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Mild COVID-19 infection does not alter the ovarian reserve in women treated with ART

BIOGRAPHY

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KEY MESSAGE

Mild COVID-19 infection did not alter the ovarian reserve in women treated with ART. Baseline AMH, AMH tested during the ART treatment, and the difference between the two AMH levels, were not statistically different between groups that tested positive for COVID-19 and those that tested negative (rapid detection test).

ABSTRACT

Research question: Does mild COVID-19 infection affect the ovarian reserve of women undergoing an assisted reproductive technology (ART) protocol?

Design: A prospective observational study was conducted between June and December 2020 at the ART unit of Tenon Hospital, Paris. Women managed at the unit for fertility issues by in-vitro fecundation, intracytoplasmic sperm injection (IVF/ICSI), fertility preservation, frozen embryo transfer or artificial insemination, and with an anti-Müllerian hormone (AMH) test carried out within 12 months preceding ART treatment, were included. All the women underwent a COVID rapid detection test (RDT) and AMH concentrations between those who tested positive (RDT positive) and those who tested negative (RDT negative).

Results: The study population consisted of 118 women, 11.9% (14/118) of whom were COVID RDT positive. None of the tested women presented with a history of severe COVID-19 infection. The difference between the initial AMH concentration and AMH concentration tested during ART treatment was not significantly different between the COVID RDT positive group and COVID RDT negative group (–1.33 ng/ml [–0.35 to –1.61) versus –0.59 ng/ml [–0.15 to –1.11], *P* = 0.22).

Conclusion: A history of mild COVID-19 infection does not seem to alter the ovarian reserve as evaluated by AMH concentrations. Although these results are reassuring, further studies are necessary to assess the effect of COVID-19 on pregnancy outcomes in women undergoing ART.

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INTRODUCTION

Since December 2019, the world has been facing a COVID-19 pandemic. Besides its effect on mortality, COVID-19 infection raises questions about short- and long-term effects on general health. Clinical manifestations are highly heterogeneous and involve many different organs (*Lai et al., 2020*).

The SARS-CoV-2 virus penetrates human cells by directly binding with angiotensin-converting enzyme 2 (ACE2) receptors present on the cell surface (*Bornstein et al., 2020*). The ACE2 receptors are present in testes (*Fan et al., 2020*; *Fu et al., 2020*; *Stanley et al., 2020*) and in ovarian tissue (*Reis et al., 2011*; *Jing et al., 2020*; *Stanley et al., 2020*). In the ovary, ACE2 plays a role in the response to gonadotrophins, steroidogenesis regulation, and in follicle development, angiogenesis and degeneration (*Domińska, 2020*; *Jing et al., 2020*). It has been suggested that SARS-CoV-2 could be responsible for testicular lesions (*Fan et al., 2020*). Analysis of testicular specimens from autopsies of men who died from COVID-19 showed modifications of the testicular structure, a thickening of the basal layer of seminiferous tubules, a decrease or absence of spermatozoa, and a decrease in the number of Leydig cells, lymphocyte infiltration and germinal cell degeneration, compared with matched controls who died from other pathologies (*Chen and Lou, 2020*; H *Li et al., 2020*; *Yang et al., 2020*; *Ma et al., 2021*). Testicular pain has been reported in about 20% of men with COVID-19 infection (*Pan et al., 2020*).

SARS-CoV-2 RNA was not found in the follicular fluid of two women who tested positive for COVID-19 and who were undergoing ovarian stimulation for IVF (*Barragan et al., 2020*). The modification of ovarian reserve by COVID-19 infection, however, has not been evaluated to date.

Therefore, the aim of this prospective study was to evaluate the effect of mild COVID-19 infection on the ovarian reserve of women undergoing an assisted reproductive technology (ART) protocol.

MATERIALS AND METHODS

Study population

This single-centre prospective observational study was conducted in the ART unit of Tenon Hospital, Paris, between June 2020 and December 2020.

Women aged 18–43 years managed for fertility issues by IVF and intracytoplasmic sperm injection (IVF/ICSI), fertility preservation, frozen embryo transfer or artificial insemination with an initial anti-Müllerian hormone (AMH) concentration tested within the 12 months preceding ART treatment, were invited to participate in the study.

Data collection

Demographic characteristics, including age, body mass index (BMI) (kg/m^2), tobacco smoking, presence of insufficient ovarian reserve, endometriosis, fallopian tube pathology and initial AMH concentration were retrieved from a prospective database. The type of ART protocol, the time between the initial (baseline) and second AMH test, as well as the oestradiol concentration on the day of AMH test, were also recorded.

As recommended by the French Agency of Biomedicine (Agence de Biomédecine), all the women completed a questionnaire about any COVID-19 infection symptoms that may have occurred during the 2 weeks before ART treatment. COVID-19 serology status was tested on the first day of ovarian stimulation monitoring with a COVID-19 rapid detection test (RDT) kit (UNCOV-40, Clinisciences, France) according to the manufacturer's instructions. Ovarian reserve was evaluated by AMH tested on the day of ovarian stimulation monitoring, and ovarian reserve modification was calculated by the difference between the baseline AMH concentration (tested within the preceding 12 months) and this new AMH concentration.

All women included in the study expressed non-opposition consent to participate in the study. The procedures used in the study were in accordance with the guidelines of the Helsinki Declaration on Human Experimentation and the Good Clinical Practice (CGP) and approved by the IRB (CEROG 2021-GYN-0508, 22/06/2021).

Statistical analysis

Quantitative variables are presented as means with SD or medians with interquartile range as appropriate. Qualitative variables are expressed as numbers with percentages (%). Differences in population characteristics between COVID positive and COVID negative women were evaluated with Student's t-test, Mann–Whitney test, chi-squared test and Fisher's exact test as appropriate. The difference in AMH concentrations between the COVID RDT positive and COVID RDT negative women was evaluated with the Mann–Whitney test.

All tests were two-sided and $P < 0.05$ was considered to be statistically significant. GraphPad Prism 7 was used for analyses.

RESULTS

Population characteristics

Of the 960 women who underwent an ART protocol in our unit during the study period (June 2020 and December 2020), 118 accepted to participate in the study. The prevalence of COVID RDT positive in the tested population was 11.9% (14/118). None of the women included in the study presented clinical manifestations of COVID during the 2 weeks preceding the beginning of the ART protocol. None of the women presented the severe form of COVID-19 infection or required hospitalization during the pandemic period.

The characteristics of the women with COVID RDT positive and COVID RDT negative are presented in TABLE 1. No significant difference was found in age, BMI, tobacco smoking, cause of infertility, baseline AMH concentration or ART protocol type. The time between the baseline and second AMH test was not significantly different between the two groups as was the oestrogen concentration on the day of the AMH test.

The median concentration of AMH tested during ART treatment was not significantly different between the two groups: 1.51 ng/ml (0.82–2.38) in COVID RDT positive group versus 1.00 ng/ml (0.49–1.99) in COVID RDT negative group ($P = 0.27$) TABLE 2.

Similarly, the difference between the baseline and second AMH concentrations was not significantly different between the groups: –1.33 ng/ml (–0.35 to –1.61) in COVID RDT positive group versus –0.59 ng/ml (–0.15

TABLE 1 CHARACTERISTICS OF THE STUDY POPULATIONS

	COVID RDT positive(n = 14)	COVID RDT negative(n = 104)	P-Value
Age, years, mean (SD)	35.7 (4.2)	34.5 (4.5)	0.32
BMI, kg/m^2, mean (SD)	23.1 (3.7)	24.3 (5.5)	0.29
Tobacco smoking, n (%)	2 (14)	18 (17)	0.78
IOP, n (%)	1 (7)	22 (21)	0.21
Endometriosis, n (%)	3 (21)	30 (29)	0.56
Fallopian tube pathology, n (%)	4 (29)	14 (13)	0.14
Initial AMH concentration, ng/ml, median (IQR)	2.87 (1.69–3.99)	1.76 (0.88–3.00)	0.13
Period between the two AMH tests, days, median (IQR)	254 (211–285)	268 (158–327)	0.77
Art protocol			0.38
IVF/ICSI, n (%)	11 (79)	64 (62)	
Fertility preservation, n (%)	0 (0)	17 (16)	
Artificial insemination, n (%)	2 (14)	18 (17)	
Embryo transfer, n (%)	1 (7)	5 (5)	
Oestrogen concentration on AMH testing day, ng/ml, median, IQR	718 (239–1534)	664 (277–1353)	0.87

AMH, anti-Müllerian hormone; ART, assisted reproductive technology; BMI, body mass index; IOP, insufficient ovarian reserve; IQR, interquartile range; IVF/ICSI, IVF and intracytoplasmic sperm injection; RDT, rapid diagnostic test.

to –1.11) in COVID RDT negative group (P = 0.22).

DISCUSSION

The results of this prospective study showed that, based on AMH concentrations, mild COVID-19 infection did not affect ovarian reserve in our population of asymptomatic women who underwent an ART protocol in our unit. The baseline AMH concentration, the concentration tested during the ART treatment, as well as the difference between the two AMH concentrations, were not significantly different between the COVID RDT positive group and COVID RDT negative group.

To date, the total number of confirmed cases of COVID-19 infection worldwide is about 71,500,000 (Santé Publique France, 2020), which represents 0.09% of the population overall. In France, 2,500,000 cases have been confirmed (Santé Publique France, 2020), which represents 3.7% of the French population overall and about 5% of the adult population. The prevalence of positive COVID RDT positive in our study population was high at 11.9%. This can be explained by the fact that serology testing is not offered systematically in France, and many asymptomatic cases remain undetected. Therefore, the number of COVID-19 cases in the general population is certainly underestimated.

Although none of the included women reported having symptoms of COVID-19 in the 2 weeks preceding their ART treatment, it is not known if they presented minor symptoms of COVID-19 infection earlier on. None of the women, however, had presented the severe form of COVID-19 infection requiring hospitalization during the pandemic period.

The extra-respiratory manifestations of COVID-19 are diverse and involve multiple organs (Lai et al., 2020). It has been suggested that COVID-19 infection could affect the female reproductive system, as the virus enters target cells by interacting with ACE2 receptors, which are expressed in the ovaries (Jing et al., 2020; Singh et al., 2020). Other viral infections, such as HIV or viral hepatitis, have been shown to potentially alter ovarian reserve (Seifer et al., 2007; Kurmanova et al., 2016; Santulli et al., 2016).

Li et al. (2020) demonstrated that sex hormone concentrations and AMH concentrations in women of reproductive age hospitalized for confirmed COVID-19 infection were comparable to the age-matched controls, even if 28% of the COVID-19 positive women in their study presented changes in their menstrual cycle and 25% changes in their menstrual volume (K Li et al., 2020). Our study confirms these results. The median concentration of AMH in the COVID RDT positive women was comparable with that found in the COVID RDT negative women (P = 0.27). Moreover, the difference between two AMH concentrations tested in the same women at different times was comparable between both groups (P = 0.22).

In contrast to the study by Li et al. (2020), which was conducted among a population of hospitalized women, mid- and long-term effects of COVID-19 infection were evaluated on ovarian reserve: AMH concentrations were tested during ART treatment sometime after a potential COVID-19 infection and in women without or with few symptoms.

TABLE 2 ANTI-MÜLLERIAN HORMONE CONCENTRATIONS IN COVID RAPID DIAGNOSTIC TEST POSITIVE AND COVID RAPID DIAGNOSTIC TEST NEGATIVE GROUPS

	COVID RDT positive (n = 14)	COVID RDT negative (n = 104)	P-value
New AMH concentration, ng/ml, median (IQR)	1.51 (0.82–2.38)	1.00 (0.49–1.99)	0.27
Change in AMH, ng/ml, median (IQR)	–1.33 (–0.35 to –1.61)	–0.59 (–0.15 to –1.11)	0.22

AMH, anti-Müllerian hormone; IQR, interquartile range; RDT, rapid diagnostic test.

COVID-19 infection by SARS-CoV-2 serology was evaluated using an immunochromatographic assay. This method is characterized by high specificity and sensitivity (98.02% and 98.81%, respectively, according to the manufacturer). It is not clear, as yet how long antibodies persist after COVID-19 infection (*Milani et al., 2020*).

The strength of our study is that AMH concentrations were tested in the same women at different time points and could, therefore, analyse any potential modification of the ovarian reserve after COVID-19 infection.

The present study, however, has some limitations. First, it has been shown that the AMH concentration is modified during ART treatment (*Peñarrubia et al., 2005*) as this hormone is secreted by granulosa cells of small growing follicles (*Moolhuijsen and Visser, 2020*), thus reflecting the granulosa cell activity. Nevertheless, the baseline and the second AMH concentrations, as well as ART protocol types and oestrogen concentrations on the day of AMH testing, were comparable between both study groups. Second, a relatively small number of women were included in the analysis and only 14 were COVID RDT positive. In addition, the both study groups were heterogenous as consecutive patients were included. The ART treatments in our unit were postponed for 3 months during the COVID epidemic after treatment was interrupted after a decision by the French government to cease treatment. Therefore, the time between the baseline AMH test and the beginning of the ART treatments was extended. It was decided, however, that only women with AMH tested within the 12 preceding months would be included because AMH concentration is age dependent and can decrease over time (*Plociennik et al., 2018*). The time between the two AMH tests was comparable between the study groups.

In conclusion, our study suggests that a history of mild COVID-19 infection does not seem to alter the ovarian reserve. Even if these results are reassuring, further studies, especially with larger samples, are required to confirm these findings.

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