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ARTICLE



The effect of SARS-CoV-2 mRNA vaccination on AMH concentrations in infertile women



BIOGRAPHY

Since 2007 Eran Horowitz has worked as a senior physician at the IVF unit, Department of Obstetrics and Gynecology, Wolfson Medical Center, Holon, and Sackler Faculty of Medicine, Tel Aviv University, Israel, where he currently holds the position of Senior Lecturer. He has published more than 40 scientific papers and book chapters.

Eran Horowitz^{1,2,*}, Yossi Mizrachi^{1,2}, Hadas Ganer Herman^{1,2}, Einat Oz Marcuschamer^{3,4}, Amir Shalev^{1,2}, Jacob Farhi^{1,2}, Elad Barber^{1,2}, Schwartz Harari Orna⁵, Arieh Raziel^{1,2}, Ariel Weissman^{1,2}

KEY MESSAGE

In this study COVID-19 vaccination was not associated with a short-term reduction of ovarian reserve in women undergoing IVF, and anti-Müllerian hormone concentrations were similar before and after vaccination. These findings may serve as a counselling tool for clinicians to reassure women undergoing fertility treatment that SARS-CoV-2 mRNA vaccination is safe.

ABSTRACT

Research question: Does SARS-CoV-2 mRNA vaccination affect the ovarian reserve of infertile women undergoing IVF?

Design: This was a prospective observational study at a single university-affiliated IVF unit that included infertile women aged 18–44 years who were undergoing IVF/intracytoplasmic sperm injection between November 2020 and September 2021, had received two doses of SARS-CoV-2 mRNA vaccination and had undergone measurement of baseline anti-Müllerian hormone (AMH) concentration within the 12 months preceding their recruitment. AMH concentrations before and after vaccination were evaluated and compared.

Results: Overall, 31 women were included in the study. The median AMH concentrations before and after COVID-19 vaccine were comparable (1.7 versus 1.6 g/ml, respectively, P = 0.96). No correlation was found between the participant's anti-COVID-19 antibody titre and the change in AMH concentration.

Conclusions: SARS-CoV-2 mRNA vaccination does not adversely affect ovarian reserve, as shown by comparing serum AMH concentrations before and after vaccination. These findings may serve as a counselling tool for clinicians to reassure women undergoing fertility treatment that SARS-CoV-2 mRNA vaccination is safe.

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KEYWORDS

Anti-Müllerian hormone COVID-19 Fertility Ovarian reserve SARS-CoV-2 mRNA vaccine

¹ IVF Unit, Department of Obstetrics and Gynecology, Edith Wolfson Medical Center, Holon, Israel

² Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

³ Department of Obstetrics and Gynecology, Assuta Medical Center Ashdod, Israel

⁴ Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

⁵ Microbiology and Immunology Laboratory, Edith Wolfson Medical Center, Holon, Israel

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INTRODUCTION

n December 11th 2020 , the Food and Drug Administration (FDA) approved the first mRNA SARS Covid-19 vaccine of Pfizer-BioNTech (FDA, 2021), Studies show that the incidence of COVID-19 infection, hospitalization and death is higher among people who are unvaccinated compared with those who are fully vaccinated (Ghasemiyeh et al., 2021). It has been clearly demonstrated that unvaccinated individuals are 11 times more likely to die from COVID-19 than people who are fully vaccinated, and those who are unvaccinated are 12 times more likely to be hospitalized with COVID-19 compared with those who are fully vaccinated.

Upon approval of the vaccine, Israel immediately initiated an anti-COVID-19 vaccination programme in December 2020, mostly using the Pfizer-BioNTech vaccine (BNT162b2 mRNA). Up to 22 January 2022, 6,683,280 Israeli residents aged 12 years and older had received a first dose of the vaccine, and 6,052,023 had received a second dose, corresponding to 72% and 66% of Israel's residents, respectively (Israeli-Ministry-of-Health).

It has been demonstrated that COVID-19 infection puts pregnant women and their fetuses at increased risk of severe complications and even death, and that COVID-19 vaccination is the best method to reduce maternal and fetal complications of COVID-19 infection without any detrimental effect of the vaccine on the pregnant woman or the fetus (Dagan et al., 2021). Therefore, individuals of reproductive age, both female and male, who were planning a pregnancy, including those who were undergoing fertility treatment, were not excluded from the national vaccination programme and were strongly encouraged to receive the vaccine.

Although the amount and quality of information available regarding the effect of COVID-19 vaccines on human fertility are fragmentary and incomplete, the available literature has so far been positive and reassuring. In male subjects, it has been shown that there is no injurious effect of COVID-19 vaccination on semen parameters (Gonzalez et al., 2021; Lifshitz et al., 2021; Safrai et al.,

2022). In female patients undergoing IVF, SARS-CoV-2 mRNA has been proved to have no detrimental effect on follicular function (Bentov et al., 2021) or performance during fresh (Orvieto et al., 2021a; Safrai et al., 2021) or frozen (Aizer et al., 2022) IVF/intracytoplasmic sperm injection (ICSI) cycles. In healthy, reproductive-aged women who were not infertile, ovarian reserve as assessed by serum anti-Müllerian hormone (AMH) concentrations was found not to be altered at 3 months following mRNA SARS-CoV-2 vaccination (Mohr-Sasson et al., 2021).

Nevertheless, there are emerging public concerns about the safety of the vaccines in general, in particular their effect on female and male fertility (Diaz et al., 2021; Robinson et al., 2021; Sajjadi et al., 2021; Turocy et al., 2021), which lead to hesitancy and delays in the uptake of vaccination. Therefore, the aim of the current study was to evaluate the effect of SARS-CoV-2 mRNA on ovarian reserve, as shown by serum AMH concentrations before and after vaccination, in a cohort of infertile patients.

MATERIALS AND METHODS

Study population

A prospective observational study was conducted at a single universityaffiliated IVF unit between November 2020 and September 2021. It included women aged 18-44 years who were undergoing IVF/ICSI in the unit's programme. Women with a baseline AMH concentration measured up to 12 months before the time of recruitment were invited to participate in the study. The study was approved on 1 June 2021 by the local Institutional Review Board (0113-21-WOMC) and was registered at the clinical trial registry and assigned the registration number NCT05034679. All the women gave their written informed consent.

Data collection

Data were retrieved from a computerized database. For every woman, the following background information was documented: age, body mass index, tobacco smoking status, obstetric history, infertility type (primary or secondary), duration and aetiology of infertility, basal FSH concentration and antral follicle count. Data were collected on the COVID-19 status of the women: whether

they were vaccinated for COVID-19, had recovered from a COVID-19 infection, or were non-infected/unvaccinated. For all participants, a blood test was taken for anti-COVID-19 antibodies and AMH concentration. Serum AMH concentrations were compared with the baseline AMH concentration that had been measured within 12 months prior to recruitment into the study.

Serum AMH concentrations were measured on a Cobas e 801 analyser (Roche Diagnostics, Germany) using an Elecsys AMH Plus Immunoassay (measurement range 0.01-23 ng/ ml). The samples for SARS-CoV-2 antibodies were sent for evaluation to an accredited laboratory and were considered positive when signal-to-cutoff values were ≥50.0 (Anti SARS-CoV-2 IgG Quant, Abbott Laboratories, USA). This assay is an automated, twostep immunoassay for the qualitative and quantitative determination of immunoglobulin G antibodies to SARS-CoV-2 in human serum and plasma using chemiluminescent microparticle immunoassay technology.

Statistical analysis

Statistical analysis was performed using SPSS software version 28.0 (IBM Corp., USA). Baseline continuous variables were presented as mean ± SD, and nominal variables as n (%). AMH concentrations were found to deviate from a normal distribution and were presented as medians. The comparison between AMH concentrations before and after COVID-19 vaccination was performed using the Wilcoxon test. Spearman's correlation coefficient was calculated in order to assess the correlation between the titre of COVID-19 antibodies and the change in AMH concentrations. A P-value < 0.05 was considered statistically significant. A post-hoc power analysis showed that the study sample size was sufficient to detect a 50% decrease in AMH concentration before and after vaccination.

RESULTS

Overall, 45 participants were recruited. Five women who had recovered from COVID-19 infection and nine who had not received the vaccine and were not infected were excluded. Therefore, 31 women who had received two doses of the mRNA COVID-19 vaccine were included in the final analysis.

TABLE 1 PATIENTS' BASELINE CHARACTERISTICS		
Characteristic		
Age (years)	35.5 ± 4.7	
BMI (kg/m²)	24.2 ± 4.5	
Smoking	8 (25.8)	
Type of infertility		
Primary	12 (38.7)	
Secondary	19 (61.3)	
Duration of infertility (years)	1.5 ± 1.1	
Nulliparity	17 (54.8)	
Baseline FSH (IU)	8.7 ± 3.9	
Baseline LH (IU)	5.5 ± 2.2	
Antral follicle count	14.2 ± 8.3	

Data are presented as mean \pm SD or n (%).

The baseline characteristics of the women who participated in the study are presented in TABLE 1. The mean age of the participants was 35.5 ± 4.7 years. Mean baseline FSH and antral follicle count were 8.7 ± 3.9 and 14.2 ± 8.3 , respectively. TABLE 2 describes the indications for IVF treatment, with male factor infertility being the prominent indication (29%).

The median time interval between the two AMH measurements (before and after vaccination) was 6 months (range 2–11 months). The median time interval between COVID-19 vaccination and the second AMH test was 4 months (range 3–9 months). The median AMH concentrations before and after COVID-19 vaccination were comparable (1.7 versus 1.6 ng/ml, P=0.96) (FIGURE 1). The mean antibody titre was 1547 \pm 1671 arbitrary units/ml (median 1070, range

234–7867 arbitrary units/ml). The titre did not correlate with the change in AMH concentration (Spearman's correlation coefficient 0.20, P = 0.18).

DISCUSSION

This study evaluated whether COVID-19 mRNA vaccine would affect serum AMH concentrations, as a reliable measure of ovarian reserve, in a cohort of infertile women. Serum AMH concentrations remained unchanged within 4 months (range 3–9 months) after vaccination compared with pre-vaccination concentrations, suggesting that the COVID-19 mRNA vaccine has no short-term deleterious effect on ovarian reserve.

Since the outbreak of the COVID-19 pandemic, concerns have been raised about potential adverse effects of the

virus on the reproductive health of both female and male patients. The potential destructive mechanism of COVID-19 infection on the male and female reproductive system is by binding of the viral spike protein (protein S) to the angiotensin-converting enzyme 2 (ACE2) receptors in the reproductive organs (Jing et al., 2020). The available evidence suggests that ACE2 is expressed in the ovary, uterus, vagina and placenta, as well as in the male testis (Chen et al., 2021; Li et al., 2020b). Previous studies have demonstrated an enhanced risk of sperm count abnormalities among men with existing COVID-19 infection and those who had recovered (Gacci et al., 2021; Li et al., 2020a).

The effect of COVID-19 infection on the female reproductive system has not been fully elucidated. Preliminary data have suggested a potential for ovarian injury during active SARS-CoV-2 COVID-19 infection, including a detrimental effect on ovarian reserve and reproductive endocrine function (Ding et al., 2021). Li and colleagues (Li et al., 2021) demonstrated that nearly one-fifth of women who recovered from active COVID-19 infection exhibited a menstrual volume decrease or cycle prolongation, which could be the consequence of transient sex hormone changes caused by a suppression of ovarian function that quickly resumes after recovery. In a prospective observational study, Kolanska and co-workers (Kolanska et al., 2021) have demonstrated that a history of mild COVID-19 infection does not seem to alter the ovarian reserve as evaluated by AMH concentrations. In a small observational study, Orvieto and collaborators (Orvieto et al., 2021b) studied nine couples before and after recovery from COVID-19 infection, who resumed IVF treatment 8-92 days after recovery. No between-cycle differences were observed in stimulation and embryological characteristics before and after recovering from the COVID-19 infection, except for a significantly lower proportion of top-quality embryos, which became a reason for caution and concern raised by the authors.

The effect of COVID-19 mRNA vaccination on human reproductive function has become a subject of great interest, and robust research efforts are currently being made in order to clarify a variety of fertility-related issues. There have been numerous reports by

TABLE 2 INDICATIONS FOR TREATMENT

Indication	
Male factor	9 (29.0)
Unexplained	5 (16.1)
Low ovarian reserve	3 (9.7)
Tubal factor	1 (3.2)
Social fertility preservation	4 (12.9)
Anovulation	1 (3.2)
PGT-M	3 (9.7)
Repeated miscarriage	1 (3.2)
Advanced maternal age	3 (9.7)
Female partner	1 (3.2)

Data are presented as n (%).

PGT-M, preimplantation genetic testing for monogenic/single gene defects.

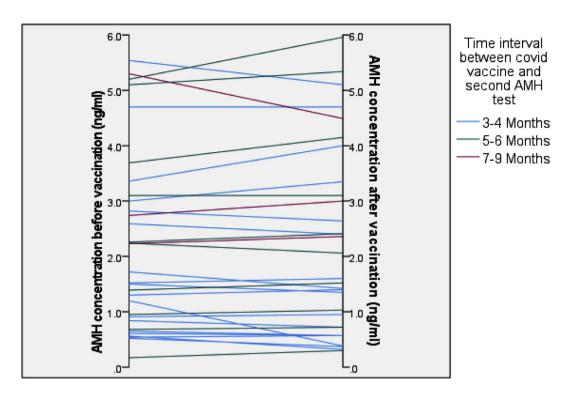


FIGURE 1 Anti-Müllerian hormone (AMH) concentrations before and after COVID-19 vaccination, according to the time interval between vaccinations.

women to primary care clinicians and those working in reproductive health that COVID-19 vaccines cause a disruption of menstrual cycles and induce unusual menstrual symptoms (Alvergne et al., 2021; Male, 2021). In a recent large UK retrospective study, following vaccination for COVID-19 menstrual disturbances occurred in 20% of participants (Alvergne et al., 2021). Although reported changes to the menstrual cycle after vaccination are short lived, such reports raise fears about the impact of vaccination on fertility and contribute to vaccine hesitancy.

Regarding natural fertility, the association of COVID-19 vaccination and SARS-CoV-2 infection with fertility among couples trying to conceive naturally was recently examined using data from an internet-based preconception cohort study (Wesselink et al., 2022). It has been demonstrated that male COVID-19 infection may be associated with a short-term decline in fertility and that COVID-19 vaccination does not impair fertility in either partner. Regarding male fertility, recent studies have shown that both BNT162b2 mRNA and mRNA-1273 (Moderna) vaccination have no influence on sperm parameters, including sperm concentration, semen volume, sperm motility, sperm volume and total number of motile spermatozoa (Gonzalez et al., 2021; Lifshitz et al., 2021; Safrai et al., 2022).

The safety of the mRNA SARS-CoV-19 vaccine for ovarian stimulation and embryological characteristics during IVF has been recently demonstrated in several independent studies (Aizer et al., 2022; Bentov et al., 2021; Orvieto et al., 2021a; Safrai et al., 2021). Orvieto and colleagues (Orvieto et al., 2021a) compared stimulation characteristics and embryological variables in 36 couples who underwent IVF before and after receiving the mRNA SARS-CoV-2 vaccine and found no impact of the vaccine on the patients' stimulation characteristics and embryological variables as well as ovarian reserve in their immediate subsequent IVF cycle (Orvieto et al., 2021a). The same group recently reported that COVID-19 infection or vaccination did not affect patients' cycle characteristics or implantation in the subsequent frozen embryo transfer cycle (Aizer et al., 2022). Furthermore, Bentov and collaborators (Bentov et al., 2021) carried out an assessment of follicular steroidogenesis and oocyte quality and did not show any measurable difference when comparing vaccinated women with unvaccinated women and with women recovering from confirmed COVID-19 infection. Taken

together, the above studies are reassuring regarding vaccine safety and female fertility.

Whether the mRNA COVID-19 vaccine has an effect on ovarian reserve is certainly of great interest. Mohr-Sasson and co-workers have recently evaluated ovarian reserve according to serum AMH concentrations in healthy, noninfertile reproductive-aged women, before and 3 months after receiving the Pfizer-BioNTech COVID-19 vaccine (Mohr-Sasson et al., 2021). Mean AMH concentrations remained unchanged within 3 months after vaccination, and no association was found between the degree of the immune response and AMH concentrations. While our conclusions are similar, there are certain differences in the findings between the two studies. Mean serum AMH concentrations in the above study were 5.3 ± 4.29 ng/ml and 5.3 ± 4.50 mg/ml at baseline and after 3 months, respectively. In the current study, mean AMH concentrations appear to be considerably lower: 1.61 ± 2.22 versus 1.69 ± 2.22 ng/ ml, before and after vaccination, respectively. This discrepancy can be explained by the younger age (29 \pm 5.23 versus 35.5 ± 4.7 years) in the above study compared with the current one, and by the fact that our study included several patients with a priori known

low ovarian reserve (FIGURE 1). Indeed, serum AMH are in the third and fourth quartiles for age in this and Mohr-Sasson and colleagues' study, respectively (Almog et al., 2011). Nevertheless, the results of the two studies are reassuring, confirming that ovarian reserve is not compromised by the mRNA SARS-CoV-2 vaccine, which may help alleviate concerns raised by infertile patients.

The strengths of this study include the measurement of AMH by a reliable automated assay in the same laboratory for all participants before and after vaccination, thus reducing possible inconsistencies between different laboratories (Nelson et al., 2015). In addition, the median time interval between the two AMH tests (before and after vaccination) was only 6 months (range 2-11), which decreases the inherent decline in the concentration of AMH with time, allowing a focus on the direct short-term effects of the vaccination (Plociennik et al., 2018). Furthermore, the SARS-CoV-2 antibody titre was also measured, and a correlation analysis with AMH concentrations was performed.

The major limitation of the study is the relatively small number of participants included. Like other publications on the same topic (Bentov et al., 2021; Orvieto et al., 2021a; Safrai et al., 2021), the small size of the study group probably represents a compromise between the authors' eagerness to shed light on issues that are urgent for their community and the difficulty of recruiting patients for whom a full set of results would be available. A second limitation of the current study is that it does not include long-term follow up on vaccinated women. Thus, a long-term adverse effect of the vaccination on ovarian reserve cannot currently be ruled out, and longitudinal surveillance of vaccinated individuals is required. In addition, because the majority of the patients were already vaccinated when the recruitment began, only a very few patients who had recovered from COVID-19 infection or were non-infected/unvaccinated were tested, so a meaningful comparison between these three patient populations could not therefore be carried out.

In summary, these results demonstrate that the mRNA SARS-CoV-2 vaccine does not compromise ovarian reserve, as shown by comparable AMH concentrations before and after vaccination. These findings may help clinicians in counselling and reassuring patients regarding the safety of the mRNA SARS-CoV-2 vaccine. Larger studies, as well as long-term follow-up, are certainly needed.

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