

## The effect of COVID-19 vaccine on ovarian reserve

Çağanay Soysal, MD, PhD, Elif Yılmaz, MD, PhD.

## ABSTRACT

**الأهداف:** تقييم تأثير لقاحات الحمض النووي الريبوزي (mRNA) المطور لمرض فيروس كورونا 19 (COVID-19) على احتياطي المبيض.

**المنهجية:** أجريت هذه الدراسة المقطعية المرتقبة على 60 مريضه خلال الفترة من يونيو وسبتمبر 2021م في عيادات أمراض النساء في مستشفى من الدرجة الثالثة، أنقرة، تركيا. اشتملت مجموعة الدراسة على نساء تتراوح أعمارهم بين 25 و30 عاماً، يراجعون العيادة الخارجية لأمراض النساء ويخططون لأخذ لقاح mRNA ضد فيروس كورونا 2 للمتلازمة التنفسية الحادة الخبيثة، وأولئك الذين لم يخططوا للتلقيح في المجموعة الضابطة. فحصنا الهرمون المضاد للمولر (AMH) قبل التطعيم (1-5 أيام قبل التطعيم) وخلال 60-90 يوماً بعد التطعيم. في المجموعة غير الملقحة، فحصنا هرمون AMH مرة واحدة.

**النتائج:** كان متوسط عمر مجموعة الدراسة  $27.30 \pm 1.66$  وللمجموعة الضابطة  $27.40 \pm 1.69$  ( $p < 0.05$ ). كانت قيم AMH بعد اللقاح في الدراسة ومجموعات المراقبة متشابهة ( $p > 0.05$ ). لم يكن هناك فرق معنوي إحصائياً بين قيم AMH قبل اللقاح وبعده في مجموعة الدراسة ( $p > 0.05$ ).

**الخلاصة:** من المهم للنساء اللواتي يخططن للحمل ويترددون وذلك لتأثير اللقاحات على احتياطي المبيض أن يعلموا بأن اللقاحات تعد واحدة من أهم وسائل مكافحة عدوى COVID-19، لا تحتوي على تأثير على مستويات هرمون AMH. هناك حاجة لدراسات مستقبلية أكبر مع فترة متابعة أطول لتأكيد نتائجنا.

**Objectives:** To Evaluate the effect of messenger ribonucleic acid (mRNA) vaccines developed for the coronavirus disease-19 (COVID-19) on ovarian reserve.

**Methods:** This prospective cross sectional study was carried out between June and September 2021, in the Gynecology Polyclinics of a tertiary hospital, Ankara, Turkey, with 60 patients. Patients between the ages of 25-30 years old, who applied to the gynecology outpatient clinic and who were considering mRNA vaccination against severe acute respiratory syndrome coronavirus 2 were included in the study group, and those who did not plan to be vaccinated were included in the control group. Anti-müllerian hormone (AMH) was examined before vaccination (1-5 days before vaccination) and within 60-90 days after vaccination. In the unvaccinated group, AMH was examined once.

**Results:** The mean age of the study group was  $27.30 \pm 1.66$  and of the control group was  $27.40 \pm 1.69$  ( $p < 0.05$ ). Post-vaccine AMH values of the study and control

groups were similar ( $p > 0.05$ ). There was no statistically significant difference between pre-vaccine and post-vaccine AMH values in the study group ( $p > 0.05$ ).

**Conclusion:** It is of great value that people who are planning pregnancy and who have hesitation regarding the effect of vaccines on ovarian reserve should be carefully informed that vaccines, which are one of the most important means of fighting against COVID-19 infection, have no effect on AMH levels. Prospective larger studies with a longer follow-up period are needed to confirm our results.

**Keywords:** mRNA, vaccine, ovarian reserve, AMH, COVID-19

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*From the Department of Obstetrics and Gynecology, Dr. Sami Ulus Women's and Children's Health Teaching and Research Hospital, Ankara, Turkey.*

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*Address correspondence and reprint request to: Dr. Elif Yılmaz, Obstetrician and Gynecologist, Department of Obstetrics and Gynecology, Dr. Sami Ulus Women's and Children's Health Teaching and Research Hospital, Ankara, Turkey. E-mail: elifakkasyilmaz@gmail.com  
ORCID ID: <https://orcid.org/0000-0001-5107-6492>*

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus, which caused the coronavirus disease-19 (COVID-19) infection, has led to a serious pandemic that negatively affected human health all over the world and has brought many systemic health problems. Angiotensin-converting enzyme 2 (ACE2), which is found in many organs in the body, is a receptor for SARS-CoV. Research articles have demonstrated that the virus can regulate ACE2 expression in host cells by invading the target cell through attachment to ACE2 in the female reproductive organs and placenta.<sup>1-3</sup> Angiotensin-converting enzyme 2 has been detected in different organs, including the respiratory tract, heart, kidney, ovaries, uterus, vagina, placenta, testis, and

gastrointestinal tract.<sup>1,4</sup> Recently, considering that ACE2 plays a prominent regulatory role in reproduction, it has been emphasized that SARS-CoV-2 may impair female fertility by invading ovarian tissue and granulosa cells or by impairing endometrial epithelial cells.<sup>1,5-8</sup>

Evaluation of ovulatory function is an important component of female fertility assessment. Reduced ovarian reserve can indicate both reduced oocyte quality and quantity, or reproductive capacity. Even though many screening tests are used for this purpose, no single test is completely trusted for evaluating ovarian reserve and hence pregnancy potential. One of the most commonly used hormones in the measurement of ovarian reserve is müllerian inhibiting hormone, known as anti-müllerian hormone (AMH). It is a dimeric glycoprotein hormone, a member of the transforming growth factor-beta family. As the follicle pool decreases throughout reproductive life, AMH released from the follicles of women of reproductive age also decreases. It falls below the limit value after menopause.<sup>7,9</sup>

Since COVID-19 infection has affected the whole world, it requires an urgent response. Mass COVID-19 vaccination is at the forefront of the planned interventions to respond to the COVID-19 pandemic. One of the vaccines developed against COVID-19 infection is the messenger ribonucleic acid (mRNA) vaccine, a cutting-edge approach that uses genetically engineered RNA particles to generate protein that creates a safe immune response. Artificially produced mRNAs made in the laboratory are aimed to work just like our own mRNAs, warning us against the virus. Although there are studies on the effect of COVID-19 infection on ovarian reserve, the effect of mRNA vaccines developed for the COVID-19 pandemic on ovarian reserve is still unknown. Reducing concerns regarding the vaccine and increasing the rates of vaccination is important in the young age group, a leading population especially in developing countries in the spread of the disease in the society. The purpose of this study was to determine the effect of mRNA vaccines developed for the COVID-19 pandemic on ovarian reserve and thus help to inform the reproductive age group experiencing this widespread concern.

**Methods.** This prospective cross sectional study was carried out in Gynecology and Obstetrics Polyclinics, Dr. Sami Ulus Training and Research Hospital, Ankara, Turkey, between June 2021 and September 2021.

**Disclosure.** Authors have no conflict of interests, and the work was not supported or funded by any drug company.

Approval was granted by the Ethics Committee of the same hospital before the study. All procedures applied in the research conformed with the ethical standards of the 1964 Helsinki Declaration and subsequent amendments or comparable ethical standards. In the design phase of the research, a literature review was carried out. For this purpose, the internet was used, the issues related to the subject were scanned from PubMed and Cochrane databases and evidence-based medicine sites were examined. After the patients were instructed in details regarding the study, informed consent was provided by the patients who agreed to take part in the study. A total of 60 patients (30 in the study group and 30 in the control group) were involved in the study. Patients aged 25-30 years old who applied to the gynecology outpatient clinic, did not have a current or previous pregnancy history, and who were considering the mRNA vaccine (BioNTech) for SARS-CoV-2, were included in the study group, and those who did not plan to be vaccinated were included in the control group. Factors that may affect fertility were taken as the exclusion criteria. Patients with a previous pregnancy history and current pregnancy, those with infertility history, conditions that may be risk factors for infertility such as menstrual irregularity, polycystic ovary, and ovarian cyst, those with systemic chronic diseases (diabetes, kidney, heart, GIS, etc.), and past or current COVID-19 infection were excluded from the study. All patients who met the inclusion criteria and stated that they would be vaccinated were included in the study group. After each patient participating in the study group, the first patient who met the criteria but stated that she would not be vaccinated was also included in the control group. The AMH test, which is used in the evaluation of ovarian reserve, was used in the study group on the same day as pre-vaccination (before vaccination) and 60-90 days after vaccination. In the control group, it was used once. It was analyzed whether there was a statistical difference between the vaccinated and unvaccinated groups and whether there was a statistical difference before and after vaccination in the study group in terms of AMH.

Anti-müllerian hormone is secreted by the preantral (<8 mm) and early antral follicles. As an indicator of ovarian function, AMH level is superior to gonadotropins (follicle-stimulating hormone and luteinizing hormone) and ovarian steroids (estradiol and progesterone) produced periodically since it indicates the extent of the primordial follicle pool, is produced constantly in the granulosa cells of small follicles during the menstrual cycle, and is not affected by pregnancy or contraceptive use can be viewed at anytime during the cycle. For all these reasons AMH is considered to be the

best biochemical marker of ovarian function in many clinical situations.<sup>10</sup> Since patients could be vaccinated on any day of their cycle, the most important criteria in the selection of the marker used to evaluate ovarian reserve in this study was one that could be measured independently of their menstrual cycle.

Many vaccines have been approved against COVID-19, each suitable for responding to the pandemic with individual protection against COVID-19. The mRNA-COVID-19 vaccines are gene-based vaccines based on a new technology. The primary antigenic target for COVID-19 vaccines is the large surface spike protein, which attaches to the ACE2 receptor on host cells. The mRNA contained in vaccines is not included in the human genome after vaccination, but is read after it enters the cell, whereupon these cells themselves produce the spike protein. The spike proteins produced by the body of the vaccinated person are perceived by the immune system as foreign proteins, thereby producing antibodies and immune cells against the spike protein of the virus. Thus, a protective immune response is induced. The vaccine (BNT162b2-Pfizer COVID-19 vaccine) is applied as 2 intramuscular injections, with the first and second vaccinations administered with a minimum of 4 weeks between doses.

**Statistical analysis.** Statistical evaluation of the data was carried out using Statistical Package for the Social Sciences, version 15.0 (SPSS Inc., Chicago, IL, USA). The results are presented as mean, standard deviation (SD) or median. The incidences are presented as a number (percentage). Paired sample t-test statistics were used in the comparison of 2 dependent groups for data having a normal distribution. Mann-Whitney-U test was used for the comparison of 2 independent groups with non-normally distributed data. Wilcoxon test statistics were used in the evaluation of 2 dependent groups. *P*-values of <0.05 were considered significant.

**Results.** Demographic data of the study group are presented in **Table 1 and 2**. The groups were similar in terms of mean age, education level, employment status, marital status, and employment status of their

partners ( $p>0.05$ ). The groups were independent and homogeneous in terms of the specified characteristics.

Comparisons of the groups in terms of AMH are presented in **Table 3**. The post-vaccine AMH values of the study group were  $4.13\pm 1.94$  and the AMH values of the control group were  $4.14\pm 2.79$ , and no statistically important difference was found ( $p>0.05$ ). Additionally, there was no statistically significant difference between the pre-vaccine ( $4.17\pm 1.87$ ) and post-vaccine ( $4.13\pm 1.94$ ) AMH values of the study group ( $p>0.05$ ).

**Discussion.** The COVID-19 pandemic is a public health threat, in which the effects are increasing exponentially all over the world and has led to the initiation of a process that needs to be worked on to find a solution. Mass COVID-19 vaccination is at the forefront of the planned interventions to respond to the COVID-19 pandemic. To date, studies have been published regarding the negative effects of COVID-19 on reproductive functions in both men and women. This study purposed to estimate the effects of mRNA vaccines developed for COVID-19 on ovarian reserve, and it was determined that there was no important difference between the vaccinated and unvaccinated groups according to AMH levels. Additionally, the pre- and post-vaccine AMH levels of the vaccinated group were also similar.

Although the respiratory system is the main affected system in COVID-19, studies showed that the cardiovascular, digestive, and urinary systems were also potential target organs.<sup>11</sup> The main host receptor for COVID-19 virus is the ACE2 system, which is the major component of the renin-angiotensin-aldosterone pathway. The virus binds to the target cell by attaching to ACE2 and modulating the expression of ACE2 in host cells. The fact that it is expressed and has a regulatory effect on follicle development and ovulation has caused concerns that the virus may impair female reproductive functions by regulating ACE2. This has been examined in many studies. Li et al<sup>6</sup> in their review of the influence of COVID-19 infection on reproductive health, stated that the potential pathogenicity of the virus on

**Table 1** - Comparison of ages between groups.

Variable	Experimental group (n=30)		Control group (n=30)		Statistical analysis <sup>*</sup>
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	Probability
Age (years)	27.30±1.66	27.0 (25.0-30.0)	27.40±1.69	27.0 (25.0-30.0)	Z= -0.233 p=0.816

<sup>\*</sup>Mann-Whitney-U test (Z-table value) statistics were used to compare the measurement values of 2 independent groups that did not have a normal distribution. SD: standard deviation, min: minimum, max: maximum

**Table 2** - Examination of the relationships between the groups and certain characteristics (N=60).

Characteristics	Experimental group (n=30)	Control group (n=30)	Statistical analysis*
			Probability
<i>Educational status</i>			
Primary school	2 (6.7)	1 (3.3)	$\chi^2=0.494$ $p=0.781$
High school	16 (53.3)	18 (60.0)	
University	12 (40.0)	11 (36.7)	
<i>Employment</i>			
Working	21 (70.0)	19 (63.3)	$\chi^2=0.075$ $p=0.784$
Not working	9 (30.0)	11 (36.7)	
<i>Marital status</i>			
Married	7 (23.3)	5 (16.7)	$\chi^2=0.104$ $p=0.747$
Single	23 (76.7)	25 (83.3)	
<i>Partner's employment status</i>			
Working	21 (91.3)	22 (88.0)	$\chi^2=0.140$ $p=0.708$
Not working	2 (8.7)	3 (12.0)	

Values are presented as a number and (%). \*Continuity correction or Pearson- $\chi^2$  crosstabs were used according to the expected value levels in examining the relationship between 2 qualitative variables.

**Table 3** - Comparison of anti-müllerian hormone values in terms of process according to groups (N=60).

Variable	Sample (n=30)		Control (n=30)		Statistical analysis*
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	Probability
<i>AMH</i>					
Pre-test	4.17±1.87	4.2 (0.9-7.9)	4.14±2.79	3.5 (0.6-11.5)	$Z= -0.843, p=0.399$ $Z= -0.591, p=0.554$
Post-test	4.13±1.94	4.3 (0.8-7.9)	4.14±2.79	3.5 (0.6-11.5)	
Statistical analysis (probability)	$t=0.275$ $p=0.785$		$Z=0.000$ $p=1.000$		

\*Paired sample t-test (t-table value), Mann-Whitney-U test (Z-table value), and Wilcoxon test (Z-table value) statistics were used. AMH: anti-müllerian hormone, SD: standard deviation, min: minimum, max: maximum

testicular and ovarian tissues and on granulosa cells, may affect testicular and ovarian function, spermatozoa, oocyte quality, and have pregnancy consequences. For this reason they stated that the evaluation of fertility in the future for people who have been infected especially with COVID-19 should be particularly emphasized. Similarly, in their research examining the potential impact of SARS-CoV-2 on the female reproductive system, Jing et al<sup>1</sup> stated that virus can infect not only the female reproductive organs but also the placenta in pregnant women through the expression of ACE2, and thus may cause infertility, menstrual irregularities, and fetal distress in pregnant women by disrupting female reproductive functions. They recommended delaying pregnancy with the evaluation and follow-up of fertility after recovery, especially in women who have had COVID-19. In their study on 100 patients to analyze the effects of COVID-19 on spermatogenesis, Kamel et al<sup>12</sup> stated that the infection negatively affected sperm count and motility, but this effect was reversible. Similarly, Omolaoye et al<sup>13</sup> emphasized

that viral orchitis may have negative effects on fertility in the long term by causing deterioration in semen parameters and recommended that people infected with the virus should be encouraged to use cryopreservation. Although the negative effects of COVID-19 on fertility are known, patients are concerned on the possible impact of mRNA vaccines on fertility since it is a new technology. However, studies examining the effects of mRNA vaccines on fertility are still very limited since the vaccines, which are the most efficient weapons in the fight against COVID-19, are still new. There was no statistically important difference between the AMH levels of the groups and the AMH values before and 2 months after the vaccine in the study group. This supports that the vaccine does not have a negative effect on AMH.

A review examining the influence of COVID-19 on the reproductive system stated that psychological distress had both direct and indirect effects on the female reproductive system and that the panic and stress caused by the COVID-19 pandemic has led to

traumatic dysfunction in women, negatively affecting oocyte quality and reproductive results.<sup>14</sup> The common opinion of the studies that investigated the effect of antioxidants on female reproductive health and fertility was that reactive oxygen species (ROS) in ovaries increase due to stress, and ROS that accumulates above the physiological level reduces follicle growth and development, leading to poor reproductive outcomes.<sup>15,16</sup> In addition, women experiencing stress have more sexual dysfunction that negatively affects reproductive outcomes.<sup>17</sup> From this point of view, it should also be considered that the vaccine may have a positive effect on reproductive outcomes by reducing stress.

**Study limitations.** The cross-sectional nature of the research, the small number of participants, being conducted with a homogeneous group, and the short follow-up period after vaccination can be considered as limitations of the study. Although the short duration of the research is a disadvantage, we believe that the study is beneficial in enlightening those concerned on this issue in a short time. Another limitation of our study was that fertility evaluated only with AMH. However, AMH is accepted as the best marker in evaluating fertility. In addition, since AMH is produced uninterruptedly during the menstrual cycle, it has been an important convenience for patients to be able to be measured on any day of the cycle. Considering these advantages, we believe that this is not a very important disadvantage of this study. Despite these limitations, this study is especially important in that it could reduce the prejudice that mRNA vaccines cause infertility, which is more common in developing countries. Since this subject has not yet been covered in the literature, there is a need for prospective studies with larger patient groups that also include a longer time frame.

In conclusion, considering both the potential negative impact of COVID-19 infection on reproductive functions through stress, and the fact that the vaccine did not have any negative effect on AMH in the light of this study results, it is suggested that people who plan fertility should not hesitate in terms of the effect of the vaccines developed with mRNA technology against COVID-19 infection on ovarian reserve. It should not be forgotten that vaccination is one of the most important interventions to overcome the COVID-19 pandemic with least damage.

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