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Case Report: Oocyte Degeneration during Thawing Procedure in a SARS-CoV-2 Positive Woman

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Abstract

An essential concern for the safe preparation of assisted reproductive techniques (ART) during the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic is the probability of viral transmission through the gametes and its potential risks on gamete function. To date, there is no evidence about the precise effects of SARS-CoV-2 on functionality of gametes derived from infected individuals and whether there are deleterious effects on IVF outcomes. In this study, we inspected the degeneration of vitrified-warmed oocytes from a SARS-CoV-2-positive woman who underwent ovarian stimulation. Although the exact reason for the degeneration of these oocytes is unknown, SARS-CoV-2 could affect oocyte performance via an increase in oxidative stress. Thus, further investigation is required regarding the effect of SARS-CoV-2 on reproductive function.

Keywords: COVID-19; Oocyte vitrification; SARS-CoV-2; Degeneration; Assisted reproductive technologies.

Introduction

In late 2019, a novel coronavirus emerged from China that resulted in the onset of a global Covid-19 outbreak. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) could have possible negative impacts on female reproductive health. Infection of the genital tract is a serious problematic issue for both natural and medically assisted procreation because of impaired gametes. It is imperative to know the risk of viral transmission by gametes in order to prevent transmission to the embryo and for laboratory safety during the assisted reproductive technique (ART) [1].

SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) receptors for host cell entry. In both reproductive-age and postmenopausal women, there is evidence that ACE2 enzyme expression, an important component of the renin-angiotensin aldosterone (RAS) system, is abundantly expressed in the ovaries. According to evidence, the RAS system is crucially involved in folliculogenesis, oocyte maturation, and ovulation [2]. It is likely that SARS-CoV-2 could theoretically decrease ovarian function and oocyte quality; however, there is limited data about indirect multiple avenues of viral damage on reproductive function [3, 4]. Therefore, researchers hypothesized that the female reproductive system might be potentially at high risk for SARS-CoV-2 infection [2]. Most recently, Barragan et al. examined in detail the expression levels of ACE2, TMPRSS2, cathepsin L, and basigin transcripts in a subpopulation of collected oocytes. Conversely, the viral RNA was undetectable in all of the mature oocytes that were analyzed from two asymptomatic positive women who were undergoing ovarian stimulation and oocyte retrieval [5]. Stanley and colleagues have asserted that the co-expression levels of ACE2 and TMPRSS2 broadly increase with oocyte maturity [6]. Based on previous studies, it could be hypothesized that SARS-CoV-2 might target granulosa cells and ovarian tissue. Thus, this could hamper both reproductive health and oocyte viability, and result in female infertility or

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miscarriage [7]. Until now, the possible risk of SARS-CoV-2 infection on cryo stored gamete quality has not been reported. Likewise, the present study aims to set the possible effect of SARS-CoV-2 infection on degenerated cryopreserved oocytes retrieved from a SARS-CoV-2 positive woman.

Case report

On June 2021, a 28-year-old Iranian woman with a body mass index (BMI) of 32.7 kg/m2, who had previously undergone four failed IVF/ICSI cycles referred to Royan Institute in Tehran, Iran. The couple was married for ≥15 years, and a secondary infertility also occurred. The patient's reproductive system was intact. Both hysteroscopy and sonography reports revealed that the uterus and ovaries were anatomically normal and had a regular size. The uterus dimensions were 88 × 45 × 51 mm and the right and left ovaries had dimensions of 38 × 14 mm and 23 × 12 mm, respectively. All of the menstrual criteria, which included duration, amount, and interval were normal. Her AMH level was 2.16 and the other hormones were within the expected ranges. The woman also has hypothyroidism.

The Royan Institute COVID-19 policy mandates that both husband and wife undergo a PCR test before commencing IVF/ICSI treatment. Prior to starting ovarian stimulation, their PCR tests were negative. The ovarian stimulation was performed with 150 IU per day Gonal-F (Merck Serono, Bari, Italy), as recombinant FSH, from the second day and Cetrotide (Merck, Idron, France), a GnRH antagonist, from day six. Ovulation was triggered by administration of 0.1 mg decapeptyl (Ferring GmbH, Kiel, Germany), a GnRH antagonist, and 250 µg ovitrelle (Merck Serono, Modugno, Italy), as recombinant hCG. There were 17 follicles. On the day of oocyte retrieval, the woman's PCR test was positive.

According to the Royan Institute COVID-19 guidelines, oocyte retrieval as well as the vitrification procedure should be planned in the safe provision ART for exceptional cases of high risk ovarian hyper-stimulation (OHSS), cancer patients, and those with diminished ovarian reserve (DOR) where cancellation was not an option for medical reasons. Therefore, in our case, the oocytes were retrieved and then vitrified for possible future use while she was involved with COVID-19. There was no exact consideration about the reason for the thawing failure. Moreover, the inflammatory factors that are present in COVID-19 infectious could be recognized as deleterious. Of note, oocytes from 12 cases were satisfactorily thawed on the same day as the oocyte retrieval for the patient with COVID-19. Written informed consent was obtained from the woman for participation in the present study.

Oocyte vitrification and warming procedures

There were 11 oocytes frozen in three cryotops from this patient with SARS-CoV-2. The vitrification/warming procedures were performed according to the Royan Institute protocol [8]. At first, denuded oocytes were placed in 50 µL equilibration drop of solution (ES: 7.5% ethylene glycol and 7.5% dimethyl sulfoxide) for 15 minutes at room temperature for oocyte equilibration. After recovery, the oocytes were transferred to a 50 µL vitrification drop of solution (VS: 15% ethylene glycol, 15% dimethyl sulfoxide, and 0.5 M sucrose) and they were kept for one minute at room temperature. Subsequently, the shrinking oocytes were loaded onto the cryotop tip (Kitazato, Japan) and then plunged directly into liquid nitrogen, where they were kept until thawing for insemination.

After the recovery time, and when the patient's PCR test for 2019-nCoV was negative, she returned for microinjection of the thawed oocytes. The cryotops were thawed one after another and the entire 11 oocytes that were loaded on the three cryotops degenerated immediately. For the thawing procedure, we used a petri dish, thawing solution (TS) medium and washing solution (WS), which were warmed to 37 °C for at least 90 minutes. Dilution solutions 1 (DS1) and 2 (DS2) were at room temperature. The cover straw was removed from the liquid nitrogen and the laminate film was submerged into 1000 µL warm TS (1 M sucrose) for one minute and then transferred to DS1 (0.5 M sucrose) for three minutes and to a DS2 drop (0.25 M sucrose) for three minutes. Afterwards, the oocytes were washed four to five times in WS drop. This is the first report on the degeneration of oocytes from a woman with a positive PCR test for SARS-CoV-2. We did not evaluate the presence of viral RNA in the 11 oocytes from this patient. Therefore, we recommend that additional confirmatory evaluations such as RT-PCR be performed for degenerated oocytes.

Discussion

At the time of ART, invasive procedures such as transvaginal oocyte retrieval increase the risk for SARS-CoV-2 infection as well as the indirect effects of viral infection in women who are candidates for IVF/ICSI [7]. Bearing in mind that viral RNA is transmitted through blood, vaginal secretions, and cumulus cells, the possibility of contamination of this virus in the oocytes cannot be entirely ruled out. The human oocyte-cumulus complex cannot be a barrier to viral entry into the oocytes, thereby raising the possibility of infection [4, 6].

Accordingly, Qiu and colleagues tried to detect viral RNA in vaginal secretions of postmenopausal women infected with SARS-CoV-2. They did not diagnose SARS-CoV-2 in all of the vaginal samples or cervical swabs [9]. Scarica et al., in 2021, presented a single case of failure to detect viral RNA in follicular fluid in a woman with an active SARS-CoV-2 infection. However, they reported the possibility of viral transmission with increasing viral loads of SARS-CoV-2 [10]. Although several studies reported negative RT-PCR test results from genital fluid samples, Scorzolini et al. observed the presence of viral RNA in a 65-year-old woman. Their finding supported the risk for SARS-CoV-2 transmission in vaginal fluid [11]. Based on these reports, it is clear that we do not have enough information about the pathophysiologic effects of SARS-Cov-2 on the female reproductive system.

The pathogenesis of SARS-CoV-2 infection is proposed to be dependent on the SARS-CoV-envelope viroporin (E). These coronavirus proteins may play a role in viral replication, ion channel formation expressed in the plasma membrane, and regulation of the pH inside the lumen of intracellular organ-

elles. Therefore, SARS-CoV-2 proteins can be trafficked to the lumen of intracellular organelles [12]. Westerbeck and Machamer were the first to demonstrate activity of the SARS-CoV-2 envelope protein (E), which corresponded to an increase in pH within the lumen of intracellular organelles (i.e., the Golgi apparatus) [13].

Intriguingly, modification of the ionic channel function of intracellular organelles by the SARS-CoV-1 envelope (E) protein is proposed to activate the cellular stress response and apoptosis pathways [14]. In this regards, it has been shown that SARS-CoV-2 could operate via numerous mechanisms such as inflammatory responses, oxidative stress, and apoptosis pathways, and result in an impaired reproductive function. Indeed, one of the main reasons for a direct, negative impact on human oocyte quality is oxidative stress. The SARS-CoV infection induces overproduction of reactive oxygen species, which might accelerate the nuclear factor kappa-light chain-enhancer of activated B cell-toll-like receptor (NF-κB–TLR) pathways. Therefore, COVID-19 results in a cytokine storm that further exaggerates the inflammatory response [7, 15, 16]. According to Timmerman et al., a case of a 54-year-old woman with inflammatory ovarian masses diagnosed during COVID-19 infection. In view of the SARS-CoV-2 infection and the signs of a systemic inflammatory response syndrome, an association with viral infection is plausible [17]. Based on the previous researches, we speculated that SARS-CoV-2 could impact oocyte performance through an increase in oxidative stress, which induces apoptosis of the oocytes [18, 19].

Thus, there is a probability of deteriorated oocyte quality, such as an alteration in oocyte membrane integrity, which is associated with oxidative stress [16]. Several other factors, which include exposure to cryoprotective agents and the cryopreservation procedure, might have a negative impact on human oocyte quality [20]. Therefore, oocytes from individuals with SARS-CoV-2 might be more prone to deleterious effects after the freezing-thawed procedure. The permeability of the oocyte membrane to cryoprotectants, and the possible stress after exposure to cryoprotectants during the cryopreservation procedure cannot be ignored. There is a probability for oocyte degeneration after the thawing procedure. Overall, due to the limited sample size, we could not draw any conclusion about the implication of SARS-CoV-2 on the quality of human oocytes. Large sample sizes are needed for future investigations.

Taken together, we present a case of a thawed-oocyte evaluation from a SARS-CoV-2-positive woman. However, the pathogenic mechanism of SARS-CoV-2 infection on gamete function is still unclear. Our small finding shows that it is possible that oxidative stress from inflammation could be responsible for oocyte degeneration in these infected patients. Nevertheless, we must consider this viral illness as a potential source of infection in the IVF practice until more information is gathered about the safety of performing ART in patients with SARS-CoV-2.

Conflict of interest: The authors declare that they have no conflicts of interests.

Ethical standards: This study was reviewed and approved by the Institutional Review Board at Royan Institute (IR.ACECR.ROYAN.REC.1400.050).

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