | No | Internal | 29; 27 | Positive | Negative | N/A[b] | Negative | Normal |

*Note:* BMI = body mass index; N/A = not applicable; NP = not performed; PCR = polymerase chain reaction.
[a] Donor sperm.
[b] In vitro fertilization cycle.

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## TABLE 2

**Embryological outcome of the cycles during SARS-CoV-2 infection.**

| Patient | Age (y) | ART | No. of COC | No. of MII (% per COC) | No. of 2PN (% per MII) | No. of embryos of quality 1 and 2 on day 3 (% per 2PN) | No. of embryos of quality 1 and 2 on day 5 (% per 2PN) | No. of embryos frozen (% per 2PN) | Treatment outcome |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 38 | ICSI | 4 | 4 (100.0) | 4 (100.0) | 3 (75.0) | N/A | 3 (75.0) | Freeze all embryos day 3 |
| 2 | 37 | ICSI | 9 | 8 (88.9) | 5 (62.5) | 4 (80.0) | 1 (20.0) | 0 | Insufficient quality for freezing day 5/6 |
| 3 | 38 | ICSI | 6 | 4 (66.6) | 4 (100.0) | 1 (25.0) | N/A | 1 (25.0) | Freeze all embryos day 3 |
| 4 | 34 | ICSI | 5 | 4 (80.0) | 4 (100.0) | 1 (25.0) | N/A | 1 (25.0) | Freeze all embryos day 3 |
| 5 | 37 | ICSI | 5 | 4 (80.0) | 3 (75.0) | 2 (66.7) | N/A | 2 (66.7) | Freeze all embryos day 3 |
| 6 | 34 | ICSI | 8 | 8 (100.0) | 8 (100.0) | 6 (75.0) | 3 (37.5) | 4 (50.0) | Freeze all embryos day 5/6 |
| 7 | 39 | ICSI | 15 | 9 (60.0) | 7 (77.8) | 6 (85.7) | 5 (71.5) | 2 (28.6) | Freeze all embryos after trophectoderm biopsy |
| 8 | 40 | ICSI | 10 | 9 (90.0) | 7 (77.8) | 3 (42.8) | 0 | 0 | Insufficient quality for freezing day 5/6 |
| 9 | 35 | ICSI | 13 | 11 (84.6) | 10 (90.9) | 9 (90.0) | 5 (50.0) | 6 (60.0) | Freeze all embryos after trophectoderm biopsy |
| 10 | 40 | ICSI | 11 | 8 (72.7) | 8 (100.0) | 8 (100.0) | 5 (62.5) | 4 (50.0) | Freeze all embryos day 5/6 |
| 11 | 33 | ICSI | 1 | 1 (100.0) | 0 | N/A | N/A | N/A | No fertilization |
| 12 | 37 | ICSI | 10 | 10 (100.0) | 6 (60.0) | 6 (100.0) | 2 (33.3) | 5 (83.3) | Freeze all embryos day 3/5 |
| 13 | 31 | ICSI | 9 | 8 (88.9) | 6 (75.0) | 3 (50.0) | N/A | 3 (50.0) | Freeze all embryos day 3 |
| 14 | 28 | ICSI | 8 | 4 (50.0) | 3 (75.0) | 2 (66.7) | 2 (66.6) | 1 (33.3) | Freeze all embryos day 5 |
| 15 | 38 | ICSI | 9 | 7 (77.8) | 6 (85.7) | 2 (33.3) | N/A | 2 (33.3) | Freeze all embryos day 3 |
| 16 | 31 | IVF | 28 | N/A | 24 (85.7)[a] | 4 (16.7) | 2 (8.3) | 0 | Insufficient quality for freezing day 5/6 |

*Note:* ART = assisted reproductive technology; COC = cumulus-oocyte complex; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; MII = metaphase II oocytes; N/A = not applicable; 2PN = fertilized oocyte.

[a] Percentage of 2PN calculated per number of COC.

*Boudry. SARS-CoV-2 RNA in assisted reproduction. Fertil Steril 2022.*

## TABLE 3

**Overview of the embryological outcome from the cycles before SARS-CoV-2 infection from the same patient.**

| Patient | Age (y) | ART | Treatment protocol | No. of COC | No. of MII (% per COC) | No. of 2PN (% per MII) | No. of embryos of quality 1 and 2 on day 3 (% per 2PN) | No. of embryos of quality 1 and 2 on day 5 (% per 2PN) | Treatment outcome |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 38 | IVF/ICSI | Agonist | 9 | N/A | 7 (77.8)[a] | 5 (71.5) | 4 (57.1) | Embryo transfer day 5 |
| 4 | 32 | ICSI | Antagonist | 12 | 8 (66.6) | 6 (75.0) | 1 (16.7) | N/A | Embryo transfer day 3 |
| 6 | 31 | ICSI | Antagonist | 2 | 1 (50.0) | 1 (100.0) | 1 (100.0) | N/A | Freeze all embryos day 3 |
| 7 | 39 | ICSI | Antagonist | 18 | 10 (55.5) | 8 (80.0) | 7 (87.5) | 2 (25.0) | Freeze all embryos after trophectoderm biopsy |
| 8 | 39 | ICSI | Antagonist | 15 | 13 (86.7) | 7 (53.8) | 2 (28.6) | N/A | Freeze all embryos day 3 |
| 11 | 31 | ICSI | Modified natural cycle | 1 | 1 (100.0) | 1 (100.0) | 1 (100.0) | N/A | Embryo transfer day 3 |
| 12 | 36 | ICSI | Antagonist | 11 | 10 (90.9) | 7 (70.0) | 3 (42.8) | N/A | Freeze all embryos day 3 |
| 13 | 31 | ICSI | Antagonist | 6 | 6 (100.0) | 5 (83.3) | 5 (100.0) | 0 | Insufficient quality for freezing day 5/6 |
| 15 | 37 | IVF | Antagonist | 5 | N/A | 4 (80.0)[a] | 2 (50.0) | N/A | Freeze all embryos day 3 |
| 16 | 27 | IVF | Antagonist | 12 | N/A | 10 (83.3)[a] | 3 (30.0) | 1 (11.1)[b] | Embryo transfer day 3 and freezing day 5 |

*Note:* ART = assisted reproductive technology; COC = cumulus-oocyte complex; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; MII = metaphase II oocytes; N/A = not applicable; 2PN = fertilized oocyte.
[a] Percentage of 2PN calculated per number of COC.
[b] Calculated per number of 2PN minus 1embryo transferred on day 3.

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## TABLE 4

**Overview of the embryologic outcome from cycles after SARS-CoV-2 infection from the same patient.**

| Patient | Age (y) | Time since the previous cycle (d) | ART | Treatment protocol | No. of COC | No. of MII (% per COC) | No. of 2PN (% per MII) | No. of embryos of quality 1 and 2 on day 3 (% per 2PN) | No. of embryos of quality 1 and 2 on day 5 (% per 2PN) | Treatment outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 39 | 307 | ICSI | Antagonist | 5 | 5 (100.0) | 4 (80.0) | 3 (75.0) | 1 (33.3)[b] | Embryo transfer day 3; freezing day 5 |
| 2 | 37 | 84 | ICSI | Antagonist | 10 | 6 (60.0) | 5 (83.3) | 3 (60.0) | 1 (25.0)[b] | Embryo transfer day 3; freezing day 5/6 |
| 3 | 38 | 127 | ICSI | Modified natural cycle | 1 | 1 (100.0) | 1 (100.0) | 1 (100.0) | N/A | Embryo transfer day 3 |
| 3 | 39 | 203 | ICSI | Modified natural cycle | 4 | 2 (50.0) | 1 (50.0) | 1 (100.0) | N/A | Embryo transfer day 3 |
| 3 | 39 | 308 | ICSI | Modified natural cycle | 4 | 2 (50.0) | 2 (100.0) | 2 (100.0) | N/A | Embryo transfer day 3 |
| 4 | 35 | 136 | ICSI | Antagonist | 10 | 9 (90.0) | 4 (44.4) | 0 | N/A | Embryo transfer day 3 |
| 7 | 40 | 73 | ICSI | Agonist | 3 | 3 (100.0) | 3 (100.0) | 3 (100.0) | 1 (33.3) | Insufficient quality for trophectoderm biopsy |
| 11 | 34 | 40 | ICSI | Modified natural cycle | 1 | 1 (100.0) | 1 (100.0) | 1 (100.0) | N/A | Embryo transfer day 3 |
| 13 | 31 | 111 | ICSI | Antagonist | 16 | 13 (81.3) | 7 (53.8) | 5 (71.4) | N/A | Embryo transfer day 3; freezing day 3 |
| 15 | 38 | 62 | IVF | Agonist | 10 | N/A | 5 (50.0)[a] | 1 (20.0) | N/A | Embryo transfer day 3 |
| 16 | 31 | 55 | IVF | Antagonist | 6 | N/A | 5 (83.3)[a] | 3 (60.0) | 1 (25.0)[b] | Embryo transfer day 3; freezing day 5 |

*Note:* ART = assisted reproductive technology; COC = cumulus-oocyte complex; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; MII = metaphase II oocytes; N/A = not applicable; 2PN = fertilized oocyte.
[a] Calculated per number of COC.
[b] Calculated per number of 2PN minus 1embryo transferred on day 3.

*Boudry. SARS-CoV-2 RNA in assisted reproduction. Fertil Steril 2022.*

blastocysts can be infected by the virus. Although SARS-CoV-2 infection of human oocytes or embryos seems plausible in theory, to our knowledge, no direct evidence of contamination has been reported yet after ART.

Because many fertility clinics reduced or discontinued their ART activities during the peak of the pandemic, access to biologic material, including follicular fluid, cumulus cells, endometrial tissue, oocytes, or preimplantation embryos from the SARS-CoV-2–positive patients was limited. As a result, there is a paucity of data available to fertility societies to develop best practice guidelines for ART in SARS-CoV-2–positive patients, and at this stage, only limited-sized studies can provide valuable information to enhance our understanding of the effect of SARS-CoV-2 infection during preimplantation embryo development and early pregnancy.

In this study, we investigated the susceptibility of follicular fluid, cumulus cells, and endometrium to SARS-CoV-2 inoculation in 16 patients who tested positive <48 hours before oocyte retrieval. None of the follicular fluid samples contained detectable levels of viral RNA. Our data confirm the results reported in a previous study by Demirel et al. (24), which reports the absence of viral RNA in follicular fluid. In another recent study, SARS-CoV-2 mRNA was not identified in follicular fluid from 8 SARS-CoV-2–positive patients undergoing ART treatment (25). To our knowledge, this is the first report on the absence of viral RNA in the cumulus cells and endometrium of patients with SARS-CoV-2. Although cumulus cells may be susceptible to SARS-CoV-2 infection based on the available machinery conducive to SARS-CoV-2 invasion, cumulus cells in our patients were not infected by the virus (19). We suggest that this observation could be explained by the existence of multiple transcript variants of ACE2 (19). Different ACE2 isoforms resulting from different glycosylation and truncation patterns have been found in oocytes, cumulus cells, and embryos. It is plausible that these variants do not have a suitable spatial conformation for SARS-CoV-2 binding. Another explanation may be that the theca cells and the basal lamina surrounding the cumulus cells limit the transmission of the virus to the cumulus cells and follicular fluid. In contrast to cumulus cells, endometrial cells are highly unlikely to be susceptible to SARS-CoV-2 invasion because ACE2 and TMPRSS2 have been identified only in very limited proportions of endometrial tissue across the menstrual cycle (26).

To evaluate the impact of acute viral infection on preimplantation embryo development, we compared embryology data from the ART cycle during SARS-CoV-2 infection with the previous and/or subsequent ART cycle in our patients. Despite the limited size of our patient cohort, these data were reassuring and led us to suggest that SARS-CoV-2 infection probably does not have a substantial impact on the quality of follicles recruited in subsequent ART cycles and ensuing fertilization and embryo development.

Our results support that a positive SARS-CoV-2 test on a nasopharyngeal swab before oocyte retrieval should lead to cycle cancelation. This information is highly relevant to couples embarking on ART treatment during the long-haul COVID-19 pandemic. We advocate shared decision making whether to proceed with oocyte retrieval; stringent safety protocols should be followed, involving collection and handling of gametes in a way that mitigates the risk of infection and embryo culture in a laboratory designed and equipped to treat patients with infectious diseases undergoing IVF/ICSI. A freeze-all approach seems justified in SARS-CoV-2–positive patients because pregnancy should be avoided in a patient who may become seriously ill. This approach should not have an impact on overall pregnancy chances (27).

There are some important limitations on the validity of conclusions from these observational data. First, this study was conducted in a small population, only included patients without severe COVID-19 symptoms, and all patients had a different viral load (as indicated by the broad range of $C_t$ values) and clinical course, which prohibits the extrapolation of these results to all patients with COVID-19. Furthermore, despite the high sensitivity of RT-PCR, PCR testing in reproductive tissue was performed using a technique validated only for use in nasopharyngeal samples. Furthermore, RNA extraction was performed using different methods. Finally, additional studies on the possibility of SARS-CoV-2 contamination during clinical and/or laboratory procedures are required to ensure maximum safety of ART during the COVID-19 pandemic. In particular, the contamination of oocytes or embryos through exposure to blood during oocyte retrieval could, at least theoretically, result in SARS-CoV-2 infection and transmission.

In conclusion, after the worldwide cessation of ART cycles, subfertile couples have gradually restarted fertility care while they are informed of the remaining uncertainties regarding safety of ART during the ongoing COVID-19 pandemic. The available embryology and clinical data indicate the absence of viral RNA in follicular fluid, cumulus cells, and endometrium. Virology studies on the embryo are needed to further investigate the potential of SARS-CoV-2 to infect human gametes. It remains of utmost importance to adequately inform patients when they have ART in fertility clinics during these unprecedented times, as many questions on the impact of SARS-CoV-2 on male and female reproduction remain unanswered.

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**ARN viral indetectable en follicular líquido, células del cúmulo y endometrio muestras de tejido en SARS-CoV-2 - mujeres positivas.**

**Objetivo:** Estudiar la presencia de ARN viral en muestras de líquido folicular, células del cúmulo y tejido endometrial en mujeres positivas a SARS-CoV-2 que se someten a tecnologías de reproducción asistida (TRA).

**Diseño:** Estudio prospectivo, unicéntrico, observacional.

**Lugar:** Hospital de tercer nivel.

**Paciente(s):** Un total de 16 pacientes que se sometieron a una extracción transvaginal de ovocitos que tuvieron una prueba de ARN de SARS-CoV-2 positiva <48 horas antes del procedimiento. Todas las pacientes se sometieron a la recuperación entre septiembre de 2020 y junio de 2021 y utilizaron fertilización in vitro o inyección intracitoplasmática de espermatozoides. Todos los embriones fueron vitrificados para evitar la concepción durante la infección por SARS-CoV-2.

**Intervención(es):** Se analizó el líquido folicular aspirado durante la recuperación de ovocitos, las células del cúmulo y las muestras endometriales en busca de ARN del SARS-90 CoV-2 con el RealStar SARS-CoV-2 RT-PCR-Kit1.0.

**Medida(s) de resultado principal:** el parámetro de resultado principal fue la detección de ARN viral en el líquido folicular, las células del cúmulo y las células endometriales. La tasa de fertilización, el potencial de desarrollo del embrión y el resultado clínico después de la transferencia de embriones congelados fueron parámetros de resultado secundarios.

**Resultado(s):** Se analizaron muestras de 16 pacientes. Los valores de umbral de ciclo de <40 se consideraron positivos. Todas las muestras fueron negativas 94 para el ARN viral del SARS-CoV-2. Histológicamente no se identificaron lesiones inflamatorias del endometrio. La tasa de fertilización, el desarrollo del embrión y los resultados clínicos después de la transferencia de embriones fueron tranquilizadores.

**Conclusión(es):** En mujeres infectadas con SARS-CoV-2 que se sometieron a TRA, el ARN viral fue indetectable en el líquido folicular, las células del cumulus y el endometrio. Se requiere precaución en vista del pequeño tamaño de la muestra y no se puede descartar el riesgo de que el SARS-CoV-2 afecte al embrión a través de las ART. La consejería adecuada de mujeres y parejas que se someten a TRA es crucial en paralelo con más investigación sobre el efecto de la exposición del embrión humano temprano al SARS-CoV-2. 99

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