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ASSISTED REPRODUCTION TECHNOLOGIES

The impact of past COVID-19 infection on pregnancy rates in frozen embryo transfer cycles

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Abstract

Purpose To study the effect of SARS-CoV-2 infection on pregnancy rates in frozen embryo transfer (FET) cycles.

Methods A retrospective cohort study including women under the age of 42 with documented SARS-CoV-2 infection up to 1 year prior to treatment, undergoing FET cycles in the first half of 2021, with transfer of embryos generated prior to the infection. Controls were SARS-CoV-2 non-diagnosed, non-vaccinated women matched by age, number, and day of embryo transfer. Demographic and cycle characteristics and outcomes were compared.

Results Forty-one recovered women and 41 controls were included. Pregnancy rates were 29% and 49% respectively ($p=0.070$). Stratification by time from SARS-CoV-2 infection to transfer into ≤ 60 and >60 days revealed a difference in pregnancy rates, with women in the COVID group having lower pregnancy rates if infected in proximity to the transfer (21% vs. 55%; $p=0.006$). In a logistic regression model, infection was a significant variable ($p=0.05$, OR 0.325, 95% CI 0.106–0.998). Logistic regression applied on the subgroup of women infected in proximity to the transfer further strengthened the univariate results, with COVID-19 remaining a significant parameter ($p=0.005$, OR 0.072, 95% CI 0.012–0.450).

Conclusions In FET cycles of patients with past SARS-CoV-2 infection, in which oocytes were retrieved prior to infection, decreased pregnancy rates were observed, specifically in patients who recovered less than 60 days prior to embryo transfer. Pending further studies, in cases of FET cycles with limited number of embryos, postponing embryo transfer for at least 60 days following recovery from COVID-19 might be considered when feasible.

Keywords COVID-19 · FET · Pregnancy · SARS-CoV-2

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the cause of coronavirus disease 19 (COVID-19), enters target host cells via the cellular receptor, angiotensin-converting enzyme 2 (ACE2), and cellular protease, transmembrane protease serine-2 (TMPRSS2) [1]. This has led to concerns that organs with a high expression of ACE2 or TMPRSS2 are vulnerable to adverse sequelae as the result of infection [2]. Most studies addressing the effect of the COVID-19 pandemic on human fertility focused on the male component, given the abundance of ACE2 receptors and TMPRSS2 in the testis tissue [3–8].

Despite the existence of ACE2 and TMPRSS2 in the female reproductive organs, the effect of COVID-19 infection on female fertility has been limitedly explored. The existence of the ACE2 axis and ACE2 markers has been confirmed in all stages of the follicular maturation in the human ovary and in the granulosa cells as well as the follicular fluid [9–12], potentially enabling SARS-CoV-2 to interfere with folliculogenesis and embryo development.

Implantation is another major step potentially impacted by SARS-CoV-2 infection. ACE2 and TMPRSS2 are expressed in the endometrium [13, 14], potentially enabling viral invasion of the cells. Unlike with bacterial

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infections, the effect of viral infections on implantation and pregnancy rates is unclear. Some evidence suggests that early embryonic and trophoblastic infection, even when caused by common viruses with low pathogenic potential, may result in impaired implantation or placentation. This has been explained by induction of an anti-trophoblast cellular immune response followed by apoptosis, reduced trophoblast invasion and remodeling of the decidua and uterine arterial vessels, and arrest of early embryonic development [15]. Another mechanism that may affect implantation is transient changes in sex hormone levels in women recently recovered from SARS-CoV-2 infection, as manifested by changes in the menstrual cycle [16].

The aim of our study was to elucidate the effect of SARS-CoV-2 infection on pregnancy rates in frozen embryo transfer (FET) cycles focusing on the implantation step.

Materials and methods

This is a retrospective cohort study, including all SARS-CoV-2 infection–infected women, aged 20–42 years that underwent FET cycles, between January 1 and June 31, 2021, at a large IVF unit in Israel (COVID group). COVID-19 status was based on polymerase chain reaction test results. Embryos transferred were the product of oocyte retrievals performed prior to SARS-CoV-2 infection. To be included in the study, maximal time from SARS-CoV-2 infection to transfer was defined as 1 year. Only the first FET cycle following recovery was included to avoid inclusion of recurrent transfers per patient. The study was approved by the Institutional Review Board.

The study group was matched by age, number of embryos transferred, and day of transfer, to unvaccinated patients with no history of past infection that underwent IVF treatments with FET at the same period (control group). Endometrial preparation protocols were individually tailored by the treating team, and included natural cycles and hormone replacement cycles, as per usual institutional routine. Embryo transfers were performed by highly experienced senior physicians. Demographic characteristics (including age at transfer and retrieval, partner's age, smoking status, number of previous pregnancies and deliveries, number of IVF treatments, and infertility cause) as well as cycle characteristics (treatment protocol, embryo grade, and endometrial width) and original retrieval characteristics (number of oocytes and number of good-quality embryos) were recorded. The primary outcome was clinical pregnancy rate, defined as an intrauterine gestational sac and fetal cardiac activity on transvaginal ultrasound imaging. Embryo grading was based on the Istanbul consensus workshop parameters [17].

Data analysis

The Shapiro–Wilk test was used to test for normality of distribution. Continuous variables were summarized with mean and 95% confidence interval (CI) and compared between groups using the Mann–Whitney test. Categorical variables were summarized using counts and percentages. The Fishers exact test or chi-square were used to compare differences between groups. A logistic regression model was applied to identify factors associated with clinical pregnancy rates in frozen cycles. Backwards elimination was applied to select the optimal model, while age and the COVID group were forced to be included in the model. No imputations for missing data were applied. A two-sided $p \leq 0.05$ was considered significant. All analyses were conducted on R package version 3.6.3 and SPSS-27 (IBM, USA).

Results

Forty-one women with past SARS-CoV-2 infection and 41 controls were included (Table 1). The mean time from infection to embryo transfer was 59 ± 68.31 days. Mean age at ovum pickup (30.72 vs. 30.69; $p = 0.929$) and at transfer (31.56 vs. 31.58; $p = 0.966$) was similar between groups, as were all other demographic characteristics. Numbers of previous retrievals and transfers were similar between groups. The predominant transfer protocol used was different between groups with higher rates of natural cycle (NC) protocol in the COVID group (61% vs. 33%; $p = 0.013$), whereas the hormonal replacement therapy (HRT) protocol was more common in the control group. All other cycle characteristics including endometrial width, number of embryos transferred, day of embryo transfer, and embryo grade were similar between groups. Similarly, there was no difference between groups in the number of oocytes retrieved, and number of vitrified embryos of the cycle from which the transferred embryo originated, and the number of patients having a fresh transfer at that retrieval. Though clinical pregnancy rates were lower in the COVID group, this difference did not reach statistical significance (29% vs. 49%; $p = 0.070$). As most patients had a single embryo transfer, implantation rates were relatively similar to pregnancy rates and did not differ between groups (27% vs. 43%, $p = 0.093$). At the time of data collection, one pregnancy in each group resulted in missed abortion while the rest were reported as ongoing pregnancy.

Stratifying the COVID group by time from SARS-CoV-2 infection to embryo transfer into ≤ 60 days and > 60 days

Table 1 Demographic and cycle characteristics and outcomes of COVID vs. control in FET cycles

Group	COVID-19 ($N=41$)	Non COVID-19 ($N=41$)	p value
Patient age at transfer (y)	31.56 (5.20) [22–42]	31.58 (5.05) [22–42]	0.966
Patient age at retrieval (y)	30.72 (5.00) [23–41]	30.69 (4.73) [22–40]	0.929
Partner's age (y)	34.79 (6.81) [25–55]	33.31 (5.26) [25–49]	0.396
Smoker	4 (10%)	11(2%)	0.399
Previous retrievals	1.15 (0.43) [0–2]	1.05 (0.47) [0–3]	0.199
Previous transfers	1.42 (0.93) [0–3]	1.11 (0.89) [0–3]	0.103
BMI	25.56 (6.09) [15.43–44.08]	24.78 (6.13) [17.63–41.40]	0.311
Infertility cause			0.738
Age related	2 (5%)	1 (3%)	
Male factor	21 (55%)	12 (40%)	
Ovulation	3 (8%)	4 (13%)	
Mechanical	1 (3%)	2 (7%)	
Unexplained	9 (24%)	10 (34%)	
Other	2 (5%)	1 (3%)	
Parity			0.297
0	18 (47%)	22 (63%)	
1	15 (40%)	9 (26%)	
≥ 2	5 (13%)	4 (11%)	
Gravidity			0.232
0	16 (42%)	21 (60%)	
1	14 (37%)	8 (23%)	
≥ 2	8 (21%)	6 (17%)	
Days from COVID to transfer	59.71 (68.31) [1–274]	NA	
≤ 60 (d)	29 (71%)	NA	
> 60 (d)	12 (29%)	NA	
Protocol			0.013
HRT	16 (39%)	26 (67%)	
NC	25 (61%)	13 (33%)	
Endometrial width (mm)	9.76 (1.83) [6–15]	9.18 (1.86) [6–13.6]	0.097
No. embryos transferred	1.10 (0.30) [1, 2]	1.12 (0.33) [1, 2]	
Day of transfer			0.515
2	1 (2%)	0	
3	18 (44%)	21 (51%)	
5	22 (54%)	20 (49%)	
Embryo grade at freezing			0.438
A	21 (52%)	18 (44%)	
B	19 (48%)	23 (56%)	
Clinical pregnancy	12 (29%)	20 (49%)	0.070
Pregnancy outcome	12	20	
Ongoing pregnancy rate	11 (92%)	19 (95%)	
Early missed abortion	1 (8%)	1 (5%)	
Implantation rate	27%	43%	0.093
No. oocytes retrieved (original retrieval)	17.58 (9.47) [3–40]	16.71 (8.29) [5–38]	0.800
No. patients who had a fresh embryo transfer (original retrieval)	30	25	0.347
Total frozen (original retrieval)	4.59 (3.31) [1–16]	4.35 (3.24) [1–12]	0.756

Data is presented as mean and (SD) and [range] or counts and (percentage)

Table 2 Cycle outcome of COVID vs. control in FET cycles, by days from COVID

Group	COVID-19 ($N=41$)	Non COVID-19 ($N=41$)	p value
≤60 days	**29**	**29**	
Clinical pregnancy	6 (21%)	16 (55%)	0.006
Ongoing pregnancy	6 (21%)	16(55%)	0.006
>60 days	**12**	**12**	
Clinical pregnancy	6 (50.0%)	4 (33%)	0.407
Ongoing pregnancy	5 (42%)	3(25%)	0.666

Data is presented as counts and (percentage)

(Table 2) revealed a significant difference in pregnancy rates, with women in the COVID group having lower pregnancy rates if infected in proximity (≤60 days) to the transfer compared to healthy women (20.7% vs. 55.2%; $p=0.006$). There were no differences in demographic and cycle characteristics between groups except for the endometrial preparation protocol with NC being more prevalent in the COVID group (69% versus 37%; $p=0.016$) (Supplementary Table 1). In the subgroup of patients with an embryo transfer > 60 days after infection, no difference in demographic and cycle characteristics nor in pregnancy rates was observed although sample size was small.

In a logistic regression model for pregnancy rates in FET cycles, including age, previous transfers, embryo grade, transfer protocol, and endometrial width, past SARS-CoV-2 infection was a significant variable ($p=0.05$, OR 0.325, 95% CI 0.106–0.998) (Supplementary Table 2). Logistic regression model for pregnancy rates applied on the subgroup of women infected in proximity to the transfer (≤60 days) further strengthened the univariate results, with past SARS-CoV-2 infection remaining a significant parameter ($p=0.005$, OR 13.88, 95% CI 2.22–83.33), together with age at OPU. The group of patients with SARS-CoV-2 infection > 60 days before transfer was too small for a separate analysis model. To support the results, we further applied the same model for patients undergoing an embryo transfer with a cutoff of 30 days from SARS-CoV-2 infection (Supplementary Table 2). A past SARS-CoV-2 infection 30 days or less from embryo transfer significantly reduced the odds for clinical pregnancy ($p=0.003$, OR 21.461, 95% CI 2.78–165.46). In the group of patients with an embryo transfer more than 30 days from SARS-CoV-2 infection, the infection did not significantly affect the odds for clinical pregnancy. As sample size in both groups (≤30, >30) was small, the confidence interval was wide affecting the stability of the model.

A difference in protocol type between the COVID and control groups was observed in the univariate analysis. It was not found to be a significant variable in the models. In order to further rule out a possible confounder effect, we analyzed a subgroup of 44 patients, 22 in each group, matched also for the protocol in addition to the age, number of embryos, and day of transfer. A univariate analysis revealed no difference in pregnancy rates for the COVID (36.4%) vs control (54.5%) group ($p=0.225$) as was for the original group, but again when stratifying by time from SARS-CoV-2 infection, pregnancy rates were significantly higher for the subgroup with a transfer < 30 days from infection ($p=0.012$) but not for the subgroup of > 30 days ($p=0.699$). The regression model applied for this subgroup of patients with stratification by time from infection was consistent with the model applied for the whole group, identifying COVID as a significant variable ($p=0.026$, OR = 23, 95% CI 1.44–326.23), although sample size was small, making the model less stable (Supplementary Table 3).

Discussion

The purpose of our study was to examine the impact on implantation by analyzing clinical and ongoing pregnancy rates in FET cycles of past SARS-CoV-2-infected women, with embryos originating from a pre-COVID treatment. In this retrospective cohort study, past infection with SARS-CoV-2, prior to frozen embryo transfer decreased pregnancy rates, especially in women with recent infection. This study is the first to report ongoing pregnancy rates in FET cycles of past SARS-CoV-2-infected women.

A recently published study examined the effect of COVID-19 immunity on FET cycle outcomes, including both recovered and vaccinated patients. The recovered group consisted of 44 patients with no difference in clinical pregnancy rates compared to the vaccinated and the control group [18]. The study included several FET cycles of the same patients and did not differentiate between embryos that were generated before and after the infection, possibly limiting the strength of its conclusions. Furthermore, there was no stratification by time from COVID-19 infection; thus, the immediate consequences of infection on pregnancy rates could not be properly evaluated.

SARS-CoV2 enters cells via the ACE2 cellular receptor and the TMPRSS2 cellular protease, expressed in all stages of follicular maturation in the human ovary, in the granulosa cells, and in the endometrium [9–12]. In order to neutralize any potential viral effect on folliculogenesis and embryo development, and isolate the implantation stage in the IVF process, we focused on FET cycles with retrieval prior to the SARS-CoV-2 infection. We found a significant reduction in pregnancy rates, especially when the transfer was performed in proximity to the SARS-CoV-2 infection. These findings may be attributed to several potential causes. First, menstrual changes following

COVID-19 have been reported, possibly due to transient changes in levels of sex hormone [16]. This may explain the short-term effect on implantation and pregnancy rates that is mainly in proximity to the acute infection. Second, like other acute viral infections, SARS-CoV-2 may impair implantation or placentation through early embryonic and trophoblastic infection [15]. This mechanism may be compared to the inflammatory orchitis caused by SARS-CoV-2 that may result in temporary reduction of testicular function [6, 19]. Evidence for trophoblastic infection comes from studies reporting an increase in intrauterine fetal demise in patients that contracted COVID-19 during pregnancy, suggesting that the hallmark of SARS-CoV-2 placental infection is trophoblastic damage [20, 21].

The main limitation of our study is its retrospective nature, with the inherent biases of collecting data that was not uniformly generated under a study protocol. Another caveat is the limited sample size, a result of the strict inclusion criteria including only patients with retrieval prior to COVID infection and only the first FET cycle. Lastly, distribution of cycle protocol (natural vs HRT) was different between groups even though age and infertility diagnosis, which may the endometrial preparation method decision, were similar between groups. However, protocol type was not a significant factor in the multivariate regression model for pregnancy rates in FET cycles, even when applying it on the subgroup of patients matched for the protocol in addition to the other matching criteria. Furthermore, according to the literature pregnancy rates are similar between protocols [22].

In conclusion, in FET cycles performed in women with past SARS-CoV-2 infection, in which oocytes were retrieved prior to the infection, decreased pregnancy rates were observed, especially in patients who recovered less than 60 days prior to embryo transfer. Further studies with larger groups are warranted to support these findings. Pending further information, in cases of FET cycles with limited numbers of embryos (advanced age, embryo donation, fertility preservation, embryos following sperm extraction), postponing embryo transfer for 30–60 days following recovery from COVID-19 might be considered, if feasible.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10815-022-02517-w.

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Declarations

Conflict of interest The authors declare no competing interests.

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