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COMMENTARY

COVID-19 and fertility: a virtual reality

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ABSTRACT

The COVID-19 pandemic is an extraordinary global situation, and all countries have adopted their own strategies to diminish and eliminate the spread of the virus. All measures are in line with the recommendations provided by the World Health Organization. Scientific societies, such as the European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine, have provided recommendations and guidance to overcome and flatten the growing curve of infection in patients who undergo IVF treatments. Although there is as yet no evidence that the virus causing COVID-19 might have negative effects on IVF outcomes, fertility treatments have been postponed in order to support healthcare systems by avoiding placing them under additional stress. The possibility of the virus affecting sperm function and egg performance cannot be excluded. In addition, an indirect effect of the virus on gametes and embryos during their manipulation cannot be ruled out. This commentary aims to provide some ideas on the possible effect of the virus on gametes and embryos, as well as how it could affect the normal functioning of the embryology laboratory.

At the time of writing, citizens in about 200 countries have been infected by the novel coronavirus SARS-CoV-2, the virus that causes COVID-19. Belonging to the family Coronaviridae (CoV), which are typically harboured in mammals and other animals, coronaviruses cause severe acute respiratory syndrome (SARS) and infect unciliated bronchial epithelial cells and type II pneumonocytes. This in turn causes fever, cough, shortness of breath and severe complications such as pneumonia (*Ashour et al., 2020*), characteristics that are typical of all common influenzas. Over the past 8 weeks, many professional bodies, including the American College of Obstetricians and Gynecologists (ACOG) and the Royal College of Obstetricians and Gynaecologists (RCOG) have provided specific recommendations regarding COVID-19 and pregnant women. The limited data cannot support the notion that pregnant women are a group with a higher risk of illness, since there are reports of women positive for SARS-CoV-2 delivering healthy infants (in Greece there are currently three cases). This could be an indication against the intrauterine transmission of the virus. Nevertheless, there is mounting evidence pointing to the possible transmission from SARS-CoV-2-positive mothers to newborns and this, together with the presence of antibodies reported in an infant born to a SARS-CoV-2-positive mother (*Dong et al., 2020*; *Zeng et al., 2020*), places pregnant women in the group with a higher risk of illness.

Apart from the above organizations, scientific societies such as the European Society for Human Reproduction and Embryology (ESRHE) and the American Society for Reproductive Medicine (ASRM) have also provided recommendations regarding assisted conception. In brief, they have recommended cancelling fertility treatments, except in poor responders, who can still undergo treatment, although this may lead to additional stress for couples who badly want to have a child. Moreover, they have recommended suspending initiation of new treatments and an alternative freeze-all protocol in cases where couples have already undergone human chorionic gonadotrophin triggering. Cryopreservation of gametes should be considered for cases of urgent fertility preservation.

At the current time, fertility centres have cancelled fertility treatments (*La Marca et al., 2020*; *Rodriguez-Wallberg and Wikander, 2020*), although the association between SARS-CoV-2 and pregnancy resulting from ART treatments is surrounded by some uncertainties.

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Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd.
*Corresponding author. E-mail address: ganif@med.uth.gr (G Anifandis). https://doi.org/10.1016/j.rbmo.2020.05.001
1472-6483/Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd.
Declaration: The authors report no financial or commercial conflicts of interest.

KEYWORDS
COVID-19
Clinical embryology
Fertility

The impact of the virus in the very early stages of embryo development, i.e. from fertilization to blastocyst and from implantation to the first trimester, is not known. Evidence-based information regarding pregnancy outcomes, including miscarriage and live birth rates, in women positive for SARS-CoV-2 would be a useful tool for creating guidelines but may never exist in assisted reproduction. Although the decision to discontinue all medical procedures related to infertility treatment was not evidence-based, the trajectory of SARS-CoV-2 has created a unique challenge in global healthcare systems, and cancelling fertility treatments will not only stop the spread of the virus, but also avoiding additional stress contribution to these healthcare systems.

To date, in the news bulletins and statements on the ESHRE website it has been announced that there is low probability of contamination of gametes or embryos by SARS-CoV-2, as washing steps, culture and freezing protocols appear to reduce the potentially transmitted viral load, although they may not eliminate it. The possible absence of SARS-CoV-2 receptors on gametes (spermatozoa, oocytes) and embryos would strongly support this assumption, while the presence of zona pellucida in the oocytes and embryos up to the sixth day of development cannot argue in favour of the notion that the virus may have a negative impact during IVF treatments.

It is not uncommon for healthcare professionals to advise couples to postpone fertility treatment when either the male or the female partner has symptoms of or has recently recovered from any of the common flus. The reason is obvious, since any kind of flu (such as Zika virus) can cause infertility (*Barzon et al., 2017*). From recent global experience, SARS-CoV-2 seems to be far more aggressive in terms of severe illness, morbidity and mortality in comparison to common influenzas. At the cellular level, common influenza viruses promote oxidant-sensitive pathways, leading to an activation of pathogenic mechanisms that the oxidative stress may cause (*Khomich et al., 2018*; *Liu et al., 2017*). Increased oxidative stress has been blamed for male infertility (*Agarwal et al., 2018*; *Bisht et al., 2017*; *Dutta et al., 2019*) through a reduction of progressive motility in spermatozoa and a simultaneous increase in sperm DNA fragmentation (*Dorostghoal et al., 2017*; *Homa et al., 2019*). Combining these data, it can be assumed that SARS-CoV-2, through an activation of pathogenic pathways, may increase sperm DNA fragmentation, which in turn may affect fertilizing potential. Along the same lines, SARS-CoV-2 may affect oocyte performance through mechanisms that increase oxidative stress. Indeed, oxidative stress has been associated with alterations in DNA methylation (*Menezo et al., 2016*), while, in combination with the IVF process itself, it may negatively influence the DNA methylation circle, with adverse neonatal outcomes (*Anifandis et al., 2015*).

Bearing in mind that SARS-CoV-2 acts through the angiotensin-converting enzyme 2 (ACE2) receptor (*Li et al., 2003*), a possible direct effect of this virus on follicles/oocytes and spermatozoa cannot be excluded. ACE2 receptors have recently been observed on human Leydig cells (*Wang and Xu, 2020*), implying a possible direct effect of the virus on the male reproductive system. Nevertheless, two recent reports suggested that the virus was not present in the semen of patients who had recently recovered from COVID-19 (*Pan et al., 2020*; *Song et al., 2020*). Another recent study has shown that testicular ACE seems to be crucial for the early stages of embryo development (*Gianzo et al., 2018*) as ACE has been found to play significant role in sperm function (*Kohn et al., 1998*). In addition, ACE has been proposed to be responsible for either reduced or total failure of fertilization during conventional IVF (*Kondoh et al., 2005*; *Li et al., 2014*). As far as the oocyte is concerned, the role of ACE was underlined some time ago (*Pan et al., 2013*), and this enzyme is present in pre-ovulatory follicles, at least in rats (*Homorato-Sampaio et al., 2012*). ACE2 receptors have been reported to be expressed in human ovaries (*Reis et al., 2011*), while angiotensin-(1–7) has been detected in measurable amounts in the follicular fluid. Therefore, a possible negative impact of the virus through an interaction between the oocyte and the somatic cells cannot be ruled out. As far as the embryo is concerned, it has been found that human germ cells and early embryos express high levels of ACE2 (*Yan et al., 2013*), and recently it has been discovered that ACE2 is enhanced by SARS-CoV-2 infection (*Li et al., 2020*). Taking these facts together, it may be true that there is a direct effect of SARS-CoV-2 on spermatozoa or follicles/oocytes, but more molecular or cellular experiments are needed to prove this.

In terms of possible contamination of the embryology laboratory and the embryology staff by SARS-CoV-2, it is highly possible that the general precautions taken during oocyte recovery or gamete/embryo manipulation will be changed during IVF treatment with virus-positive asymptomatic patients. First, a separate area in the laboratory will be needed. The use of physical barriers, such as glass windows, can be implemented in screening areas or around registration desks in fertility clinics, as well as in embryology laboratories, to prevent spaces and staff being exposed to the COVID-19 virus. Additionally, the already in-use areas of the clinics (oocyte recovery rooms, sperm recovery rooms, etc.) should be cleaned and disinfected with specific products after every case. All staff should be trained in specific protocols for dealing with individuals with SARS-CoV-2, and should wear specific filtering face masks. Where a physical presence is not necessary, the use of telemedicine for any fertility inquiries, including embryological results, will minimize the spread of the virus. The usual work uniform would be better replaced by one similar or even identical to the uniform for working with SARS-CoV-2 infection, for instance protective coverall shoes, and isolation clothing in a combination with a head cover. This would produce a physical barrier to protect the body from any possible contamination. Gloves and face masks should continue to be worn but always on a single-use basis (i.e. not only in SARS-CoV-2-positive but also positive asymptomatic patients). Such personal protective equipment, including masks with respirators for healthcare workers in fertility clinics, is likely to be added to the daily work situation. In the case of clinics with many stimulation cycles, it would be wise to restrict the number of the staff who come in close contact with COVID-19 patients.

Contamination of the embryology laboratory seems to be a realistic expectation, despite the negative pressure that exists inside laboratories. Because the virus can survive for several hours in the air and at temperature above 22°C (the usual temperature

in most laboratories), it can easily be speculated that incubators could become contaminated. SARS-CoV-2 seems not to follow seasonal patterns, as seen in most flu outbreaks, and thus the virus may still have the ability to contaminate at temperatures above 25°C. Therefore, it might be useful to re-evaluate laboratory temperatures and pressures, to provide reassurance that the virus is not present in the air or does not have a chance to survive. Separation of incubators from those for patients not infected with COVID-19 may be used to avoid any potential cross-contamination. It has been found that SARS-CoV-2 may still contaminated plastics for about 72 hours (*van Doremalen et al., 2020*; *Kampf et al., 2020*), which means that non-embryotoxic dishes may not be free of the virus. It may be that, in the coming years, the quality control of culture dishes will include SARS-CoV-2-free sheets to ensure that exposable dishes and all related equipment are free of the virus. Although the SARS-CoV-2 virus has not yet been tested for its resistance to cooling rates during vitrification, the resistance of other viruses to cryogenic liquid nitrogen temperatures was reported a couple of years ago (*Merrill et al., 2018*). Due to the similarity of SARS-CoV-2 to other common viruses, it would be safer to have separate liquid nitrogen cryostorage containers for SARS-CoV-2-positive women. Moreover, another separate but vapour cryostorage tank for COVID-19-positive sperm samples could be a good choice. In a similar way, all donors will need to declare results of a SARS-CoV-2 test. All liquids should be tested for the virus, and all suppliers should submit a statement that the ingredients are free of the virus.

Despite early discussions in the absence of serious scientific evidence, it can be concluded that the unexpected state of the SARS-CoV-2 explosion has brought unique challenges to the global reproductive healthcare community, with possible detrimental consequences for couples seeking infertility treatment. In the next few years, we may experience reduced fertilization, implantation and live birth rates, while at the same time novel challenges will arise for embryology laboratories in neutralizing any virus present while carrying out delicate procedures.

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Received 13 April 2020; received in revised form 22 April 2020; accepted 1 May 2020.