IVF under COVID-19: treatment outcomes of fresh ART cycles

Running title: SARS-CoV-2 infection and ART treatment outcomes

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Abstract

Study question: Does prior SARS-CoV-2 infection in women undergoing fertility treatments affect the outcomes of fresh ART cycles?

Summary answer: SARS-CoV-2 infection does not affect fresh ART treatment outcomes, except for a possible long term negative effect on oocyte yield (>180 days post infection).

What is known already: A single previous study suggested no evidence that a history of asymptomatic or mild SARS-CoV-2 infection in females caused impairment of fresh ART treatment outcomes.

Study design, size, duration: Retrospective cohort study, including all SARS-CoV-2 infected women who underwent fresh ART cycles within a year from infection (the first cycle post infection), between October 2020 and June 2021, matched to non-diagnosed controls.

Participants/materials, setting, methods: Patients from two large IVF units in Israel who were infected with SARS-CoV-2 and later underwent fresh ART cycles were matched by age to non-diagnosed, non-vaccinated controls. Demographics, cycle characteristics and cycle outcomes, including oocyte yield, maturation rate, fertilization rate, number of frozen embryos per cycle, and clinical pregnancy rates, were compared between groups.

Main results and the role of chance: One hundred and twenty-one infected patients and 121 controls who underwent fresh ART cycles were included. Oocyte yield (12.50 versus 11.29; p=0.169) and mature oocyte rate (78% versus 82%; p=0.144) in all fresh cycles were similar between groups, as were fertilization rates, number of frozen embryos per cycle and clinical pregnancy rates (43% versus 40%; p=0.737) in fresh cycles with an embryo transfer. In a logistic regression model, SARS-CoV-2 infection more than 180 days prior to retrieval had a negative effect on oocyte yield (p=0.018, Slope=-4.08, 95% CI -7.41 – -0.75), although the sample size was small.

Limitations, reasons for caution: A retrospective study with data that was not uniformly generated under a study protocol, no antibody testing for the control group.

Wider implications of the findings: The study findings suggest that SARS-CoV-2 infection does not affect treatment outcomes, including oocyte yield, fertilization and maturation rate, number of good quality embryos, and clinical pregnancy rates, in fresh ART cycles, except for a possible long term negative effect on oocyte yield when retrieval occurs > 180 days post SARS-CoV-2 infection. Further studies are warranted to support these findings.

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Key words: IVF, COVID-19, infertility, SARS-CoV-2, pregnancy, oocytes

Introduction

Corona Virus disease 19 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). SARS-CoV-2 enters target host cells via the cellular receptor angiotensin-converting enzyme 2 (ACE2) and the cellular transmembrane protease serine-2 (TMPRSS2) (Lukassen *et al.*, 2020). In theory, organs with a high expression of ACE2 or TMPRSS2 are more vulnerable to infection (Zou *et al.*, 2020). The COVID-19 pandemic has raised concerns regarding the possible effect on human fertility, especially for couples undergoing fertility treatment. The male component has been the focus of most studies investigating the virus' effect on fertility, given the abundance of ACE2 receptors and TMPRSS2 in the testis tissue (Anifandis *et al.*, 2020; Jing *et al.*, 2020; Li *et al.*, 2020a, 2020b; Gacci *et al.*, 2021; Guo *et al.*, 2021).

There is evidence that the renin–angiotensin–aldosterone system (RAS) is involved in female reproductive processes such as folliculogenesis, steroidogenesis, oocyte maturation and ovulation. The existence of the ACE2 axis and ACE2 markers were confirmed in all stages of follicular maturation in the human ovary, including the granulosa cells and follicular fluid (Reis *et al.*, 2011; Jing *et al.*, 2020; Anifandis *et al.*, 2021; Choi *et al.*, 2021). ACE2 and TMPRSS2 are also expressed in the endometrium, possibly affecting implantation (Vaz-Silva *et al.*, 2009; Henarejos-Castillo *et al.*, 2020).

Furthermore, as with other viral infections, it can be assumed that SARS-CoV-2 may promote oxidative stress through oxidant-sensitive pathways, leading to activation of pathogenic mechanisms (Barzon *et al.*, 2017; Liu *et al.*, 2017; Khomich *et al.*, 2018). Increased oxidative stress may affect male fertility through reduction in motility and an increase in sperm DNA fragmentation (Bisht *et al.*, 2017; Agarwal *et al.*, 2018; Homa *et al.*, 2019). Similarly, SARS-CoV-2 may affect oocyte performance through mechanisms that increase oxidative stress which has been associated with alterations in DNA methylation (Menezo *et al.*, 2016).

Given these considerations, it is reasonable to suspect that COVID-19 may affect oocyte performance or early implantation. Nevertheless, to date, the possible effects of COVID-19 on female fertility are largely unknown, and the effects on IVF outcomes has yet to be elucidated (Setti *et al.*, 2021; Wang *et al.*, 2021). In this study we aimed to evaluate the effect of female SARS-CoV-2 infection on the outcomes of IVF treatments in fresh cycles.

Materials and methods

A retrospective cohort study, including all SARS-CoV-2 infected women aged 20-42 years that underwent fresh IVF treatment cycles between January 1, 2021, and June 31, 2021, at Shamir Medical Center and Herzliya medical center, Israel (COVID group). To be included in the study, maximal time from SARS-CoV-2 infection to treatment was defined as one year. Only the first cycle following recovery was included. The study was approved by the Institutional Review Board of both participating medical centers.

The study group was matched by age to the first following non-vaccinated patient with no history of past infection, who underwent IVF treatments at the same time period (October 2020- June 2021) (control group). Stimulation protocols, fertilization methods and embryo transfer parameters were individually tailored by the treating team, as per usual institutional routine. Demographic characteristics (including age, partner's age and COVID status, smoking status, number of previous pregnancies, deliveries and IVF treatments and infertility cause) as well as cycle characteristics (treatment protocol, overall gonadotropins (GT) administered, Estrogen levels on day of ovulation triggering (maximal E2), fertilization method and endometrial thickness) were recorded. Primary outcome measures were the mean number of retrieved oocytes per cycle and clinical pregnancy rates (defined as an intrauterine gestational sac on ultrasound imaging). Secondary outcomes included MII (mature oocyte) rates (MII/oocytes retrieved – in ICSI only cycles), fertilization rates (2PN/oocytes retrieved), and mean number of vitrified embryos. As varying time from infection to retrieval may have a different pathophysiologic effect on cycle outcomes, further stratification by time from SARS-CoV-2 infection to retrieval into groups of ≤ 90, 90-180 and > 180 days was performed. For the purpose of pregnancy rates, fertilization rates and number of vitrified embryos, only women undergoing embryo transfer were included. Embryo grading was based on the Istanbul consensus workshop parameters (Balaban *et al.*, 2011).

Data analysis

Shapiro & Wilk test was used to test for normality of distribution. Continuous variables were summarized with mean and 95% CI and compared between groups using the Mann-Whitney test. Categorical variables were summarized using counts and percentages. The Fisher Exact Test or Chi-square test were used to compare differences between groups.

A logistic regression model was applied to identify factors associated with clinical pregnancy rates. Backwards elimination was applied to select the optimal model, while the age & COVID group were forced to be included in the model. To confirm the adequacy of the model we have applied the models including the minimal selected variables with similar results.

A linear regression model was applied to identify factors related to the total number of oocytes retrieved. No imputations for missing data were applied.

A two-sided $P<0.05$ was considered significant. R core team (2021). R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. Multivariate analyses were conducted using SPSS-27 software, IBM, Armonk, NY, USA.

Results

All cycles

One hundred and twenty-one women in the study group and 121 women in the control group were included (Table I). The mean time from SARS-CoV-2 infection to oocyte retrieval was 84.5 days (SD 78.02; range 8-348). Mean age was similar in the study and control groups (33.3 versus 33.2 years respectively), as were mean partner's age, smoking rates and BMI. No differences were observed in the obstetrical history, infertility cause and number of prior IVF treatments. Patients in the study group and the control group had similar cycle characteristics in terms of stimulation protocol, total GT dosage, maximal E2 levels and endometrial thickness. The mean number of oocytes retrieved per cycle (12.50 versus 11.29; p=0.169) and the rate of mature oocytes in ICSI cycles (78 versus 82; p=0.144) were similar between groups.

Similarly, a univariate analysis, with stratification by time from SARS-CoV-2 infection to treatment (≤90 day, 90-180 days and > 180 days), revealed no differences between groups in any of the parameters (Supplementary Table SI).

A linear regression model for oocyte yield in all patients including patients' age, previous transfer and past SARS-CoV-2 infection, demonstrated no effect of COVID-19 status on oocyte yield (p=0.104), while age remained a significant factor, reducing the number of oocytes by 0.64 for every additional year (p<0.001) (Table II). In a sub analysis of the linear regression model according to time from SARS-CoV-2 infection (Table II), while age remained a significant factor, the COVID status was not significant in the first two groups (≤90 day, 90-180 days). In the small subgroup (29 patients) with a past infection > 180 days, a negative effect on oocyte yield was observed (p=0.018, slope=-4.08, 95% CI -7.41 – -0.75). A Bonferroni correction for multiple comparisons attenuated this result (p=0.054).

Cycles with an embryo transfer

Ninety-one of 121 women in the COVID group and 94 of 121 women in the control group underwent embryo transfer and were included in the pregnancy rate analysis (Table III). Of the 57 patients who did not undergo embryo transfer and were excluded from this analysis the majority were treated for fertility preservation (medical or social), underwent genetic testing, or had a hyper-response preventing embryo transfer. Only one patient from each group, both with an infertility diagnosis of premature ovarian insufficiency, did not undergo embryo transfer, without a pre-planned indication.

Demographic characteristics were similar in both groups (Supplementary Table SII). Partners' COVID status significantly differed between groups with higher rates of recovered and vaccinated partners in the COVID group ($p<0.001$). Cycle characteristics were similar between groups except for the fertilization method, with a higher intra-cytoplasmic sperm injection (ICSI) rate in the COVID group (70% versus 50%; $p=0.009$). Number of oocytes retrieved, mature oocytes, fertilization rates and number of vitrified embryos were similar between groups. Number of embryos transferred, and the day of transfer did not differ, but significantly more embryos graded C were transferred ($p=0.007$) in the control group with no difference in grade A and B embryos. Clinical pregnancy rates were similar between groups (43% versus 40%; $p=0.737$).

Stratifying by time from SARS-CoV-2 infection to treatment ≤90 day, 90-180 days and > 180 days (Supplementary Table SIII), pregnancy rates (41% versus 30%, $p=0.19$; 38% versus 67%, $p=0.063$; 58% versus 46%, $p=0.54$ respectively), mature oocytes, fertilization rate and number of vitrified embryos were similar between the COVID and control groups.

A backwards multivariate logistic regression model for pregnancy rate (Table IV) (including age, previous transfers, number of embryos transferred, day of transfer, embryo grade, endometrial thickness, number of oocytes retrieved, and fertilization method) was performed showing no effect of past SARS-CoV-2 infection on pregnancy rates ($p=0.889$). Patient age and endometrial thickness were the only significant variables. The same model

199 was applied for patients having an embryo transfer within 90 days of SARS-CoV-2 infection,
200 with patient age being the only significant variable (Table IV). The groups of patients with
201 SARS-CoV-2 infection 90-180 days and > 180 days before transfer were too small for
202 inclusion in the model.

203

Discussion

In this retrospective cohort study, past infection with SARS-CoV-2 had no impact on fresh IVF treatment outcomes in terms of oocyte yield, maturation rate, fertilization rate, number of vitrified embryos and clinical pregnancy rates, except for a possible long-term effect on oocyte yield (retrieval > 180 days post infection).

The COVID-19 pandemic has had a profound psychosocial impact on fertility patients, which was especially apparent at the beginning of the pandemic, when fertility treatments were suspended in many countries (Ben-Kimhy *et al.*, 2020; Boivin *et al.*, 2020; Marom Haham *et al.*, 2021). The purpose of our study was to examine whether, in addition, there was a measurable effect of SARS-CoV-2 infection on fertility treatment outcomes. To the best of our knowledge, this is the largest study to date reporting the effect of prior SARS-CoV-2 infection on fertility treatment outcomes in fresh ART cycles.

SARS-CoV2 enters cells via the ACE-2 cellular receptor and the TMPRSS2 cellular protease. Those are expressed in all stages of follicular maturation in the human ovary, in the granulosa cells and in the endometrium (Reis *et al.*, 2011; Jing *et al.*, 2020; Anifandis *et al.*, 2021; Choi *et al.*, 2021). In this study, the effect on the follicular development, maturation and ovulation was evaluated by oocyte yield, maturation and number of good quality embryos (vitrified). Further effects on embryo development and implantation were evaluated by pregnancy rates.

We found that recent past infection with SARS-CoV-2 (<180 days) had no influence on treatment outcomes in terms of oocyte yield and maturation. This result is in line with the limited literature published to date (Setti *et al.*, 2021; Wang *et al.*, 2021). As the dominant follicle originates from a primordial follicle that has been recruited up to one year earlier (Gougeon, 1986; Erickson and Shimasaki, 2001), we stratified the participants by time from infection to evaluate different possible mechanisms of influence. Shortly after the infection, in addition to direct viral cell invasion, possible oxidative stress may affect ovarian function,

compromising antral and preovulatory follicular growth and development. Furthermore, endometrial cellular damage seems more likely in proximity to the acute infection. We hypothesized that a possible differential effect in oocyte yield and maturation would be apparent for more than 90 days after infection in case the growing follicle was damaged in its earlier developmental stages. Nevertheless, no difference was observed in either outcome when the infection occurred up to six months prior to treatment (sub-groups (≤ 90 and 90-180 days). These results were consistent in all regression models performed, including the linear model for oocyte yield and the logistic regression model for pregnancy rates, providing reassurance that recent SARS-CoV-2 infection does not compromise IVF treatment outcomes.

Further analysis of women infected more than 180 days prior to treatment was performed to assess for long-term effects as a result of possible damage to the primordial follicles or during the initial recruitment process. In this subgroup of patients, lower oocyte yields were observed, while all other parameters were unaffected. It should be noted however that the sample size was small, thus cautious interpretation of the results is warranted.

In univariate analyses of cycles with embryo transfer, a significant difference was observed in the fertilization method. ICSI was more commonly used in the COVID group whereas IVF was more commonly used in the control group, despite the fact that there was no difference in infertility causes. This may possibly be explained by the lab evaluation of partner sperm prior to fertilization. Partners' COVID status was, as expected, more likely to show past infection in the COVID group. Prior studies have reported a decrease in sperm parameters, potentially explaining the higher ICSI rates in the COVID group. However, fertilization method was not found to be significantly associated with the pregnancy rate in the multivariate regression model.

The strength of our study is our relatively large sample of women that tested positive for SARS-CoV-2, allowing us to evaluate different stages of the IVF procedure, including

oocyte development, maturation and embryo implantation, at different time points after the acute infection. The main limitation of the 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 lack of sperm analyses, which, especially given the possible effect of SARS-CoV-2 infection on sperm parameters and significant difference in rates of recovered partners between groups, may explain the significant difference in the fertilization method utilized. Even so, fertilization rates and embryo grade were similar between groups. Another limitation is the fact that women in the control group were chosen based on medical history and did not undergo antibody testing.

In conclusion, recent past SARS-CoV-2 infection, less than 180 days prior to IVF treatment, did not affect oocyte yield, fertilization, maturation and clinical pregnancy rates in fresh IVF cycles. A possible long-term effect (>180 days) compromising oocyte yield was observed. Further studies are warranted in order to support these findings with special attention to the long-term effects.

Data availability: The data underlying this article will be shared on reasonable request to the corresponding author.

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373 **Table I:** Demographic and cycle characteristics and outcomes of COVID versus control group in fresh
374 cycles. Data are presented as mean and (SD) and [range] or counts and (percentage)
375

Group	COVID-19 (N=121)	Non COVID-19 (N=121)	p value
Patient age (y)	33.3 (5.37) [21-42]	33.23 (5.33) [22-42]	0.896
Partners age (y)	35.78 (6.90) [22-55]	34.39 (5.45) [21-48]	0.2
Smoker	14 (12%)	17 (15%)	0.445
Previous retrievals	1.07 (1.60) [0- 8]	1.19 (1.48) [0.00 - 8.00]	0.156
Previous transfers	1.11 (2.18) [0 – 12]	1.25 (1.92)	0.087
BMI	25.25 (5.55) [16.23 - 42.97]	25.48 (5.86) [16.53 - 42.45]	0.959
Infertility cause (N)	110	102	0.209
Age related	14 (13%)	19 (19%)	
Male factor	32 (29%)	35 (34%)	
Ovulation	6 (5%)	11 (11%)	
Mechanical	11 (10%)	4 (4%)	
Unexplained	27 (25%)	17 (16%)	
Fertility preservation	12 (11%)	8 (8%)	
Other	8 (7%)	8 (8%)	
Parity (N)	104	95	0.519
0	71 (68%)	66 (70%)	
1	19 (18%)	21 (22%)	

>=2	14 (14%)	8 (8%)	
Gravidity (N)	105	93	0.993
0	65 (62%)	55 (59%)	
1	18 (17%)	21 (23%)	
>=2	22 (21%)	17 (18%)	
Days from COVID to oocyte retrieval	84.54 (78.02) [8-348]	NA	
<=90	77 (64%)	NA	
>90-180	29 (24%)	NA	
>180	15 (12%)	NA	
Protocol			0.177
Antagonist	106 (88%)	104 (87%)	
Long luteal	6 (5%)	6 (5%)	
MNC	1 (1%)	6 (5%)	
Short (flare)	8 (6%)	4 (3%)	
Gonadotropins dosage (IU)	2524 (1317)600- 7800	2335 (1220)348 - 6600	0.255
Max. E2 (pmol/L)	8584 (6191) [1337–31650]	8842 (6415) [456–35898]	0.824
Endometrial thickness (mm)	10.53 (2.29) [4.5 - 17.1]	9.97 (2.29) [4.6 – 16]	0.108
Oocytes retrieved	12.50 (7.83) [0 – 40]	11.29 (7.60) [1 – 39]	0.169
Fertilization method (N)	112	113	0.004
ICSI	82 (73%)	60 (53%)	0.002*
ICSI/IVF	25 (22%)	39 (34%)	0.043*
IVF	5 (5%)	14 (12%)	0.033*
MII /oocytes(%)(ICSI only)(N)	80	60	0.144
	78 (18.03) [25 – 100]	82 (18.86) [33.33- 100]	
Total available embryos	3.41 (2.71) [0-13]	3.72 (2.77) [0-16]	0.398
Partner COVID status (N)	76	84	<0.001
Recovered	48 (63%)	0	
Vaccinated	17 (22%)	9 (11%)	0.046*
Non	11 (15%)	75 (89%)	<0.001*

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377 *Post hoc analysis

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Table II: Linear Regression model for number of oocytes retrieved – total sample and subdivided by time from COVID-1***9***

Patients included	N	Variables	P value	Slope	Lower 95% CI	Upper 95% CI
All	227	Group	0.164	1.285	-0.53	3.10
		Patient age	<0.001	-0.638	-0.82	-0.46
		Previous retrievals	0.810	-0.075	-0.69	0.54
Days ≤ 90	142	Group COVID versus control	0.172	1.70	-0.75	4.14
		Patient age	<0.001	-0.61	-0.83	-0.39
		Previous retrievals	0.815	-0.10	-0.98	0.77
90-180	56	Group COVID versus control	0.125	2.81	-0.805	6.432
		Patient age	0.001	-0.69	-1.086	-0.302
		Previous retrievals	0.362	0.54	-0.638	1.721
>180	29	Group COVID versus. control	0.018	-4.08	-7.41	-0.75
		Patient age	0.055	-0.51	-1.04	0.01
		Previous retrievals	0.061	-0.96	-1.97	0.05

Table III: Cycle characteristics and outcomes of COVID versus control group - fresh embryo transfer cycle. Data are presented as mean and (SD) and [range] or counts and (percentage).

Group	COVID-19 (N=91)	Non COVID-19 (N=94)	p value
Gonadotropin dosage (IU)	2529.42 (1418) [600-7800]	2334.70 (1269) [600-6600]	0.365
Max. E2 (pmol/L)	7598 (5375) [1337-28382]	7510 (5151) [456-25022]	0.939
Endometrial thickness (mm)	10.75 (2.25) [6-17]	9.99 (2.22) [5.4-15]	0.062
Oocytes rerieved	11.26 (6.19) [1-33]	10.04 (6.90) [1-31]	0.085
Fertilization method	91	93	0.009
ICSI	64 (70%)	46 (50%)	
ICSI/IVF	22 (24%)	33 (35%)	
IVF	5 (6%)	14 (15%)	
Percent MII /oocytes (ICSI) (N)	64	46	0.072
	77.60 (18.87) [25-100]	83.08 (19.98) [33-100]	
Fertilization rate	0.59 (0.24) [0.07-1]	0.62 (0.26) [0-1]	0.365
Total frozen embryos	1.71 (2.40) [0-15]	2.12 (2.34) [0-11]	0.168
No. of embryos transferred			0.545
1 2 3	57 (63%) 30 (34%) 3 (3%)	63 (67%) 30 (32%) 1 (1%)	
Day of transfer			0.252
2	16 (17%)	26 (28%)	
3	57 (63%)	53 (56%)	
5	18 (20%)	15 (16%)	
Embryo grade			0.015
A	52 (58%)	54 (57%)	
B	35 (39%)	26 (28%)	
C	3 (3%)	14 (15%)	0.007*
Clinical pregnancy	39 (43%)	38 (40%)	0.737
Partner COVID status (N)	63	68	<0.001
Recovered	40 (63%)	0	
Vaccinated	13 (21%)	7 (10%)	
Non	10 (16%)	61 (90%)	

*Post hoc analysis

Table IV: Logistic regression model for clinical pregnancy rates in fresh embryo transfer – Whole group and subgroup of patients with a recent infection

Patients included	N	%	Variables	P value	Odds Ratio	Lower 95% CI	Upper 95% CI
All	154	83	Previous transfers	0.302	0.895	0.724	1.105
			Embryo grade	0.226			
			Embryo grade B versus A	0.267	0.659	0.315	1.377
			Embryo grade C versus A	0.135	0.330	0.077	1.414
			Endometrial thickness	0.031	1.194	1.017	1.402
			Group COVID vs control	0.889	1.052	0.517	2.140
			Patient age >39y versus <=39y	0.040	0.249	0.066	0.939
			Number of embryos transferred (1 versus 2+3)	0.351	1.457	0.661	3.215
≤90 days from COVID	95	81	Embryo grade	0.260			
			Embryo grade B versus A	0.960	0.975	0.366	2.602
			Embryo grade C versus A	0.104	0.162	0.018	1.452
			Endometrial thickness	0.122	1.173	0.958	1.435
			Group COVID versus control	0.320	1.596	0.636	4.008
			Patient age >39y versus <=39y	0.037	0.179	0.036	0.903
			Number of embryos transferred (1 versus. 2+3)	0.933	1.045	0.376	2.904