

Case Report

Failure to Detect Viral Ribonucleic Acid in Follicular Fluid of a Severe Acute Respiratory Syndrome Coronavirus 2-Infected Female – A Report from the Indian Subcontinent

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ABSTRACT

Till today, we are still not clear about the impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on reproductive tissues and its vertical transmission through the gametes and embryos. There are few case reports that aim to detect the presence or absence of SARS-CoV-2 viral ribonucleic acid (RNA) in the follicular fluid of the infected females. We hereby present the case report of a 33-year-old woman who was undergoing intracytoplasmic sperm injection with testicular sperm aspiration and was found to be positive for SARS-CoV-2 infection. The follicular fluid analysis failed to detect the presence of viral RNA in the sample. Ours is the first case report from India trying to detect the viral RNA from the follicular fluid of a female with SARS-CoV-2 positive status.

KEYWORDS: *Follicular fluid, intracytoplasmic sperm injection, severe acute respiratory syndrome coronavirus 2, testicular sperm aspiration*

INTRODUCTION

The year 2019 saw the fast emergence of the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which jeopardised the whole world. On 30 January 2020 following the recommendations of the Emergency Committee, the WHO Director-General declared the outbreak a Public Health Emergency of International Concern. Its emergence led to strict measures to limit the spread of the infection. Fertility treatments were suspended during the pandemic as per the guidelines issued by various societies. Further, the fertility treatments were restarted as the waves of the disease settled.^[1]

Little is known about the SARS-CoV-2 on fertility, *in vitro* fertilisation (IVF) cycles and their outcome as most of the work had been put on hold during the pandemic. Only a few case reports have been there to detect the presence or absence of viral RNA in the follicular fluid of the infected patients, but no such study has been reported from India, we hereby present

probably the first case of a SARS-CoV-2- infected patient who underwent oocyte pickup at a level 2 fertility clinic based in Hisar, Haryana. Whose follicular fluid was analysed for the presence of viral RNA.

CASE REPORT

A 33-year-old female presented to our infertility centre with secondary infertility. She had been married for 13 years. A baseline Transvaginal sonography (TVS) carried out for her revealed the presence of an endometrial polyp for which she underwent hysteroscopic polypectomy. The husband's semen analysis revealed azoospermia on two occasions for which he was evaluated. His further evaluation pointed out towards obstructive pathology for azoospermia. Her antral follicle count was two in the right ovary and six in the left ovary. The patient was counselled regarding her low reserve and was counselled to

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Received: 02-11-2022
Accepted: 19-12-2022

Revised: 17-12-2022
Published: 30-12-2022

Access this article online

Quick Response Code:



Website:
www.jhrsonline.org

DOI:
10.4103/jhrs.jhrs_156_22

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How to cite this article: Bhayana DP, Raja KA, Chitra J, Sonu B. Failure to detect viral ribonucleic acid in follicular fluid of a severe acute respiratory syndrome coronavirus 2-infected female – A report from the Indian subcontinent. *J Hum Reprod Sci* 2022;15:396-8.

undergo intracytoplasmic sperm injection (ICSI) with Testicular sperm aspiration (TESA). As the male partner was not willing to trial TESA, the use of donor sperm was explained in the event of failure to retrieve the mature sperm from the testes on the day of ovum pickup. On day 2 of her period, the patient was started on the antagonist protocol with 450 IU of human menopausal gonadotropin. An antagonist was added when her follicles reached 12–13 mm during the stimulation. She needed a total of 11 days of stimulation utilisation. A total of 7 follicles were seen them on the last day of stimulation the patient complained of malaise, a fever with a mild cough. She was advised of COVID-19 Reverse transcription-polymerase chain reaction (RT-PCR) to rule out COVID-19 infection. As the symptoms were mild, decision was taken to trigger ovulation using 0.2 mg of Decapeptyl subcutaneously along with 2000 IU of human chorionic gonadotropin via the intramuscular route. Blood samples were sent on the day of the trigger approximately 42 h before the ovum pickup.

In our setting, the reports for COVID-19 are available after 24 h, and the next day her report was found to be positive. Her husband had been asymptomatic and she had been staying away from her husband at her mother's place. Subsequently, a thorough discussion was held with the couple regarding the cancellation of the cycle explaining to them the risks involved. However, as the couple had already undergone the complete stimulation and the symptoms were very mild, the couple wished to proceed with the oocyte retrieval procedure. Following evaluation by a physician, she underwent oocyte pickup with all due precautions to limit the spread of COVID-19 infection.

Oocyte pickup and laboratory procedure

We planned the oocyte retrieval in our laparoscopic Operation theater (OT) so as not to expose our laboratory area and embryologists to the infection. Follicular fluid was aspirated after 35 h of trigger injection and then transferred to the IVF laboratory and we obtained seven oocytes. Follicular fluid was sent for RT-PCR to detect the presence or absence of SARS-CoV-2. Her follicular fluid report showed a negative result for the viral RNA. Meanwhile, oocytes were incubated for 4 h after which denudation was carried out and five mature eggs were identified. The husband underwent surgical sperm retrieval on the same day and ICSI was performed. The couple was counselled for an all-freeze cycle in view of ongoing COVID-19 infection. She was subsequently discharged to be remained in home isolation for 14 days. Her symptoms subsided after 2 days of ovum pickup. Fertilisation check revealed a total of three fertilised.

However, no vitrification was performed due to poor quality of embryos.

DISCUSSION

We are still unclear about the possible impact of active SARS-CoV-2 infection on female gametes. Viral transmission occurs predominantly through respiratory droplets, but transmission to gametes cannot be ruled out. Coronaviruses are RNA viruses that utilise angiotensin-converting enzyme 2 (ACE2) receptor and transmembrane serine protease 2 (TMPRSS2) for their attachment and entry into the host cell. Rajput *et al.* have shown the co-expression of ACE2 and TMPRSS2 proteins in the mature eggs (MII oocytes), embryos and blastocysts, indicating that these gametes and embryos are potentially susceptible to SARS-CoV-2 infection if exposed to the virus.^[2] We could not find any study evaluating the lag period between blood positivity and follicular fluid positivity.

Case reports have been published for the detection of viral RNA in the follicular fluid samples. One study failed to identify any viral RNA in both the follicular fluid and the embryo culture medium in a 24-year-old woman with SARS-CoV-2 infection.^[3] Similar are the results of another study where they failed to detect viral RNA in the follicular fluid of SARS-CoV-2-infected women.^[4] Viral RNA of SARS-CoV-2 was not detected in 16 oocytes from two women who were positive for SARS-CoV-2.^[5] Another report also studied the follicular fluid from 300 patients and these all samples were negative for SARS-CoV-2 infections.^[2] Another report on SARS-CoV-2 infected women undergoing assisted reproductive technology (ART) also showed that, the absence of viral RNA in the cumulus cells, endometrial tissue and follicular fluid samples.^[6] COVID-19 infection or the BNT162b2 mRNA vaccination has no measurable detrimental effect on the function of the ovarian follicle.^[7]

Ours is the first case report from india looking into the presence of viral RNA in the follicular fluid of a SARS CoV 2 infected female who underwent oocyte pickup. Until we have abundant evidence for the safety, strict precautions in the form of prevention of droplet spread by using N95 masks, and personal protective equipment kits to prevent the spread of infection need to be followed.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their

names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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