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The effect of coronavirus disease 2019 immunity on frozen-thawed embryo transfer cycles outcome

Adva Aizer, Ph.D.,^{a,b} Meirav Noach-Hirsh, Ph.D.,^a Olga Dratviman-Storobinsky, M.Sc,^a Ravit Nahum, M.D.,^{a,b} Ronit Machtinger, M.D.,^{a,b} Yuval Yung, Ph.D.,^a Jigal Haas, M.D.,^{a,b} and Raoul Orvieto, M.D., M.M.Sc.^{a,b,c}

^a Infertility and IVF Unit, Department of Obstetrics and Gynecology, Chaim Sheba Medical Center (Tel Hashomer), Ramat Gan, Israel; ^b Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; and ^c The Tarnesby-Tarnowski Chair for Family Planning and Fertility Regulation, at the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Objective: To study the effect of patients' immunization after coronavirus disease 2019 (COVID-19) infection or messenger ribonucleic acid (mRNA) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine on frozen-thawed embryo transfer (FET).

Design: Cohort retrospective study.

Setting: Tertiary university affiliated medical center.

Patient(s): All consecutive patients undergoing FET cycles in our center. The study group (immune group) consisted of patients treated during the COVID-19 pandemic (between January 2021 and August 2021) who either recovered from COVID-19 infection or received the mRNA SARS-CoV-2 vaccine. The control groups consisted of patients treated during the COVID-19 pandemic (between January 2021 and August 2021) but were not infected or did not receive the mRNA SARS-CoV-2 vaccine (not-immune2021 group) and those treated between January 2019 and August 2019 (before the pandemic) (not-immune2019 group).

Intervention(s): Frozen-thawed embryo transfer cycles.

Main Outcome Measure(s): Ongoing pregnancy rates and FET cycles' characteristics. Data on patient age and variables related to infertility treatment were collected from the patient records.

Result(s): During the study periods, 428 patients underwent 672 FET cycles. The immune group consisted of 141 patients who underwent 264 FET cycles (44 in postinfection and 220 in postvaccination), whereas the not-immune2021 and not-immune2019 groups consisted of 93 and 194 patients undergoing 125 and 283 FET cycles, respectively. Patients' characteristics and the types of endometrial preparations were comparable between the study groups. The implantation rate and clinical and ongoing pregnancy rates per transfer were similar between the study groups (immune group, postinfection and postvaccination; not-immune2021 group; not-immune2019 group).

Conclusion(s): Coronavirus disease 2019 infection or vaccination did not affect patients' performance or implantation in their subsequent FET cycle. (Fertil Steril® 2022;117:974–9. ©2022 by American Society for Reproductive Medicine.)

El resumen está disponible en Español al final del artículo.

Key Words: COVID-19, vaccination, frozen-thawed embryo transfer, IVF



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Coronavirus disease 2019 (COVID-19), first reported in Wuhan, China, in December 2019, rapidly spread globally. In March 2020, it was declared by the World Health Organization as a public health emergency pandemic of international concern (1–3).

Coronavirus disease is caused by a single, positive-strand ribonucleic acid (RNA) virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which comprises a spike protein, a membrane protein, an envelope protein, nucleocapsids, hemagglutinin-esterase dimers, and its genetic material

(4). The spike protein is responsible for the high affinity of SARS-CoV-2 for human angiotensin-converting enzyme 2 (ACE2) receptor after the viral entry into cells (5). Some studies focused on the gene expression of the ACE2 receptor in the endometrium to determine the risk of endometrial infection resulting in the low expression of ACE2 in the uterus surrounding (6, 7).

Israel was one of the first countries that widely vaccinated its population using the messenger RNA (mRNA) vaccines (Pfizer-BioNTech COVID-19 Vaccine), allowing a large portion of the population to become vaccinated along with the population recovering from

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Reprint requests: Adva Aizer, Ph.D., Infertility and IVF Unit, Department of Obstetrics and Gynecology, Sheba Medical Center, Derech Sheba, Ramat Gan 52621, Israel (E-mail: adva.aizer@sheba.health.gov.il).

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SARS-CoV-2. This enabled a comprehensive examination of the effect of the vaccine or COVID-19 on a woman's fertility potential. Recently, we assessed the influence of COVID-19 infection and mRNA SARS-CoV-2 vaccine on the stimulation characteristics and embryological variables of patients undergoing in vitro fertilization (IVF) treatments (8, 9). Although both COVID-19 infection and mRNA SARS-CoV-2 vaccine did not affect patients' performance or ovarian reserve in their immediate subsequent IVF cycle, COVID-19 infection was demonstrated to cause a significant reduction in the proportion of top-quality embryos (TQEs) (8).

Prompted by the aforementioned information and the possible detrimental effect of SARS-CoV-2 on the endometrium, we aimed to evaluate the effect of patients' acquisition of antibodies after COVID-19 infection or mRNA SARS-CoV-2 vaccine on frozen-thawed embryo transfer (FET) cycle outcome.

MATERIALS AND METHODS

This is a single-center cohort, retrospective analysis of all consecutive patients attending our IVF center because of different causes of infertility who underwent FET cycles between January 2019 and August 2019 and January 2021 and August 2021. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The study was approved by the institutional research ethics board of Sheba Medical Center.

The study group (immune group) consisted of patients treated during the COVID-19 pandemic (between January 2021 and August 2021) who either recovered from COVID-19 infection or received the mRNA SARS-CoV-2 vaccine. The control group consisted of patients who were treated during the COVID-19 pandemic (between January 2021 and August 2021) but were not infected or did not receive the mRNA SARS-CoV-2 vaccine (not-immune2021 group) and those who were treated between January 2019 and August 2019 (before the pandemic) (not-immune2019 group).

The type of endometrial preparation used was decided by the treating physician and largely depended on the fashion at the time. Endometrial preparation and transfer procedure were performed as previously described (10). Patients aged 18–38 years were included. None of the embryos underwent preimplantation genetic testing for aneuploidy, which is not a common practice in our country.

Embryos were cryopreserved by vitrification, using a vitrification kit (SAGE Vitrification Kit; SAGE Media, Trumbull, CT), on cleavage or blastocyst stages. The vitrification-warming method was performed as previously described (11). Only high-quality embryos were considered for vitrification. The number of embryos transferred complied with the Israeli National Ministry of Health guidelines and was confirmed by patient approval or request. According to our laboratory procedure, high-quality embryos eligible for vitrification had the following morphological criteria: cleaved embryos; 6–8 cells on day 3; with up to 10% fragmentation; absence of vacuoles; and up to minimal asymmetric blasto-

meres. Grading of blastocyst was according to the Gardner method (12, 13), based on the assessment of the inner cell mass and trophectoderm appearance. Only type A and B blastocysts were vitrified.

Data on patient age and variables related to infertility treatment were collected from the patient records. Clinical outcomes were assessed and compared between the study groups (immune group, not-immune2021 group, and not-immune2019 group). Clinical pregnancy was defined as the visualization of a gestational sac and fetal cardiac activity on transvaginal ultrasound. Embryo implantation rate was defined as the number of gestational sacs observed divided by the number of embryos transferred. The primary outcome measure was the clinical pregnancy rate. The secondary outcome measures were FET cycle characteristics and implantation rate.

To investigate the effect of SARS-CoV-2 vaccination/infection on FET cycles outcome, a sample size of 290 participants was estimated to be sufficient to reveal a difference in ongoing pregnancy rate from a baseline of 20% to a proposed 30% after vaccination/infection with a type 1 error (α) of 5% and a power of 80%.

Continuous variables were tested for normal distribution before using parametric statistics. For multiple comparisons, one-way analysis of variance with post hoc Bonferroni and Kruskal-Wallis tests were used whenever appropriate. Categorical variables were analyzed using the chi-square test. Significance was set at $P < .05$. Statistical analyses were conducted using the IBM Statistical Package for the Social Sciences (IBM SPSS v.23; IBM Corporation Inc., Armonk, NY)

RESULTS

During the study periods, 428 patients underwent 672 FET cycles. Patients were divided accordingly into 3 groups: the immune group consisted of 141 patients who underwent 264 FET cycles, whereas the not-immune2021 and not-immune2019 groups consisted of 93 and 194 patients undergoing 125 and 283 FET cycles, respectively.

Tables 1 and 2 detail the baseline clinical characteristics, etiology of infertility, and clinical outcomes of the 5 different FET groups in the 2 study periods. One-way analysis of variance with post hoc Bonferroni was used for normal distributed parameters (age and progesterone), and the Kruskal-Wallis test was used for parameters with abnormal distributions (body mass index, time interval, endometrial thickness, peak estradiol levels, and number of embryos transferred). The mean patient age, body mass index, and prevalence of smoking were comparable between the study groups. The types of endometrial preparation were based mostly on spontaneous natural cycles (86% across all groups) and were comparable between the different study groups. Moreover, the mean endometrial thickness, peak estradiol, and progesterone levels were similar in the 3 study groups.

The implantation rates (23.2% vs. 24.1% vs. 20.3%), positive β -human chorionic gonadotropin (28% vs. 28.8% vs. 26.5%), clinical pregnancy rates (25.6% vs. 26.4% vs. 23%), and ongoing pregnancy rates (25% vs. 22.7% vs. 23%) per

TABLE 1

Patients' baseline clinical characteristics.

Clinical characteristics	Postinfection	Postvaccination	Immune group	Not-immune2021 group	Not-immune2019 group	P value
Number of patients	26 (18.4% of immuned)	115 (81.6% of immuned)	141	93	194	
Number of cycles	44	220	264	125	283	
Mean interval between COVID-19–positive test/second vaccine to postinfection FET cycle, d (mean ± SD)	153 + 93	79 + 46	100 + 63	—	—	
Age, y (mean ± SD)	28.9 + 4.5	30.4 + 4.4	30.1 + 4.4	30.7 + 4.1	31 + 3.9	.135
Body mass index, kg/m ² (mean ± SD)	24.9 + 4.6	25.2 + 5.9	25.2 + 5.6	24.6 + 5.5	21.6 + 8.9	.08
Smoking (%)	2/26 (7.7)	11/115 (9.6)	13/141 (9.2)	10/93 (10.8)	31/194 (16.0)	.26
Type of infertility						
Male (%)	11/26 (42.3)	40/115 (34.8)	51/141 (36.2)	37/93 (39.8)	91/194 (47.0)	.2
Tubal (%)	2/26 (7.6.9)	11/115 (9.6)	13/141 (9.2)	8/93 (8.6)	25/194 (12.9)	.71
Endometriosis (%)	0	9/115 (7.8)	9/141 (6.4)	5/93 (5.4)	11/194 (5.7)	.91
Unexplained infertility (%)	3/26 (11.5)	14/115 (12.2)	17/141 (12.1)	11/93 (11.8)	27/194 (13.9)	.97
Ovulatory disorder (%)	3/26 (11.5)	10/115 (8.7)	13/141 (9.2)	11/93 (11.8)	24/194 (12.4)	.82
Uterine factor (%)	1/26 (3.8)	1/115 (0.9)	2/141 (1.4)	1/93 (1.08)	5/194 (2.6)	.68
Others (%)	8/26 (30.8)	46/115 (40.0)	54/141 (38.3)	42/93 (45.2)	61/194 (31.4)	.18

Note: COVID-19 = coronavirus disease 2019; FET = frozen-thawed embryo transfer. *Alizer. Coronavirus 2019 in Frozen-thawed cycles. Fertil Steril 2022.*

transfer were similar between the immune and not-immune2021 and not-immune2019 groups (respectively).

Immunized patients were further divided into 2 subgroups, postinfection (44 FET cycles) and postvaccination (220 FET cycles). No in-between subgroup differences or differences between these subgroups and the 2 not immune control groups were observed in patients' clinical characteristics or in FET cycle characteristics and clinical outcomes (Tables 1 and 2).

DISCUSSION

In the present study of patients undergoing FET before and during the COVID-19 pandemic, we observed no difference in clinical outcome, reflecting no detrimental effect of previous infection or vaccination on the FET cycle outcome, with an acceptable ongoing pregnancy rate (25% per transfer).

These observations are in accordance with what is already known regarding vaccination during pregnancy. Pregnant women receiving flu vaccination, for example, were found to benefit beyond the simple prevention of maternal infection including the reduction of stillbirth (14). This may be secondary to the immune activation. The induction of the immune system pathways that favors immunologic tolerance has long been considered a possible path to improved embryo implantation and reducing miscarriages, although the topic is still somewhat controversial (15–17). Accordingly, patients undergoing an IVF cycle after mRNA SARS-CoV-2 vaccination showed no detrimental effect on the ovarian stimulation characteristics, embryological variables, or proportion of TQEs (9).

Nowadays, the impact of SARS-CoV-2 infection on endometrial receptivity is unknown. Coronavirus disease 2019 may damage endometrial epithelial cells and affect early embryo implantation (18). Moreover, a study by Henarejos-Castillo et al. (7) assessed endometrial susceptibility to SARS-CoV-2 infection by measuring several endometrial genes expression and observed that overall, the endometrium appears to have low susceptibility to SARS-CoV-2 infection because of low ACE2 and transmembrane serine protease-2 expression. A recent study by our group could not demonstrate any effect of COVID-19 infection on the ovarian stimulation characteristics and embryological variables of patients undergoing IVF treatments, except for a reduced proportion of TQEs (8).

Following the previous studies on the effect of COVID-19 infection and vaccination on folliculogenesis and embryonic development, the present study concentrates on implantation during FET cycle. It further clarifies the safety of assisted reproductive technology treatment after infection and vaccination. Of notice, we could not observe the differences in FET cycles outcome between the postinfection and postvaccination subgroups.

The limitations of our study are the small sample size and the short period of follow-up. The strength of the study is that it was conducted in a single center by a professional consistent team on a large study group. Moreover, to our knowledge, this is the first study to assess the influence of COVID-19 infection or vaccination on FET cycles.

TABLE 2

Frozen-thawed embryo transfer cycle characteristics and clinical outcomes.

Cycle characteristics	Postinfection	Postvaccination	Immune group	Not-immune2021 group	Not-immune2019 group	P value
FET cycle characteristics						
Number of ET	44	220	264	125	283	
Spontaneous natural cycle (%)	38/44 (86.3)	191/220 (86.8)	229/264 (86.7)	108/125 (86.4)	245/283 (86.6)	.99
Mean endometrial thickness, mm (mean ± SD)	9.9 + 1.9	9.5 + 2	9.6 + 2	9.5 + 1.9	9.7 + 1.9	.68
Mean peak E2, pmol/L (mean ± SD)	773 + 323	910 + 574	886 + 540	895 + 420	1112 + 645	< .001
Mean peak P, nmol/L (mean ± SD)	2.9 + 1.7	2.79 + 1.6	2.81 + 1.6	2.87 + 1.7	2.94 + 1.9	> .99
Number of embryos transferred	50	255	305	141	349	
Median number of embryos transferred (IQR)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	
Cleavage-stage embryo transfer rate per transfer	29/44 (65.9)	119/220 (54.0)	148/264 (56.1)	67/125 (53.6)	179/283 (63.2)	.13
Clinical outcomes						
Number of positive β-hCG	16	58	74	36	75	
Positive β-hCG per transfer (%)	16/44 (36.4)	58/220 (26.4)	74/264 (28.0)	36/125 (28.8)	75/283 (26.5)	.71
Positive β-hCG per patient (%)	16/26 (61.5)	58/115 (50.4)	74/141 (52.5)	36/93 (38.7)	75/194 (38.7)	.017
Number of sacs observed	14	57	71	34	71	
Implantation rate (%)	14/50 (28.0)	57/255 (22.3)	71/305 (23.2)	34/141 (24.1)	71/349 (20.3)	.71
Number of clinical pregnancies	13	55	68	33	65	
Clinical pregnancy rate per transfer (%)	13/44 (29.5)	55/220 (25.0)	68/264 (25.6)	33/141 (26.4)	65/283 (23.0)	.86
Number of ongoing pregnancies	12	54	66	32	65	
Ongoing pregnancy rate per transfer (%)	12/44 (27.3)	54/220 (24.5)	66/264 (25.0)	32/141 (22.7)	65/283 (23.0)	.95

Note: β-hCG = β-human chorionic gonadotropin; E2 = estradiol; ET = embryo transfer; FET = frozen-thawed embryo transfer; IQR = interquartile range; P = progesterone.

Aizer. Coronavirus 2019 in frozen-thawed cycles. *Fertil Steril* 2022.

In conclusion, COVID-19 infection or vaccination did not affect patients' performance or implantation in their subsequent FET cycle. Unfounded claims in popular media linked a possible correlation between the SARS-CoV-2 vaccine and potential infertility. Such false claims by antivaccine activists aim to incite fear and deter public opinion from vaccination, consequently jeopardizing the vaccination plan and the end of the pandemic. Our results refute such claims and strengthen the notion that the SARS-CoV-2 vaccine is safe and should be recommended to fertility-seeking couples. Future larger studies with longer follow-up will be needed to validate our observations.



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El efecto de la inmunidad por la enfermedad del coronavirus 2019 en el desenlace de los ciclos de transferencia de embriones congelados – descongelados.

Objetivo: Estudiar el efecto de la inmunidad después de la infección enfermedad por coronavirus 2019 (COVID-19) o de inmunización con vacuna de ácido ribonucleico mensajero (mARN) para el síndrome respiratorio agudo por coronavirus 2 (SARS-CoV-2) en los ciclos de transferencia de embriones congelados y descongelados (TEC).

Diseño: Estudio de cohorte retrospectivo.

Escenario: Centro medico terciario con afiliación universitaria.

Pacientes: Todos los pacientes consecutivos de TEC de nuestro centro. El grupo de estudio (grupo inmune) consistió en pacientes tratados durante la pandemia de COVID -19 (entre Enero 2021 y Agosto 2021) quienes se recuperaron de la infección por COVID -19 o recibieron la vacuna mARN contra SARS-CoV-2. Los grupos controles correspondieron a pacientes que fueron tratados durante la pandemia COVID-19 (entre Enero 2021 y Agosto 2021) pero que no estuvieron infectados o no habían recibido la vacuna mARN contra SARS-CoV-2 (grupo no inmune 2021) y aquellas pacientes tratadas entre Enero 2019 y Agosto 2019 (antes de la pandemia) (grupo no inmune 2019).

Intervención(es): Transferencia de embriones congelados-descongelados (TEC).

Medida(s) de desenlace principal: Tasas de embarazo en curso y características de ciclos de TEC . Datos sobre la edad de las pacientes y variables relacionadas con el tratamiento de su infertilidad fueron recolectados de las historias clínicas.

Resultado(s): Durante el período del estudio a 428 pacientes se les practicaron 672 ciclos de TEC. El grupo inmune consistió en 141 pacientes quienes recibieron 264 ciclos de TEC (44 post-infección y 220 post-vacunación) , mientras que los grupos no inmune 2021 y no inmune 2019 consistieron en 93 y 194 pacientes que recibieron 125 y 283 ciclos de TEC, respectivamente. Las características de las pacientes y las modalidades de preparación endometrial fueron comparables entre los grupos del estudio. Las tasas de implantación, de embarazo clínico y de embarazo en curso por transferencia , fueron similares entre los grupos del estudio (grupo inmune, post-infección y post-vacunación; grupo no inmune 2021; grupo no inmune2019).

Conclusión(es): La enfermedad por infección por Coronavirus 2019 o la vacunación, no afectó el desempeño de las pacientes o su tasa de implantación en los ciclos subsecuentes de TEC. (Fertil Steril[®] 2022; [©]2022 por la Sociedad Americana de Medicina Reproductiva)