Impact of Covid – 19 mRNA vaccine on Ovarian Reserve

Original Article

Mahmoud Soliman and Jehan Sadek

Department of Obstetrics and Gynecology, Kasr Al-Ainy University Hospital, Cairo University, Egypt

ABSTRACT

Background: Coronavirus disease 2019 is a serious global pandemic resulting in rising morbidities and mortalities. Global vaccination programs, using rapidly developed vaccines, resulted in widespread controversy regarding whether they may negatively impact the ovarian reserve, due to a lack of rigorous clinical trials.

Objective: To evaluate the effect of the mRNA Pfizer-BioNTech Covid-19 on anti-mullerian hormone (AMH) and antral follicle count (AFC).

Study Design: A prospective study, consisting of 115 eligible women with normal ovarian reserve, conducted from May to December 2021, at Cairo University. The women were vaccinated by two Pfizer-BioNTech Covid-19 vaccines (21 days apart). Baseline and three-month post vaccine blood samples and pelvic ultraound, were taken to determine AMH and AFC. Primary outcome was defined as the absolute and percentage change in AMH and AFC levels. A decrease of 10% or more in the serum level of AMH or AFC was considered clinically significant.

Results: A significant difference was found between baseline and three-month post vaccination AMH and AFC levels for the whole study group. The proportion of women with a greater than 10% decrease in levels of AMH and AFC following vaccination were less than those with a less than 10% decrease, in the total cohort of women, and within the sub analyses performed for three age groups: <30, 30-35 and >35 years. No significant association between adjusted risk factors (age, BMI and baseline AMH or AFC values) and percentage change of AMH or AFC.

Conclusion: The mRNA SARS–CoV–2 Pfizer/BioNTech vaccine, is not associated with a clinically important change in ovarian reserve. These findings may reassure women hesitant to take the vaccine. Further studies with large sample sizes and with longer follow up periods are needed to ensure the safety of these vaccines.

Key Words: Anti-Müllerian hormone, covid-19, SARS-CoV-2 mRNA vaccine, fertility, ovarian reserve.

INTRODUCTION

The Coronavirus disease 2019 (COVID – 19), identified as a global pandemic by the World Health Organisation (WHO)[1], is mediated by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[2]. By the second half of 2021 it has infected more than 250 million worldwide, and has taken the lives of more than five million people[1]. Although the CoV-2 pathogen is air borne, with the respiratory system being the main target[3,4], it could result in damage to other systems such as the nervous, immune and reproductive systems[5-7].

Attachment of the viral spike protein S to the Angiotensin Converting Enzyme 2 (ACE2) is recognised as the primary method for the virus to gain access to the host cell[8,9]. With ovaries expressing ACE2 receptors[10], reports to date have suggested ovarian damage by SARS-CoV2[11,12] though further studies are required to assess the impact of this damage on ovarian function[13].

In response to the pandemic, the race to develop effective, yet safe vaccines resulted in the quick introduction of new generation mRNA vaccines against SARS-CoV-2. These vaccines have been found to produce an immune response that has high efficacy against the disease, with the ability to prevent hospitalisation and death[14]. However, given the potential damage of SARS-CoV-2 to the reproductive system, the mRNA vaccine, which mimics the virus, has been hypothesised to also affect fertility via the same mechanism[15]. The mRNA vaccine, BNT162b2 Pfizer/BioNTech uses the spike protein S of SARS-CoV-2, rather than the whole pathogen, to mediate an immune response, and this has been suggested to have a higher safety profile[16,17]. Although the Centers for Disease Control and Prevention (CDC) have stated that vaccine ingredients or antibodies made as a result of the vaccine would not affect

the ability to become pregnant now or in the future[18], with ACE2 receptors highly expressed within the ovary, a negative impact of the vaccine on the ovaries, and thus on ovarian reserve has not yet been ruled out[9,10,19-21], and current studies are still underway[18].

With the ongoing SARS – CoV-2 pandemic, the need to promote global vaccination programs had been apparent to reduce the ever-rising morbidities and mortalities[22,23]. However, following the declaration of the authorised emergency use of these vaccines by the Food and Drug Administration (FDA), wide spread concern regarding the adverse effects on human fertility surfaced across social media, evident by more than an alarming 200% increase in fertility related searches by the general public[24] With misinformation, lack of adequate research, conspiracy theories, and with rapidly developed, approved and manufactured vaccines, there is apparent vaccine hesitancy among reproductive age women[25,26].

Female fertility can be evaluated by assessing the ovarian reserve, with common indicators such as anti-Mullerian hormone (AMH), sex hormones and basal antral follicle count (AFC)[27]. AMH, a glycoprotein produced by ovarian preantral and small antral follicles[28], is considered to now be the chosen measurement as, in contrast to other reproductive hormones, is not influenced by the menstrual cycle[29,30]. However, assessment of the effect of the mRNA vaccines on female fertility by studying these well researched indicators, requires caution. AFC, FSH and inhibin B have been demonstrated to vary greatly between cycles[31-33], and despite these inter – cycle variations being reported to be smaller with AMH[31,35], some studies have also documented high levels of variability[36,37].

Recently, the mRNA vaccine Pfizer/BioNTech Covid–19 vaccine, has become widely accessible in Egypt. Only a few studies, to our knowledge, has evaluated the possible effect of this recently developed vaccine on female fertility [38-43]. With a lack of data, and anxieties about the newness of this vaccine, we find the need to evaluate the effect of the mRNA Pfizer-BioNTech Covid-19 on ovarian reserve, important and relevant to increase public trust and vaccine confidence.

METHODS

Approval from the ethics committee of the institute was attained before starting the enrolment. The trial is a prospective, single centre study, conducted at the Department of Obstetrics and Gynecology, Faculty of Medicine, Cairo University. The study population consisted of 115 eligible women of reproductive age, attending the outpatient gynaecology clinic, from May to December 2021.

Eligible women were those between the age of 18 – 42 years, with preserved menstrual function, normal ovarian

reserve and about to receive the first dose of Pfizer – BioNTech Covid – 19 vaccine. Pregnant women or those with past Covid 19 infection or vaccination were excluded from the trial.

Women were asked to be seen on the first week of their menstrual cycle and baseline characteristics were recorded at recruitment. Blood samples were taken to determine AMH levels and concentration of SARS-CoV-2 IgM and IgG antibodies. A pelvic ultrasound was performed to assess the number of antral follicles. The AMH assay was performed using electrochemiluminescence technology on cobas e 601 module, Roche using Elecsys AMH kit, with results expressed in ng/mL. Detection of SARS-CoV-2 IgM and IgG antibodies was done using the lateral flow immunochromatographic assay Artron One Step COVID-19 IgM/IgG Antibody Test (Artron Laboratories Inc., Canada). Women with serum AMH levels below 1.2ng/ml or an AFC less than a total of five follicles in both ovaries were excluded from the trial. A positive antibody test against SARS – CoV2 would suggest prior infection or vaccination and these women were also excluded from the trial.

As recommended by the Food and Drug Administration (FDA), a positive antibody test result was used to identify those with a previous SARS-CoV2 infection, however not to determine the level of immunity following COVID 19 vaccine[44]. As a result of these recommendations, a repeat test for antibodies against SARS-CoV2 following vaccination was dismissed.

The second mRNA vaccine was administered three weeks after the first, with a follow up appointment scheduled at three months after the first visit. A blood sample was taken for a repeat AMH serum level and a pelvic ultrasound was performed to assess the AFC. Adverse effects, such as local site reactions, headache, myalgia or a raised temperature, related to vaccine administration, were also documented.

The primary study outcome was the absolute and percentage change in AMH levels three months following double vaccination. Any change in the number of antral follicles was also documented. A decrease of 10% or more in the serum level of AMH or AFC was considered clinically significant[38]. The secondary study outcome was any vaccine related adverse events, appearing within seven days of either dose of vaccine.

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Because the groups are large enough, comparison between the 3 age groups was done using One Way Analysis of Variance (ANOVA) test. Within group comparison between pre- and post-vaccination was done using paired t test. Multivariate linear regression analysis was used to

test for the preferential independent predictors of change in AMH and AFC. Two- sided *p values* less than 0.05 was considered statistically significant. IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows was used for all statistical analyses.

RESULTS

A total of 150 women were recruited at the start of the study, of which 17 were excluded following analysis of the blood tests and pelvic ultrasound. Baseline investigations suggested an ovarian reserve of below average in six women (4%), and an immune result suggestive of a previous infection by SARS – CoV2 in 11 women (7.3%). Of the 133 women recruited, 115 (86.5%) attended the three month follow up.

Baseline characteristics of the studied participants are presented in (Table 1). The average age was 31 (± SD 4.66) years and the average body mass index (BMI) was 23 (± SD 1.66) kg/m2. Contraception was used in 55.7 % of all women, with 19.1% using a form of hormonal contraception.

Further sub analyses of the data were conducted by comparing the demographic characteristics and dynamics of AMH and AFC for three separate age groups; below 30 years (n = 50), between 30 and 35 years (n = 39) and above 35 years (n = 26). BMI values were not found to be significantly different between the different age groups.

Table 1: Baseline characteristics of total participants

	Total Vaccinated N = 115	
	Mean ± SD	Median (range)
Age (years)	31 ± 4.66	31(22-39)
BMI (kg/m^2)	22.99 ± 1.66	23.3 (19.7-28.2)
Menstruation frequency (days)	28.62 ± 1.23	28 (26-31)
AMH baseline (ug/l)	4.53 ± 1.87	4.7 (1.3 – 8.4)
AMH post vaccine (ug/l)	4.34 ± 1.8	4.3 (1.2 – 8.5)
Delta AMH (ug/l)	-0.19 ± 0.42	-0.3 (-0.9-0.8)
% change in AMH	-3.68 ± 9.14	-5.88 (-16.3-16)
AFC baseline	6.74 ± 1.42	7 (5-10)
AFC post vaccine	6.26 ± 1.469	6 (3-10)
% change in AFC	-6.95 ± 11.42	0 (-40-20)
	n	%
Contraception		
None	51	44.3
Condom	24	20.9
OCP	19	16.5
IUD	17	14.8
Mirena	3	2.6
Other	1	0.9

Although AMH and AFC values were significantly lower for women older than 35 years compared to those younger than 35 years, both in baseline and post vaccination levels, the percentage change in AMH and AFC values were not significantly different (Table 2).

Table 2: Comparison of demographic characteristics, AMH and AFC values between age groups

	Age group						p value
	<30 (n=50)		30-35 (n=39)		>35 (n=26)		
	Mean ± SD	Median (range)	Mean ± SD	Median (range)	Mean ± SD	Median (range)	
Age (years)	26.44 ± 1.85	27 (22-29)	33.05 ± 1.61	33 (30-35)	37.15 ± 1.01	37 (36-39)	
BMI (kg/m^2)	23.06 ± ± 1.94	23.35 (19.8-28.2)	23.22 ± 1.33	23.5 (19.9 – 25.1)	22.53 ± 1.45	22.6 (19.7-25.1)	0.237
Menstruation frequency (days)	28.76 ± 1.08	29 (27-30)	28.87 ± 1.26	29 (26-31)	27.96 ± 1.28	28 (26 – 31)	0.007*
AMH baseline (ug/l)	5.934 ± 1.21	5.9 (2.9-8.4)	4.41 ± 1.25	4.1 (2.5-7.4)	2.012 ± 0.473	2 (1.3-2.9)	0.000*
AMH post vaccine (ug/l)	5.654 ± 1.27	5.75 (2.6-8.5)	4.267 ± 1.13	4.2 (2.4-7.1)	1.94 ± 0.406	2(1.2-2.8)	0.000*
Delta AMH (ug/l)	-0.282 ± 0.482	-0.4 (-0.9-0.8)	-0.144 ± 0.39	-0.2 (-0.8-0.5)	-0.073 ± 0.234	-.10 (-0.4-0.3)	0.081
% change in AMH	-4.854 ± 8.067	-7.63 (-16.3-12.9)	-2.39 ± 9.077	-3.85 (-14.3-16)	-3.348 ± 11.067	-6.275 (-15-15.8)	0.446
AFC baseline	7.64 ± 1.32	8 (5-10)	6.33 ± 1.155	6 (5-9)	5.62 ± 0.752	5 (5-7)	0.000*
AFC post vaccine	7.08 ± 1.32	7 (5-10)	5.9 ± 1.29	6 (4-9)	5.23 ± 1.107	5 (3-8)	0.000*
% change in AFC	-7.05 ± 9.22	-10.56 (-29-20)	-6.85 ± +- 11.596	0.00 (-33-20)	-6.9 ± 14.94	0.00 (-40-20)	0.997

The multivariate logistic regression analysis shows no significant association between adjusted risk factors (age, BMI and baseline AMH or AFC values) and percentage change of AMH or AFC (Tables 3,4).

Table 3: Multiple logistic regression analysis of adjusted risk factors for AMH % change

Constant	95% Confidence Interval		p value
	Upper	Lower	
Age	0.369	-0.679	0.558
BMI	1.078	-0.990	0.933
AMH baseline	0.370	-2.250	0.158

Table 4: Multiple logistic regression analysis of adjusted risk factors for AFC % change

Constant	95% Confidence Interval		p value
	Upper	Lower	
Age	0.359	-0.712	0.514
BMI	1.271	-1.318	0.972
AMH baseline	0.962	-2.557	0.371

The baseline serum AMH and AFC levels were compared with those at three months after vaccination and did reveal a significant difference for the whole study group (Table 5). However, once the total study group was divided into the different age groups, the change in AMH was not found to be statistically significant at the above 35 years group (Table 6).

Table 5: Comparison of total AMH levels and AFC at baseline versus post vaccination

	Paired Differences			p value
	Mean ± SD	95% Confidence Interval of the Difference		
		Upper	Lower	
AMH baseline - AMH Post	0.19 ± 0.41	0.11	0.26	0.000*
AFC baseline - AFC Post	0.48 ± 0.73	0.34	0.61	0.000*

Table 6: Comparison of AMH levels at baseline versus post vaccination in different age groups

Age Group (y)	Paired Differences			p value
	Mean ± SD	95% Confidence Interval of the Difference		
		Upper	Lower	
< 30	0.28 ±0.48	0.14	0.42	0.000*
30 - 35	0.14 ± 0.40	0.01	0.27	0.030*
> 35	0.07 ± 0.23	-0.02	0.17	0.124

To account for inter-cycle variations in AMH and AFC levels, a clinically significantly decreased serum AMH and AFC level was defined as a greater than 10% decrease in the levels respectively, following vaccination. The proportion of women with a greater than 10% decrease in levels of AMH and AFC following vaccination were less than those with a less than 10% decrease, in the total cohort of women (40% versus 60% in AMH and 47.8% versus 52.2% in AFC), and also within each age group. The incidence of women with significantly decreased AMH or AFC levels after vaccination between the age groups showed no differences (Tables 7,8,9).

Table 7: Comparison of AFC values at baseline versus post vaccination in different age groups

Age Group (y)	Paired Differences			p value
	Mean ± SD	95% Confidence Interval of the Difference		
		Upper	Lower	
< 30	0.56 ± 0.68	0.37	0.75	0.000*
30 - 35	0.44 ± 0.72	0.20	0.67	0.001*
> 35	0.39 ± 0.85	0.17	0.73	0.030*

Table 8: Number and percent of women with a >10% decrease of AMH levels following vaccination by age groups.

Age groups (y)	Number (%) women with AMH change						P value
	>10% decrease		No or =<10% decrease		Total		
	n	%	n	%	n	%	
<30	22	44	28	56	50	100	
30-35	13	33.3	26	66.7	39	100	
>35	11	42.3	15	57.7	26	100	0.573
Total	46	40	69	60	115	100	

Table 9: Number and percent of women with a >10% decrease of AFC values following inoculation by age groups.

Age groups	Number (%) women with AMH change						P value
	>10% decrease		No or =<10% decrease		Total		
	n	%	n	%	n	%	
<30	25	50	25	50	50	100	
30-35	18	46.2	21	53.8	39	100	
>35	12	46.2	14	53.8	26	100	0.92
Total	55	47.8	60	52.2	115	100	

Unfavourable effects related to the vaccine were recorded at the three month follow up visit. A local reaction to the injection site was seen in 79 women (68.7%), a raised temperature in 80 women (69.6%) and symptoms of intoxication such as myalgia or headache were found in 72 women (62.6%). None of the women reported a serious adverse effect that needed hospitalisation.

DISCUSSION

This prospective study supports the hypothesis that the mRNA SARS–CoV–2 Pfizer/BioNTech vaccine, despite being associated with a statistically significant change in AMH and AFC, when comparing baseline values to three month post vaccination, is not considered a clinically important change in ovarian reserve. This is reflected by a lower proportion of women, in the total cohort of women, and across all age groups, with a greater than 10% decrease in AMH and AFC values three months following vaccination.

This study has found, using multivariate analysis, that factors such as baseline levels of AMH and AFC, age or BMI, do not affect the percentage change of AMH levels or of AFC. Sub analyses of three age groups; below 30 years, 30-35 years and above 35 years, was also carried out to account for potential age specific differences in AMH and AFC. Lower baseline AMH and AFC were found in older age groups (P = 0.001 prior to and post vaccine AMH and AFC levels) in comparison to younger women. This is in agreement with a well-recognised strong inverse association of AMH and AFC with age[45]. Despite this finding, the percentage change in AMH and AFC values across the separate age groups were not significantly different.

These findings agree with the limited number of studies that have explored the relation of SARS-CoV-2 infection and mRNA vaccine with ovarian fertility, though only a single study, to our knowledge, has focused their attention on the effect of the mRNA Pfizer/BioNTech vaccine on AMH.

Mohr-Sasson *et al.*, conducted a study on 129 women who had received two doses of mRNA vaccine. Mean levels of AMH were not significantly different at baseline and three months post vaccine (P= 0.11). Sub analyses performed for separate age groups, to account for any possible age specific difference in AMH, still failed to show any significant difference (P=0.46)[38]. Another recent study by Orvieto *et al.*, could not demonstrate any difference in ovarian response, ovarian stimulation or embryo parameters, in addition to an acceptable pregnancy rate of 30% per transfer, in 36 couples undergoing IVF treatment cycle before and after SARS-CoV-2 vaccine[39]. Likewise, Chandi A and Jain N documented a similar quality of follicles when comparing those of Pfizer/BioNTech vaccinated women to those that were not vaccinated[40]. A study by Ahoron *et al* also demonstrated no associated adverse effect of the COVID 19 mRNA vaccines on ovarian stimulation or early pregnancy outcomes after IVF in 222 vaccinated women[41]. Morris *et al* and Wesselink AK conducted studies to asses the impact of the virus or vaccine on fecundity however no focus was on the impact on indicators of ovarian reserve[42,43]. Female fertility

following mRNA vaccine has been studied in rats in the study conducted by Bowman C *et al.*, with no detrimental effects detected between both the control group and that of the vaccine[46].

Other studies explored the effect of infection with SARS-CoV-2 on ovarian integrity. The virus, as with the new mRNA vaccine, have been hypothesised to compromise female fertility by a similar pathway of attacking cells though the binding of their S protein to ACE receptors, expressed within the ovaries. Additionally, with the ovaries being a common target for autoimmune attacks, the immune response to the infection as with the vaccine, could also potentially influence fertility negatively[47-48]. Only one study however, to our knowledge, has found a possible detrimental effect of SARS-CoV2 infection on the ovary. Orvieto *et al.*, compared the ovarian response to ovarian stimulation, prior to and after infection, in nine couples that were undergoing IVF. Although ovarian response was similar, a reduced proportion of top quality embroys were reported by the authors suggesting a possible negative effect on folliculogensis[39]. This result needs to be interpreted with caution due to the small sample size in this study. This finding was not observed in the recent studies conducted by Wang *et al.*, or Barragan *et al.*, with similar ovarian responses detected before and after SARS-CoV-2 infection in women undergoing ART treatment[49,50].

The incidence of unfavourable effects within seven days of the Pfizer/BioNTech vaccine were comparable to those reported in the literature[51-53]. Menni *et al.*, reported a 66.7% (188,178 of 208,103) and 54.03% (15,241 of 28,207) incidence of adverse effects within eight days of the first and second dose of the mRNA vaccine, respectively[52]. Local pain, injection site reaction, fatigue, headache and myalgia were found to be the most frequent unfavourable outcomes in the recent systemic review of Amanzio *et al*[53]. These findings are comparable to the findings in this study.

These limited studies with restricted sample sizes have evaluated the ovarian function in response to SARS-CoV-2 infection and the new mRNA vaccines. Although these vaccines have been authorised for use by the FDA, the safety profile regarding female fertility is not yet supported by strong research.

This study is a prospective study, that has evaluated more than one indicator for ovarian reserve (AMH and AFC) in a large cohort of reproductive aged Egyptian women of normal ovarian function. AMH levels were tested in the same central laboratory. Additional studies with larger sample sizes in different populations and with longer term follow up, to account for inter-cyclic variations in ovarian indicator values, and possible unfavourable effects of the vaccine over a longer period of time, are needed.

CONCLUSION

We are in the era where the administration of vaccines against SARS-CoV-2, to the general public, is globally encouraged. The possible impact of the virus and these vaccines on human fertility cannot be ignored and deserves our attention and concern. Anti-vaccination advocates still hold that data available are not enough to justify the safety and effectiveness of these vaccines, with the concern that large scale vaccination programs can result in unpresented issues. With Pfizer/BioNTech vaccine available now in Egypt, this study is significant and essential. The findings of this study may provide reassurance for hesitant women, who would have otherwise refused to take this vaccine, due to concerns on the potential effect on their future fertility. Importantly, the potential side effects of vaccination on fertility should also be compared with the adverse outcome resulting from SARS-CoV-2 infection rather than being considered alone[15]. Further studies with large sample sizes and with longer follow up periods are needed to ensure the safety of these vaccines.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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