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

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ABSTRACT

Research Question

Does mild COVID-19 infection impact the ovarian reserve of women undergoing an Assisted Reproductive Technology (ART) protocol?

Design

We conducted a prospective observational study between June and December 2020. We included women managed in our ART unit for fertility issues by in vitro fecundation / intracytoplasmic sperm injection (IVF/ICSI), fertility preservation (FP), frozen embryo transfer (ET) or artificial insemination (AI) and with an AMH test performed within 12 months preceding ART treatment. All the women underwent a COVID rapid detection test (RDT) and we compared AMH concentrations between those who tested positive (RDT+) and those who tested negative (RDT-).

Results

The study population consisted of 118 women, 11.9% (14/118) of whom were COVID RDT+. 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+ group and COVID RDT- group [-1.33 ng/ml (-0.35 – -1.61) versus -0.59 ng/ml (-0.15 – -1.11), p=0.22].

Conclusion

Our study suggests that a history of mild COVID-19 infection does not seem to alter the ovarian reserve as evaluated by AMH concentrations. While these results are reassuring, further studies are necessary to assess the impact of COVID-19 on pregnancy outcomes in women undergoing ART.

KEY WORDS:

COVID; ovarian reserve; ART; AMH

1 INTRODUCTION

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

6 The SARS-CoV-2 virus penetrates human cells by directly binding with angiotensin-
7 converting enzyme 2 (ACE2) receptors present on the cell surface (Bornstein et al.,
8 2020). ACE2 receptors are present in testis (Fan et al., 2020; Fu et al., 2020; Stanley
9 et al., 2020) and in ovarian tissue (Jing et al., 2020; Reis et al., 2011; Stanley et al.,
10 2020). In the ovary, ACE2 plays a role in the response to gonadotropins,
11 steroidogenesis regulation, and in follicle development, angiogenesis and
12 degeneration (Domińska, 2020; Jing et al., 2020). It has been suggested that SARS-
13 CoV-2 could be responsible for testicular lesions (Fan et al., 2020). Analysis of
14 testicular specimens from autopsies of men who died from COVID-19 showed
15 modifications of the testicular structure – a thickening of the basal layer of
16 seminiferous tubules, a decrease or absence of spermatozoa, decrease in the
17 number of Leydig cells, lymphocyte infiltration, and germinal cell degeneration –
18 compared with matched controls who died from other pathologies (Chen and Lou,
19 2020; H. Li et al., 2020; Ma et al., 2021; Yang et al., 2020). Testicular pain has been
20 reported in about 20% of men with COVID-19 infection (Pan et al., 2020).

21 SARS-CoV-2 RNA was not found in the follicular fluid of two women who tested
22 positive for COVID-19 and who were undergoing controlled ovarian hyperstimulation
23 for in vitro fertilization (IVF) (Barragan et al., 2020). However, the modification of
24 ovarian reserve by COVID-19 infection has not been evaluated to date.

25 Thus, the objective of this prospective study was to evaluate the impact of mild
26 COVID-19 infection on the ovarian reserve in women undergoing an Assisted
27 Reproductive Technology (ART) protocol.

28

29 **MATERIEL AND METHODS**

30 **Study Population**

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

33 Women aged 18-43 years managed for fertility issues by IVF / intracytoplasmic
34 sperm injection (IVF/ICSI), fertility preservation (FP), frozen embryo transfer (ET), or
35 artificial insemination (AI) with an initial AMH concentration tested within the 12
36 months preceding ART treatment, were invited to participate in the study.

37

38 **Data collection**

39 Demographic characteristics including age, body mass index (BMI, kg/m²), tobacco
40 smoking, presence of insufficient ovarian reserve (IOP), endometriosis, fallopian tube
41 pathology and initial AMH concentration were retrieved from a prospective database.
42 The type of ART protocol, the time between the initial (baseline) and second AMH
43 test as well as the oestradiol concentration on the day of AMH test were also
44 recorded.

45 As recommended by the French Agency of Biomedicine (*Agence de Biomédecine*),
46 all the women completed a questionnaire about any COVID-19 infection symptoms

47 that may have occurred during the 2 weeks prior to ART treatment. COVID-19
48 serology status was tested on the first day of ovarian stimulation monitoring with a
49 COVID-19 rapid detection test (RDT) kit (UNCOV-40, Clinisciences, France)
50 according to the manufacturer's instructions. Ovarian reserve was evaluated by AMH
51 tested on the day of ovarian stimulation monitoring and ovarian reserve modification
52 was calculated by the difference between the baseline AMH concentration (tested
53 within the preceding 12 months) and this new AMH concentration.

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

58

59 **Statistical analysis**

60 Quantitative variables are presented as means with standard deviation (SD) or
61 medians with interquartile range (IQR) as appropriate. Qualitative variables are
62 expressed as numbers with percentages (%). Differences in population
63 characteristics between COVID+ and COVID- women were evaluated with Student's
64 t-test / Mann-Whitney test or chi squared / Fisher exact test as appropriate. The
65 difference in AMH concentrations between the COVID RDT+ and COVID RDT-
66 women was evaluated with the Mann-Whitney test.

67 All tests were two-sided and $p < 0.05$ was considered to be statistically significant.

68 Analyses were done with GraphPad Prism 7.

69

70 **RESULTS**

71 **Population characteristics**

72 Of the 960 women who underwent an ART protocol in our unit during the study
73 period (June 2020 and December 2020), 118 accepted to participate in the study.
74 The prevalence of COVID RDT+ in the tested population was 11.9% (14/118). None
75 of the women included in the study presented clinical manifestations of COVID during
76 the 2 weeks preceding the beginning of the ART protocol. Neither had any of the
77 women presented the severe form of COVID-19 infection or required hospitalisation
78 during the pandemic period.

79 The characteristics of the women with COVID RDT+ and COVID RDT- are presented
80 in Table 1. There was no significant difference in age, BMI, tobacco smoking,
81 infertility aetiology, baseline AMH concentration, or ART protocol type. The time
82 between the baseline and second AMH test was not significantly different between
83 the two groups as was the oestrogen concentration on the day of AMH test.

84 The median concentration of AMH tested during ART treatment was not significantly
85 different between the two groups: 1.51 ng/ml (0.82-2.38) in COVID RDT+ group
86 versus 1.00 ng/ml (0.49-1.99) in COVID RDT- group ($p=0.27$).

87 Similarly, the difference between the baseline and second AMH concentrations was
88 not significantly different between the groups: -1.33 ng/ml (-0.35 – -1.61) in COVID
89 RDT+ group versus -0.59 ng/ml (-0.15 – -1.11) in COVID RDT- group, ($p=0.22$).

90

91 **DISCUSSION**

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

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

107 While none of included women reported having symptoms of COVID-19 in the 2
108 weeks preceding their ART treatment, it is not known if they presented minor
109 symptoms of COVID-19 infection earlier on. However, none of the women had
110 presented the severe form of COVID-19 infection requiring hospitalisation during the
111 pandemic period.

112 The extra-respiratory manifestations of COVID-19 are diverse and involve multiple
113 organs (Lai et al., 2020). It has been suggested that COVID-19 infection could impact
114 the female reproductive system, as the virus enters target cells by interacting with
115 ACE2 receptors which are expressed in the ovaries (Jing et al., 2020; Singh et al.,

116 2020). Other viral infections, such as HIV or viral hepatitis, have been shown to
117 potentially alter ovarian reserve (Kurmanova et al., 2016; Santulli et al., 2016; Seifer
118 et al., 2007).

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

128 In contrast to the study by Li *et al.* (K. Li et al., 2020), which was performed in a
129 population of hospitalised women, we evaluated mid- and long-term effects of
130 COVID-19 infection on ovarian reserve: AMH concentrations were tested during ART
131 treatment some time after a potential COVID-19 infection and in women without or
132 with few symptoms.

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

138 The strength of our study is that we tested AMH concentrations in the same women
139 at different time points and could thus analyze any potential modification of the
140 ovarian reserve after COVID-19 infection.

141 However, the study has some limitations. Firstly, it has been shown that the AMH
142 concentration is modified during ART treatment (Peñarrubia et al., 2005) as this
143 hormone is secreted by granulosa cells of small growing follicles (Moolhuijsen and
144 Visser, 2020) thus reflecting rather the granulosa cell activity. Nevertheless, the
145 baseline and the second AMH concentrations, as well as ART protocol types and
146 oestrogen concentrations on the day of AMH testing were comparable between both
147 study groups. Secondly, a relatively small number of women were included in the
148 analysis and only 14 were COVID RDT+. In addition, the group with COVID-19 RDT+
149 and the control group were heterogenous due to the methodology of inclusion
150 consisting in a consecutive patients' inclusion. ART treatments in our unit were
151 postponed for 3 months during the COVID epidemic following interruption of activity
152 due to a decision by the French government. Thus, the time between the baseline
153 AMH test and the beginning of the ART treatments were extended. However, we
154 decided to include only women with AMH tested within the 12 preceding months
155 because AMH concentration is age dependent and can decrease over time
156 (Plociennik et al., 2018). The time between the two AMH tests was comparable
157 between the study groups.

158

159 **CONCLUSION**

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

163

164

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169

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173

174 **DECLARATIONS OF INTEREST**

175 None

176

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