The effect of Covid-19 mRNA vaccine on serum anti-Müllerian hormone levels

Running title: Covid-19 vaccine and ovarian reserve

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Abstract

Study question: Does the administration of the acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccine have an association with ovarian reserve as expressed by circulating anti Müllerian hormone (AMH) levels?

Summary answer: Ovarian reserve as assessed by serum AMH levels is not altered at three months following mRNA SARS-CoV-2 vaccination.

What is known already: A possible impact of SARS-CoV-2 infection or vaccination through an interaction between the oocyte and the somatic cells could not be ruled out, however, data is limited**.**

Study design, size and duration: This is a prospective study conducted at a university affiliated tertiary medical center between February to March 2021.

Participants/materials, setting, methods: Study population included reproductive aged women (18 – 42 years) that were vaccinated by two Pfizer-BioNTech Covid-19 vaccines (21 days apart). Women with ovarian failure, under fertility treatments, during pregnancy, previous Covid-19 infection or vaccinated were excluded from the study. Blood samples were collected for AMH levels before the first mRNA vaccine administration. Additional blood samples after three months were collected for AMH and anti Covid-19 antibody levels. Primary outcome was defined as the absolute and percentage change in AMH levels.

Main results and the role of chance: The study group consisted of 129 women who received two mRNA vaccinations. Mean AMH levels were 5.3 (±SD 4.29) μg/L and 5.3 (±SD 4.50) μg/L at baseline and after three months, respectively (p=0.11). To account for possible age-specific changes of AMH, sub-analyses were performed for three age groups: <30, 30-35 and >35 years. AMH levels were significantly lower for women older than 35 years at all times (p=0.001 for pre and post vaccination AMH

levels versus younger women). However, no significant differences for the changes in AMH levels before and after vaccinations (Delta AMH) were observed for the three age groups (p=0.46). Additionally, after controlling for age, no association was found between the degree of immunity response and AMH levels.

Limitations, reasons for caution: Although it was prospectively designed, for ethical reasons we could not assign a priori a randomized unvaccinated control group. This study examined plasma AMH levels at three months after the first vaccination. It could be argued that possible deleterious ovarian and AMH changes caused by the SARS-CoV-2 mRNA vaccinations might take effect only at a later time. Only longer-term studies will be able to examine this issue.

Wider implications of the findings: The results of the study provide reassurance for women hesitant to complete vaccination against Covid 19 due to concerns regarding its effect on future fertility. This information could be of significant value to physicians and patients alike.

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Trial registration number: The study protocol was approved by the "Sheba Medical Center" Ethical Committee Review Board (ID 8121-21 -SMC) on the 8th of February 2021 and was registered at the National Institutes of Health (NCT04748172).

Keywords: Covid-19, SARS-CoV-2 mRNA vaccine, ovarian reserve, anti-Müllerian hormone, fertility

Introduction

The Covid-19 pandemic exerted tremendous pressure on scientists to develop safe and effective vaccines. A few delivery systems for next-generation vaccines against Covid-19 were introduced (Wang et al. 2020). The new-generation vaccines consist of either a specific antigen or antigens of the pathogen, instead of the whole pathogen, thus suggesting a better safety profile (Vartak and Sucheck 2016).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has four main structural proteins (Wrapp et al. 2020). The spike protein (S) which is located at the outer surface of the virus particles is considered to have a strong binding affinity to the human cell surface receptor of angiotensin-converting enzyme 2 (ACE2) (Hamming et al. 2007). This interaction causes receptor-mediated endocytosis of the virus as well as of the S protein particle in the vaccine and is the main target to evoke the self-immune system (Lan et al. 2020). Since the mRNA molecules have low apparent transfection efficacy, lipid nanoparticles (LNPs) are often used to facilitate the incorporation of the mRNA molecules for transfection purposes (Schlake et al. 2012).

During the ongoing global Covid-19 pandemic, many countries around the world have promoted vaccination programs to reduce morbidity and mortality (Billon-Denis and Tournier 2020; Tregoning et al. 2020). Various anti vaccination groups have publicly questioned its safety (Robson 2021). A special concern, that was spread quickly via the social media and had implications on the decision whether to undergo vaccinations, surrounded the concern whether SARS-CoV-2 mRNA vaccine could negatively influence future fertility (Health Care 2021). Reproductive age women and

parents became hesitant and reluctant to get vaccinated due to this information that was not evidence based.

Is there any potential association between SARS-CoV-2 mRNA vaccine to future fertility expressed by the ovarian reserve? High expression of ACE2 receptors in testicular, uterine, placental, and sperm cells might suggest a potential detrimental effect of SARS-CoV-2 infection on human reproductive organs (Jing et al. 2020). Specifically, as ACE2 receptors are expressed in human ovaries, and angiotensin has been detected in measurable amounts in the follicular fluid (Reis et al. 2011), a possible impact of SARS-CoV-2 infection or vaccination through an interaction between the oocyte and the somatic cells cannot be ruled out (Anifandis et al. 2020; Li et al. 2003).

Anti Müllerian hormone (AMH) is a glycoprotein produced by the granulosa cells of the ovarian preantral and small antral follicles in women. As such, AMH is only present in the ovary until menopause (Pellatt et al. 2010). Its circulating level has been proposed as a predictor of ovarian response to ovarian stimulation and as a measure of the ovarian follicular reserve (Themmen 2005). In contrast to other reproductive hormones, AMH levels are not influenced by the state of the menstrual cycle. and are nowadays considered as the measurement of choice for ovarian reserve estimation (Practice Committee of the American Society for Reproductive Medicine 2015; Themmen 2005).

As Israel was the first country to widely vaccinate its population using the mRNA vaccines (Pfizer-BioNTech Covid-19 Vaccine), and due to all the aforementioned, the aim of this study was to evaluate a possible effect of the mRNA SARS-CoV-2

vaccines on ovarian reserve as estimated by the change in AMH levels before and three months after the first vaccination.

Material and Methods

This is a prospective study conducted at a university affiliated tertiary medical center including reproductive aged women (18 – 42 years) that were about to receive first vaccine by the Pfizer-BioNTech Covid-19 vaccine, between February to March 2021. Women with ovarian failure, under infertility treatments or during pregnancy were excluded from the study. Past Covid-19 infection based on women's report and previous vaccination were also causes for exclusion.

All participants completed a computerized questionnaire about their general medical, gynecological and obstetrical background at recruitment and three months later. Blood samples for AMH plasma levels were collected at recruitment. The second mRNA vaccine was given 21 days after the first. A follow up visit was scheduled at three months after the first vaccination. During this visit, blood samples were collected for AMH levels and for anti Covid-19 antibody levels (Serology). Additionally, women were asked to complete a second computerized questionnaire which focused on possible adverse effects following vaccinations. Adverse effects were defined by a specific local or systemic adverse event or any use of antipyretic or pain medication within 7 days after the receipt of each dose of vaccine.

Plasma concentrations of AMH were determined in the Sheba Medical Center accredited Endocrine Lab. Blood samples were centrifuged and serum was preserved at -30 °C Only after completing the collection of all samples, they were analyzed using the same batch for each two samples of a woman using Beckman Gen II ELISA kit with normal range values of 0.3-10.8 (μg/L) (Beckman coulter 2021).

Immunization was evaluated by the Access SARS-CoV-2 IgG Immunoassay, that is a two-step enzyme immunoassay using for the reaction paramagnetic particles coated with recombinant SARS-CoV-2 specific for the receptor binding domain (RBD) of the S1 protein (Beckman coulter 2021). All samples were send immediately after withdrawn for evaluation in an accredited Mega Lab and was considered positive when signal to cut off (S/CO) values were >1 (Beckman coulter 2021).

Primary outcome was defined as the change in AMH levels at three months following the first vaccine minus the first AMH levels (Delta AMH=Second AMH- first AMH). Changes were also expressed as percentage changes (Delta AMH*100) / First AMH). Secondary outcomes included: anti-Covid 19 antibody levels.

The study protocol was approved by the "Sheba Medical Center" Ethical Committee Review Board (ID 8121-21 -SMC) on the 8th of February 2021 and was registered at the National Institutes of Health (NCT04748172).

Statistical Analysis

Sample size calculation was performed for the primary outcome (change in AMH levels during the study period; a priori analysis). For a two-tailed test, standardized effect size (mean percentage change / SD of percentage change; dz) = 0. 25, α=0.05 and 1-β = 0.80 a sample size of 128 was required.

We calculated the percent difference between the second and the first AMH value and defined a significant decline in AMH levels when the second AMH decreased by more than 10% than the first AMH. Comparisons between age groups and women with decreased and not decreased AMH level were conducted with Student's t-test, Mann–Whitney U test, or Chi-square and Fisher's exact tests as appropriate for normally distributed, not normally distributed or categorical variables respectively. We used

paired t-test for comparing AMH level between first and second blood sample. ANOVA or non-parametric Kruskal Wallis tests were used to compare continuous variables among the three age groups.

Sample size calculation was performed with the G*Power 3.1. software. All additional statistical analyses were performed using the IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp. Two-sided P <0.05 was considered statistically significant.

Results

A total of 163 women were recruited for the study, of them complete follow up was achieved in 132/163 (81.0%). All women completed two vaccinations. Two women with undetectable levels of AMH at recruitment were excluded from analysis. One woman had an exceptionally high second AMH measurement (first AMH 5.48 versus second AMH 26.40) therefore a statistical decision was made to exclude her as an outlier (Delta 20.92). Analysis was made for 129 women that constituted the final study group (Figure 1).

The clinical characteristics of the women included in the study are presented in Table I. The mean age was 29 (±SD 5.23) years. Menstrual irregularity or known diagnosis of polycystic ovarian syndrome (PCOS) were detected in 22 (19.0%) of the women. 103 (79.8%) of the women were nullipara. Contraception was not used by 50 (42%) of the women. Among the 27 (23.1%) women who used hormonal contraception, five reported on follow-up a change in the brand used, however, none of them quit treatment. Mean AMH levels were 5.30 (±SD 4.29) μg/L and 5.30 (±SD 4.50) μg/L at baseline and after three months, respectively (p=0.11).

Additional sub-analyses for AMH results were made for three age groups: <30, 30-35 and >35 years old (Table II). As expected, AMH levels were significantly lower for women older than 35 years than for those younger than 35 years (p=0.001 for the comparison of pre and post vaccination AMH levels). No significant difference for the changes in AMH levels before and after vaccination (Delta AMH) were observed for any of the three groups (p=0.29).

Figure 2 presents scatter plots of the second AMH levels (y axis) as a function of the first AMH levels (x axis) and the percent changes in AMH levels according to the first AMH level in the different age groups. The plot demonstrates that nearly all values in the study group are close to the diagonal line, reflecting no statistical difference for the change in AMH values at recruitment and at three months after vaccination.

Evaluation of the immunity response achieved by the vaccinations in the three subgroups did not show differences between groups [Median: 17.26 (IQR11.56-25.86) versus 16.82 (IQR 9.59-24.69) versus 12.48(IQR 8.42-23.07) S/CO, respectively; p=0.23]. After controlling for age, no association was found between the degree of immunity response (as expressed by anti-covid antibody levels) and AMH levels (partial correlation 0.005 and 0.035 for first and second AMH blood test respectively).

We conducted additional sub-analyses in order to evaluate whether individual changes in AMH levels were associated with any of the baseline characteristics of the participating women. We defined “decreased AMH levels” if a larger than 10% decrease in personal AMH levels was found. No differences in women's characteristics were observed between women with decreased AMH levels compared to “no decreased” levels. Table III demonstrates no differences between the age

groups in the incidence of women with decreased AMH levels following vaccination (p=0.63).

Adverse effects following vaccinations were reported via computerized questionnaire completed on follow-up visit. Eighty-one (62.8%) and 90 (69.8%) women reported suffering any kind of adverse effect following the first and second vaccine shot, respectively (p=0.12). Main symptoms included: local pain, muscle pain, general malaise, headache and fever. None of the women reported a serious adverse effect or need for hospitalization. As local pain was the most frequent symptom experienced after the first vaccine shot [44/129 (34.1%) versus 17/129 (13.2%) women; p=0.001], elevated fever was more commonly reported after the second [4/129 (3.1%) versus 35/129 (27.1%); p=0.001].

Discussion

Principle findings

The main findings of our study are: 1. No significant interpersonal changes in plasma AMH levels were found at three months following two mRNA SARS-CoV-2 vaccinations. 2. AMH levels remained unchanged following vaccination also after sub analyses of different age groups. 3. No differences in mean antibody levels at three months following vaccinations were observed between age groups nor were they associated with AMH levels.

Clinical implications

Our study demonstrated that at three months after SARS-CoV-2 mRNA vaccinations AMH levels did not change irrespective of baseline levels and age. In sub-analyses by age groups, baseline AMH levels (first AMH) were lower in the older group (≥35

years old). This finding reflects a well-known decrease in ovarian reserve that occurs with age (Broer et al. 2014). Despite the different baseline AMH levels in the different age groups, the changes in AMH levels after three months still did not differ significantly between the sub age groups.

Data on the influence of SARS-CoV-2 infection and of SARS-CoV-2 mRNA vaccinations on fertility and ovarian function are limited. The SARS-CoV-2 attacks human cells through binding of the viral S protein to the ACE2 receptor. This S-protein is used in the mRNA vaccines as a presenting antigen and it was questioned whether such a pathway might negatively affect ovarian integrity (Hamming et al. 2007; Reis et al. 2011).

Few small studies examining the potential association between SARS-CoV-2 infection and its influence on the female reproductive system have been recently published (Barragan et al. 2021; Orvieto et al., 2021; Wang et al. 2021). One study described two women who underwent controlled ovarian stimulation after a positive PCR test to SARS-CoV-2 infection. On the day of oocyte collection viral RNA was not detectable in the ovarian follicular fluid analyzed (Barragan et al. 2021). Another study reported results of nine couples undergoing in vitro fertilization (IVF) treatments post documented Covid-19 infection (seven after female infection and two after male infection). This study found similar ovarian response to ovarian stimulation when compared to pre infection cycles. However, the authors reported on a reduced proportion of top-quality embryos (TQEs) (Orvieto et al. 2021), suggesting a possible detrimental effect of Covid-19 infection on folliculogenesis or spermatogenesis.

Wang et al. compared 65 women with asymptomatic or mild severe SARS-CoV-2 infection to 195 controls undergoing assisted reproductive technology (ART)

treatments and found that after matching, the ovarian reserves and ovarian responses were similar between groups (Wang et al. 2021).

Recently, one study examined the effect of SARS-CoV-2 mRNA vaccine on IVF cycles outcome. Orvieto et al. (Orvieto et al. 2021) reported on 36 couples undergoing IVF treatment cycle before and 8-92 days after receiving mRNA SARS-CoV-2 vaccine. No influence of mRNA SARS-CoV-2 vaccine on patients' performance during their immediate subsequent IVF cycle was observed, reflecting no detrimental effects of the vaccine on patients' ovarian reserve, nor the developing gametes/embryos, with an acceptable pregnancy rate (30% per transfer). Our findings are in concordance with previous studies demonstrating no significant influence on ovarian reserve following SARS-CoV-2 mRNA vaccination**.**

In order to ascertain active immunization in our study group we determined anti Covid-19 antibody levels in our participants. We found in all vaccinated women after three months a good immunological response to the SARS-CoV-2 mRNA vaccinations at all ages. As claims regarding the potential association between immunity response to the vaccine effect on AMH levels could have been raised, our study found no such association. Although it is well known that the ovaries are a common target for autoimmune attacks, data regarding the potential negative influence of immunity response following vaccination on fertility are limited (Schmuhl et al. 2020; Zhu et al. 2021; Szeliga et al. 2021). Similar concerns were raised when Human Papilloma vaccine was introduced, especially as it was recommended for adolescents and young adults. These concerns were refuted by a population-based cohort study of nearly 200,000 women that found no association between the HPV vaccine and premature ovarian insufficiency (Naleway et al. 2018).

Our results are in concordance to previous studies reporting no influence of immunity response on potential fertility following vaccination, estimated in this study by AMH. The characteristics of adverse effects in our study population following vaccination were similar to those reported in the literature (Amanzio et al. 2021; Menni et al. 2021; Bauernfeind et al. 2021). Systematic review including two studies with 37,590 participants receiving mRNA vaccines reported that the most frequent adverse effects were fatigue, headache, local pain, injection site reactions, and myalgia (Amanzio et al. 2021). A prospective observational study, examining the proportion of adverse effects within eight days of mRNA (Pfizer) vaccination reported 66.70% (188,178 of 208,103) following first dose and 54.03% (15,241 of 28,207) following the second dose (Menni et al. 2021). Thus, the adverse effects rates observed in our study are in concordance with previous reports, reflecting similarity to the general population reaction to the vaccine, therefore, strengthening the external validity of our findings.

Limitation and strength

The study has strengths that should be acknowledged. To the best of our knowledge this is the first study evaluating by an objective parameter (AMH levels) the potential influence of the new SARS-CoV-2 mRNA vaccine on ovarian reserve on large cohort group. Each woman served as her own control. Follow-up was possible in 80% of the women. All women were diagnosed in a single medical center, were evaluated by the same team and AMH levels were determined in one central laboratory during the same time period. The two personal AMH evaluations were performed during the same laboratory run. Furthermore, inclusion of serological data allowed us to ascertain the vaccination status of each participant.

This study has also limitations that need to be mentioned. Although it was prospectively designed, for ethical reasons we could not assign a priori a randomized unvaccinated control group as primarily planned. In times of a worldwide pandemic, in the peak of a national vaccination project, and when the amounts of available vaccines were not certain, recruiting women who are quasi encouraged or obliged to remain unvaccinated was regarded by all involved as unethical. Therefore, before launching the study, we decided to abandon the recruitment of the unvaccinated control group and to remain with the primary group, where the women served as their own controls. Additionally, this study examined plasma AMH levels at three months after the first vaccination. It could be argued that possible deleterious ovarian and AMH changes caused by the SARS-CoV-2 mRNA vaccinations might take effect only at a later time. Only longer-term studies will be able to examine this issue. Further, data on medical gynecological and obstetrical characteristics, including changes on follow up, were gathered via computerized questionnaires that were completed by the women and were not based on medical records. This could have exposed the results to recall bias.

Conclusion

In the present study, we found that plasma AMH levels before and three months following two mRNA SARS-CoV-2 vaccinations did not change significantly. This finding was consistent also after analyzing different age groups. All vaccinated women in this study demonstrated elevated anti Covid-19 antibody levels at three months. We did not find any association between antibody levels and AMH levels.

Therefore, we conclude that SARS-CoV-2 mRNA vaccinations are not associated with a decrease in ovarian reserve at three months. This information could be of

significant value to physicians and patients alike. Additional studies and long term follow-up could further strengthen our findings.

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Aya Mohr-Sasson- Conception and design, acquisition of data, analysis and interpretation of data, statistical analysis, writing, editing.

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Shlomi Abuhasira- Acquisition of data

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Estela Derazne- Statistical analysis, interpretation of data

Rina Hemi- Acquisition of data, reviewing manuscript

Raoul Orvieto- Interpretation of data, writing, editing

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Conflict of Interest

All authors have nothing to disclose

Data availability

Data available on request

Figure legends

Figure 1: Study population

Figure 2: Scatter plots of second anti-Müllerian hormone (AMH) and the change in AMH as a function of first AMH by age groups

Reference

Amanzio, M., D. D. Mitsikostas, F. Giovannelli, M. Bartoli, G. E. Cipriani, and W. A. Brown. 2021. "Adverse events of active and placebo groups in SARS-CoV-2 vaccine randomized trials: A systematic review." *Lancet Reg Health Eur*: 100253. https://doi.org/10.1016/j.lanepe.2021.100253. https://www.ncbi.nlm.nih.gov/pubmed/34729549.

Anifandis, G., C. I. Messini, A. Daponte, and I. E. Messinis. 2020. "COVID-19 and fertility: a virtual reality." *Reprod Biomed Online* 41 (2): 157-159. https://doi.org/10.1016/j.rbmo.2020.05.001. https://www.ncbi.nlm.nih.gov/pubmed/32466995.

Barragan, M., J. J. Guillén, N. Martin-Palomino, A. Rodriguez, and R. Vassena. 2021. "Undetectable viral RNA in oocytes from SARS-CoV-2 positive women." *Hum Reprod* 36 (2): 390-394. https://doi.org/10.1093/humrep/deaa284. https://www.ncbi.nlm.nih.gov/pubmed/32998162.

Bauernfeind, S., B. Salzberger, F. Hitzenbichler, K. Scigala, S. Einhauser, R. Wagner, A. Gessner, J. Koestler, and D. Peterhoff. 2021. "Association between Reactogenicity

and Immunogenicity after Vaccination with BNT162b2." *Vaccines (Basel)* 9 (10). https://doi.org/10.3390/vaccines9101089. https://www.ncbi.nlm.nih.gov/pubmed/34696197.

Beckman coulter. 2021. https://www.beckmancoulter.com/products/immunoassay/access-sars-cov-2-igg-antibody-test

Billon-Denis, E., and J. N. Tournier. 2020. "[COVID-19 and vaccination: a global disruption]." *Med Sci (Paris)* 36 (11): 1034-1037. https://doi.org/10.1051/medsci/2020203. https://www.ncbi.nlm.nih.gov/pubmed/33151866.

Broer, S. L., F. J. Broekmans, J. S. Laven, and B. C. Fauser. 2014. "Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications." *Hum Reprod Update* 20 (5): 688-701. https://doi.org/10.1093/humupd/dmu020. https://www.ncbi.nlm.nih.gov/pubmed/24821925.

Hamming, I., M. E. Cooper, B. L. Haagmans, N. M. Hooper, R. Korstanje, A. D. Osterhaus, W. Timens, A. J. Turner, G. Navis, and H. van Goor. 2007. "The emerging role of ACE2 in physiology and disease." *J Pathol* 212 (1): 1-11. https://doi.org/10.1002/path.2162. https://www.ncbi.nlm.nih.gov/pubmed/17464936.

Health Care. 2021. https://www.muhealth.org/our-stories/does-covid-19-vaccine-affect-fertility-heres-what-experts-say.

Jing, Y., L. Run-Qian, W. Hao-Ran, C. Hao-Ran, L. Ya-Bin, G. Yang, and C. Fei. 2020. "Potential influence of COVID-19/ACE2 on the female reproductive system." *Mol Hum Reprod* 26 (6): 367-373. https://doi.org/10.1093/molehr/gaaa030. https://www.ncbi.nlm.nih.gov/pubmed/32365180.

Lan, J., J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang, and X. Wang. 2020. "Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor." *Nature* 581 (7807): 215-220. https://doi.org/10.1038/s41586-020-2180-5. https://www.ncbi.nlm.nih.gov/pubmed/32225176.

Li, W., M. J. Moore, N. Vasilieva, J. Sui, S. K. Wong, M. A. Berne, M. Somasundaran, J. L. Sullivan, K. Luzuriaga, T. C. Greenough, H. Choe, and M. Farzan. 2003. "Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus." *Nature* 426 (6965): 450-4. https://doi.org/10.1038/nature02145. https://www.ncbi.nlm.nih.gov/pubmed/14647384.Menni, C., K. Klaser, A. May, L. Polidori, J. Capdevila, P. Louca, C. H. Sudre, L. H. Nguyen, D. A. Drew, J. Merino, C. Hu, S. Selvachandran, M. Antonelli, B. Murray, L. S. Canas, E. Molteni, M. S. Graham, M. Modat, A. D. Joshi, M. Mangino, A. Hammers, A. L. Goodman, A. T. Chan, J. Wolf, C. J. Steves, A. M. Valdes, S. Ourselin, and T. D. Spector. 2021. "Vaccine side-effects and SARS-CoV-2 infection after vaccination in users of the COVID Symptom Study app in the UK: a prospective observational study." *Lancet Infect Dis* 21 (7): 939-949. https://doi.org/10.1016/S1473-3099(21)00224-3. https://www.ncbi.nlm.nih.gov/pubmed/33930320.

Naleway, A. L., K. F. Mittendorf, S. A. Irving, M. L. Henninger, B. Crane, N. Smith, M. F. Daley, and J. Gee. 2018. "Primary Ovarian Insufficiency and Adolescent Vaccination." *Pediatrics* 142 (3). https://doi.org/10.1542/peds.2018-0943. https://www.ncbi.nlm.nih.gov/pubmed/30131438.

Orvieto, R., M. Noach-Hirsh, A. Segev-Zahav, J. Haas, R. Nahum, and A. Aizer. 2021. "Does mRNA SARS-CoV-2 vaccine influence patients' performance during IVF-ET cycle?" *Reprod Biol Endocrinol* 19 (1): 69. https://doi.org/10.1186/s12958-021-00757-6. https://www.ncbi.nlm.nih.gov/pubmed/33985514.

Orvieto, R., A. Segev-Zahav, and A. Aizer. 2021. "Does COVID-19 infection influence patients' performance during IVF-ET cycle?: an observational study." *Gynecol Endocrinol*: 1-3. https://doi.org/10.1080/09513590.2021.1918080. https://www.ncbi.nlm.nih.gov/pubmed/33974475.

Pellatt, L., S. Rice, and H. D. Mason. 2010. "Anti-Müllerian hormone and polycystic ovary syndrome: a mountain too high?" *Reproduction* 139 (5): 825-33. https://doi.org/10.1530/REP-09-0415. https://www.ncbi.nlm.nih.gov/pubmed/20207725.

Practice Committee of the American Society for Reproductive Medicine. 2015. "Testing and interpreting measures of ovarian reserve: a committee opinion." *Fertil Steril* 103 (3): e9-e17. https://doi.org/10.1016/j.fertnstert.2014.12.093. https://www.ncbi.nlm.nih.gov/pubmed/25585505.Reis, F. M., D. R. Bouissou, V. M. Pereira, A. F. Camargos, A. M. dos Reis, and R. A. Santos. 2011. "Angiotensin-(1-7), its receptor Mas, and the angiotensin-converting enzyme type 2 are expressed in the human ovary." *Fertil Steril* 95 (1): 176-81. https://doi.org/10.1016/j.fertnstert.2010.06.060. https://www.ncbi.nlm.nih.gov/pubmed/20674894.

Robson, David. 2021. " Social media is rife with posts disparaging the vaccine hesitant – but these reactions to a complex and nuanced issue are doing more harm than good" https://www.bbc.com/future/article/20210720-the-complexities-of-vaccine-hesitancy

Schlake, T., A. Thess, M. Fotin-Mleczek, and K. J. Kallen. 2012. "Developing mRNA-vaccine technologies." *RNA Biol* 9 (11): 1319-30. https://doi.org/10.4161/rna.22269. https://www.ncbi.nlm.nih.gov/pubmed/23064118.

Schmuhl, N. B., K. E. Mooney, X. Zhang, L. G. Cooney, J. H. Conway, and N. K. LoConte. 2020. "No association between HPV vaccination and infertility in U.S. females 18-33 years old." *Vaccine* 38 (24): 4038-4043. https://doi.org/10.1016/j.vaccine.2020.03.035. https://www.ncbi.nlm.nih.gov/pubmed/32253100.

Szeliga, A., A. Calik-Ksepka, M. Maciejewska-Jeske, M. Grymowicz, K. Smolarczyk, A. Kostrzak, R. Smolarczyk, E. Rudnicka, and B. Meczekalski. 2021. "Autoimmune Diseases in Patients with Premature Ovarian Insufficiency-Our Current State of Knowledge." *Int J Mol Sci* 22 (5). https://doi.org/10.3390/ijms22052594. https://www.ncbi.nlm.nih.gov/pubmed/33807517.

Themmen, A. P. 2005. "Anti-Müllerian hormone: its role in follicular growth initiation and survival and as an ovarian reserve marker." *J Natl Cancer Inst Monogr* (34): 18-21. https://doi.org/10.1093/jncimonographs/lgi026. https://www.ncbi.nlm.nih.gov/pubmed/15784815.

Tregoning, J. S., E. S. Brown, H. M. Cheeseman, K. E. Flight, S. L. Higham, N. M. Lemm, B. F. Pierce, D. C. Stirling, Z. Wang, and K. M. Pollock. 2020. "Vaccines for COVID-19." *Clin Exp Immunol* 202 (2): 162-192. https://doi.org/10.1111/cei.13517. https://www.ncbi.nlm.nih.gov/pubmed/32935331.

Vartak, A., and S. J. Sucheck. 2016. "Recent Advances in Subunit Vaccine Carriers." *Vaccines (Basel)* 4 (2). https://doi.org/10.3390/vaccines4020012. https://www.ncbi.nlm.nih.gov/pubmed/27104575.

Wang, J., Y. Peng, H. Xu, Z. Cui, and R. O. Williams. 2020. "The COVID-19 Vaccine Race: Challenges and Opportunities in Vaccine Formulation." *AAPS PharmSciTech* 21 (6): 225. https://doi.org/10.1208/s12249-020-01744-7. https://www.ncbi.nlm.nih.gov/pubmed/32761294.

Wang, M., Q. Yang, X. Ren, J. Hu, Z. Li, R. Long, Q. Xi, L. Zhu, and L. Jin. 2021. "Investigating the impact of asymptomatic or mild SARS-CoV-2 infection on female fertility and." *EClinicalMedicine* 38: 101013. https://doi.org/10.1016/j.eclinm.2021.101013. https://www.ncbi.nlm.nih.gov/pubmed/34250457.

Wrapp, D., N. Wang, K. S. Corbett, J. A. Goldsmith, C. L. Hsieh, O. Abiona, B. S. Graham, and J. S. McLellan. 2020. "Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation." *Science* 367 (6483): 1260-1263.

https://doi.org/10.1126/science.abb2507.
https://www.ncbi.nlm.nih.gov/pubmed/32075877.

Zhu, X., J. Liu, H. Pan, Z. Geng, W. Huang, T. Liu, and B. Zhang. 2021. "Thymopentin treatment of murine premature ovarian failure via attenuation of immune cell activity and promotion of the BMP4/Smad9 signalling pathway." *Int J Med Sci* 18 (15): 3544-3555. https://doi.org/10.7150/ijms.61975.
https://www.ncbi.nlm.nih.gov/pubmed/34522181.

Table I: Women's characteristics

	Vaccinated (n=129)	
	Mean ±SD	**Median (25^{th}-75^{th}percentile)**
Age (years)	29.3±5.2	29.0 (26.0-33.0)
BMI (kg/m²)	22.6±4.0	21.9 (20.0-24.5)
Menstruation Frequency (days)	28.1±3.7	28.0 (28.0-30.0)
Menstruation Length (days)	4.8±1.3	5.0 (4.0-5.0)
AMH First (µg/L)	5.3±4.2	4.2 (2.4-7.1)
AMH Second (µg/L)	5.3±4.5	4.2 (2.4-6.5)
Delta AMH(µg/L)	-0.1±1.9	-0.2 (-0.8-0.7)
Change (%) in AMH	2.5±38.9	-4.9 (-17.5-19.9)
Interval between AMH examination (days)	98.8±12.7	94.0 (91.0-108.0)
	n	**%**
Gravidity		
0	87	75.0
1	10	8.6
2	19	16.4
Parity (≥1)	26	22.2
S/P Cesarean delivery	11	9.4
Irregular Menstruation/PCOS	22	19.1
Contraception		
None	50	42.7
Condom	32	27.4
Hormonal contraception	27	23.1
IUD Hormonal	2	1.7
IUD Non-Hormonal	3	2.6
Other	3	2.6

AMH- Anti Müllerian Hormone, µg/L- microgram per liter, S/P- Status Post, PCOS-Poly cystic ovary syndrome, IUD-Intra Uterine Device

(*) Fisher's exact test for variable dichotomized into yes or no

Table II: Women's characteristics by age group

	Age group			
	<30 (n=79)	**30-35 (n=31)**	**>=35 (n=19)**	
	Mean±SD Median (25^{th}-75^{th}percentile)	Mean±SD Median (25^{th}-75^{th}percentile)	Mean±SD Median (25^{th}-75^{th}percentile)	p
Age (years)	25.9±3.1 26.0 (24.0-28.0)	32.8±1.2 33.0 (32.0-34.0)	37.8±1.5 38.0 (36.0-39.0)	1.2 E-21
BMI (kg/m^2)	22.6±3.6 22.1 (20.1-24.8)	21.4±2.6 21.1 (19.6-23.2)	24.6±6.4 23.4 (21.0-24.8)	0.166
Menstruation Frequency (days)	28.1±3.5 28.0 (28.0-30.0)	28.7±4.3 28.0 (28.0-30.0)	26.9±3.4 28.0 (26.0-28.0)	0.369
Menstruation Length (days)	4.6±1.2 5.0 (4.0-5.0)	5.2±1.6 5.0 (4.0-6.0)	4.6±1.5 4.0 (4.0-5.5)	0.281
AMH First (µg/L)	6.3±4.6 4.9 (3.2-8.1)	4.9±2.9 4.9 (2.7-6.4)	2.0±1.5 1.7 (1.1-2.4)	1.5 E-06
AMH Second (µg/L)	6.1±4.9 4.7 (2.8-7.4)	5.1±3.5 4.4 (2.7-6.4)	2.1±1.7 1.6 (0.6-3.3)	3.4 E-05
Delta AMH(µg/L)	-0.2±2.1 -0.3 (-0.9-0.5)	0.2±1.7 0.2 (-0.6-0.8)	0.1±0.8 -0.1 (-0.4-0.6)	0.288
Change (%) in AMH	-1.6±28.6 -6.0 (-17.2-16.8)	-10.8±43.9 2.9 (-13.9-27.4)	5.8±61.5 -2.3 (-36.6-28.5)	0.459
Interval between AMH examination (days)	99.8±12.7 94.0 (91.0-110.0)	96.8±12.3 93.0 (89.0-103.0)	97.5±13.4 94.0 (91.0-105.0)	0.639
Serology (S/CO)	19.8±11.5 17.3 (11.6-25.5)	19.4±12.4 16.8 (11.0-24.0)	14.5±8.6 12.5 (8.4-23.1)	0.229

AMH- Anti Mullerian Hormone, µg/L- microgram per liter, S/CO- signal to cut off

Table III: Number and percent of women with a >10% decrease of anti-Müllerian hormone (AMH) levels following inoculation by age groups

		Number (percent) of women with change in AMH						
		>10% decrease		**No or ≤ 10% decrease**		**Total**		
		n	%	n	%	n	%	P value
Age	<30	33	41.77	46	58.23	79	100.00	0.63
groups	30-35	10	32.26	21	67.74	31	100.00	
	>35	8	42.11	11	57.89	19	100.00	

Figure 1: Study population

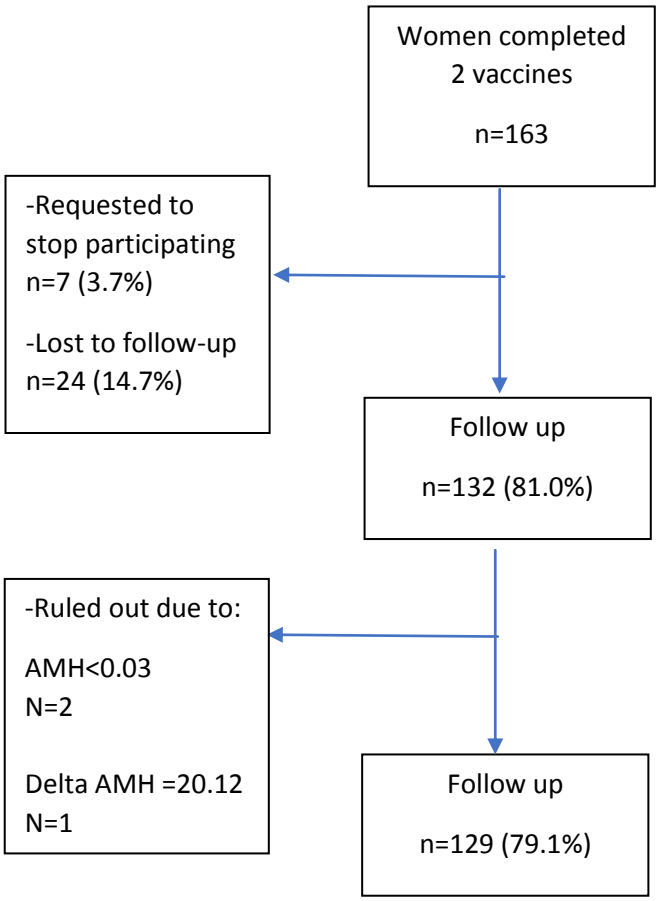
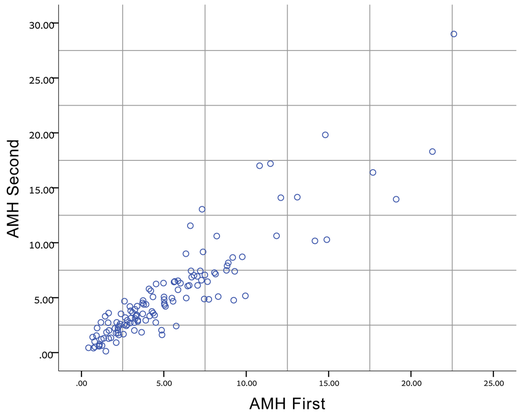
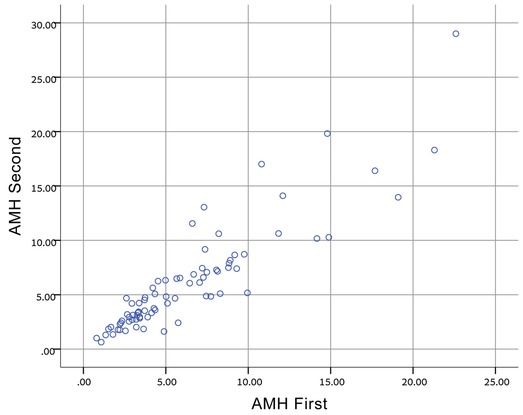


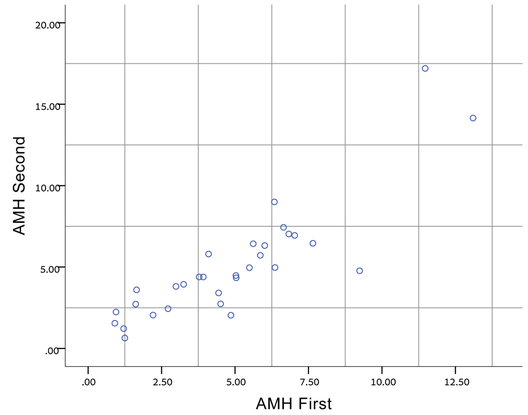
Figure 2: Scatter plots of second AMH and the change in AMH as a function of first AMH by age groups



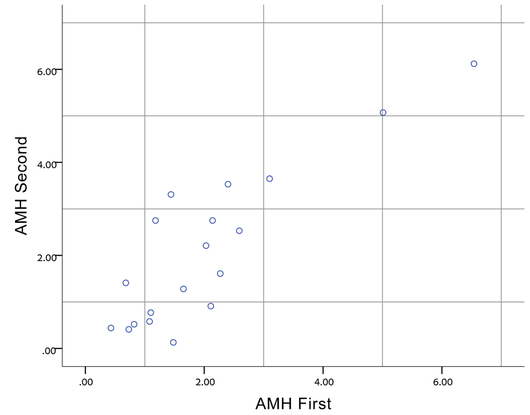
AMH Second=0.05 + 0.97 (AMH First)



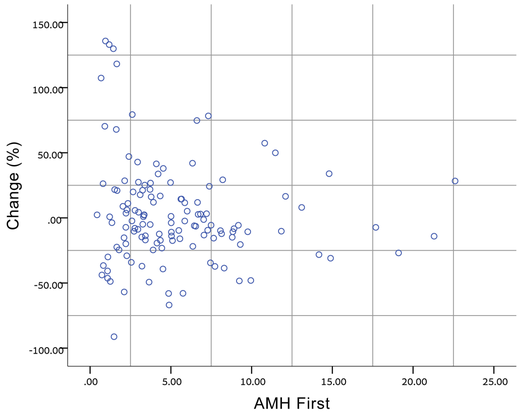
AMH Second=0.006 + 1.03 (AMH First)



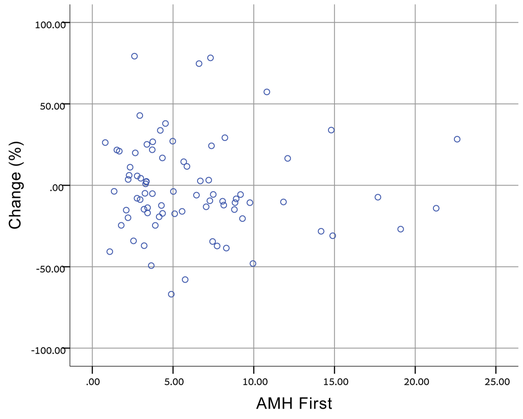
AMH Second=0.16 + 0.95 (AMH First)



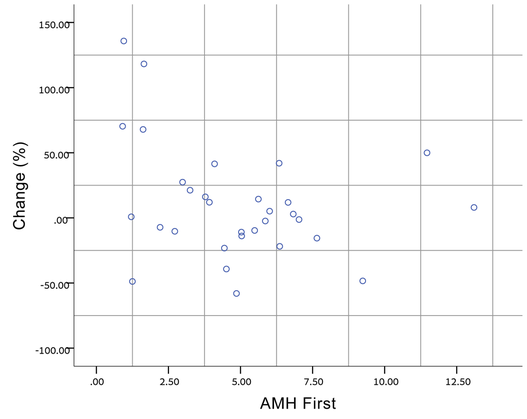
AMH Second=0.16 + 0.95 (AMH First)



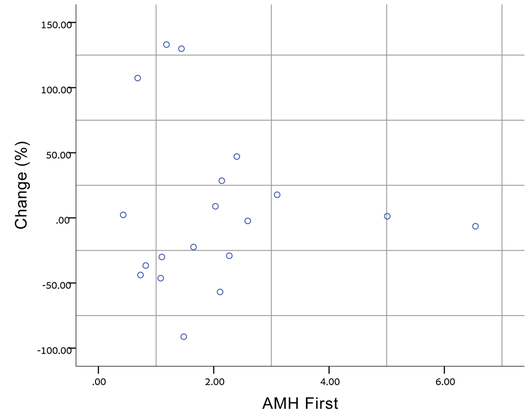
Total Study Population



Age<35



Age 30-35



Age35<