

# Serum SARS-COVID-19 IgG Level Effect on Oocyte and Embryo Quality in Women Underwent ICSI

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## Abstract

**Background:** SARS-COVID-19 pandemic become a major health problem and impinge a large burden on health systems. SARS-CoV-2 utilize ACE2 receptors as a main port for its entry to the cells. These are widely spread in the body including ovaries. They are important for ovarian development. Therefore SARS-CoV-2 infection may have long term consequences of female's fertility and on parameters that determine ART outcomes. This study designed to display if there is a long term effect of this disease on ova and embryo qualities during ART procedures.

**Material and methods:** This is a cohort study enrolling 54 infertile women attending our institute for ICSI procedure. Parameters determine oocytes and embryo quality are recoded. Serum SARS-CoV-2 IgG measured and patient classified in those with negative (<1) and positive (≥1) groups.

**Results:** There are no significant differences between cases with negative and positive SARS-CoV-2 IgG in parameters for oocytes and embryo quality: p value for oocytes no. 0.84, abnormal oocytes 0.25, germinal vesicles 0.46, MI 0.52, MII 0.75, number of embryo transfer 0.86, G1 embryo 0.82, maturation rate 0.79, and fertilization rate 0.61.

**Conclusion:** There are no long term effect of SARS-CoV-2 infection on embryo and ova quality in patient have ART procedures.

**Keywords:** Embryo and Oocytes Quality, ICSI, SARS-CoV-2.

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## INTRODUCTION

Infertility in a major issue that worried newly married couples <sup>[1]</sup>. The first case of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had been reported in Wuhan, china in December 2019 <sup>[2]</sup>. The WHO announced SARS-COVID-19 as global pandemic on March 2020 and this them rapidly spread to more than 220 countries and territories <sup>[3]</sup>. It was believed that angiotensin-converting enzyme 2 (ACE2) receptors found on cell surfaces are the main receptors for SARS-CoV-2 entry into the cells <sup>[4]</sup>. Hence, any tissue

It is prerequisite for development of ovarian follicles, oocytes maturation, ovulation, sex hormone production, follicular atresia and angiogenesis of ovaries and corpus luteum. Angiotensin-(1-7) found in the theca cells is important for steroidogenesis, follicular development, resumption of oocytes meiosis and enhancement of ovulation <sup>[11, 12]</sup>.

Angiotensin-(1-7), Mas-receptors and ACE2 found in altages of follicular development and there is evidence that it has potential role in infertility. The proportion of mature oocytes

harboring such receptors are potential targets for this virus. The lung express such receptor in highest concentration. However, other organs express such receptors as kidney <sup>[5]</sup>, intestine <sup>[6]</sup> and cardiovascular system <sup>[7, 8]</sup>, and male reproductive system <sup>[9]</sup> and female reproductive organs <sup>[10, 11]</sup>. Although ACE2 are widely spread through female reproductive organs, the ovaries harvest the highest concentration of such receptors. Meanwhile, ovaries harbor all component of renin-angiotensin system (RAS) and this make them the principal target for damaging effect of SARS-CoV-2. Angiotensin-II found mainly in granulosa cell.

is correlated well with follicular fluid Ang-(1-7) level obtained during ovarian stimulation as a part of in vitro fertilization. On other hand there is evidence to suggest that Ang-(1-7) is a maturation factor of human oocytes <sup>[13]</sup>. Down regulation of ACE2 due to SARS-CoV-2 affect normal ovarian physiology including follicular development and oocytes maturation. Ang-II have pro-inflammatory effect and thereby increased oxidative stress and this may impinge a detrimental damaging effect of human fertility <sup>[12]</sup>.

## Study hypothesis

SARS-COVID-19 is a major health pandemic. The disease display multi-organs involvement with a potential damaging effect including female reproductive organs this damaging effect is theoretically related to interplay between viral replication and host immune response including IgG production. The effect of SARS-COVID-19 on oocytes and embryo quality during ICSI need to be clarified.

This study is designed to declare the effect of virus and its immune response represented by SARS-CoV-2 serum IgG on oocytes and embryo quality during ICSI procedure.

## MATERIAL AND RESEARCH METHODS

This is a prospective cohort study done in the high institute for infertility diagnosis and assisted reproductive techniques in one year period. Fifty-four patients recovered for COVID-19 (IgG+ and IgM -) infections enrolled in this study. All patients informed about participation in the study and written informed consent obtained from them. All procedures performed in studies involving participants were in accordance with the ethical standards of the institutional research committee and with the Helsinki Declaration (as revised in 2013).

Full history taken as fertility history (monitory of ovulation, period of unprotected intercourse, coitus frequency, previous attempts for conception, history of anteceded evaluation and treatment for infertility), gynecologic history (onset of menarche, characteristics of the cycle as length and duration, presence of dysmenorrhea and dyspareunia, chronic pelvic pain, history of thyroid disease, hirsutism and others as indicated), medical and surgical history as history related to autoimmune diseases and endocrine history, family and social history.

Physical examination done for all patients including neck examinations for goiter, breast examinations, speculum examination for cervical and vaginal lesions. All patients' weights and lengths are measured. Investigation include hormonal assays as measurement of FSH, LH, prolactin, estradiol-2, PRI, TSH and AMH. Serum SARS-CoV-2 IgG measured by minividas as directed by manufacturer <sup>[14]</sup>.

Controlled ovarian stimulation done using GnRH antagonist protocol. Recombinant human chorionic gonadotropin (HCG) injected intramuscularly when there are two to three follicles having a diameter more than 18 mm. oocytes retrieved 34-36 hours after trigger by HCG. The presence of two pronuclei (2PN) considered normal fertilization. Embryos cultured to cleavage stage G1-plus medium until day 3. One or two embryos that have highest quality are freshly transferred (9).

## Statistical analysis

The Statistical Package for Social Sciences software (version 26.0; SPSS, IBM, USA) wa used for analyzing data. Independent sample t-test and analysis of variance (ANOVA) tests used to evaluate numerical data. On other hand, chi-squared test and Fischer exact test used to analyze categorical data as appropriate.

## RESULTS

### Demographic data

Mean age of enrolled cases is  $32.460 \pm 5.740$  and ranged from 18 to 40 years. Mean BMI is  $28.40 \pm 5.54$  and ranged 19.920 to 51.730.

Studies cases divided into those who are negative for Serum SARS-CoV-2 IgG level [ $<1$  negative (no detection of IgG anti SARS-CoV-2)] and those who are positive [ $\geq 1$  positive (detection of IgG anti SARS-CoV-2)].

No significant differences observed between groups for basal characteristics (Table 1)

For those patient negative for SARS-COVID-19 IgG, 16 (29.600%) aged  $<35$  years and 14 (25.900%) aged  $\geq 35$  years. Meanwhile patient positive for SARS-COVID-19 IgG16 (29.600%) are  $<35$  while 8 (14.800%) patients aged  $\geq 35$  years. No significant differences between these groups (( $p=0.32$ ) (Table 4.3).

Six (11.100%) patient are normal weight, 11(20.400%) overweight, and 13 (24.100%) obese and are negative for serum SARS-CoV-2 IgG. On other hand, BMI distribution in those patients positive for serum SARS-CoV-2 IgG level as follow: 9 (16.700%) normal weight, 9 (16.700%) overweight, and 6 (11.100%) are obese. no significant differences observed between two groups in relation to BMI ( $p=0.25$ ) Table 1.

Table 1: Demographic data, hormonal levels, and maturation and fertilization rates in the studied groups

parameters	serum SARS-COVID-19 IgG		P-value
	negative(<1) (N=30)	positive (≥1) (N=24)	
E2	34.270±13.570	35.780±22.390	0.76
AMH	1.230±0.690	1.730±1.450	0.09
FSH	6.180±3.660	6.030±3.530	0.82
LH	4.460±2.460	4.580±2.540	0.86
PRL	19.480±8.260	21.510±14.450	0.52
E2 at trigger	970.180±660.030	1115.340±1089.640	0.54
age group (yrs.) (N)			
<35 (%)	16 (29.60%)	16(29.60%)	0.32
≥35 (%)	14 (25.90%)	8(14.80%)	
BMI (kg/m2) (N)			
normal	6(11.10%)	9(16.70%)	0.25
overweight	11(20.40%)	9(16.70%)	
obese	13(24.10%)	6(11.10%)	
Occupations (%)			
housewife	23(42.60%)	23(42.60%)	0.06
employee	7(13.00%)	1(1.90%)	
Infertility type (%)			
primary	22(40.70%)	19(35.20%)	0.61
secondary	8(14.80%)	5(9.30%)	
Female infertility causes (%)			
normal	10(18.50%)	10(18.50%)	0.35
ovulatory	12(22.20%)	6(11.10%)	
tubal	0(0.00%)	2(3.70%)	
unexplained	8(14.80%)	6(11.10%)	

\*P-value <0.05 indicate significant differences. BMI: Body mass index.

### Embryo and oocytes quality

In patient negative for serum SARS-COVID-19 IgG level the following parameters assess oocytes quality as follow:

Studied cases negative for serum SARS-CoV-2 IgG: Mean oocyte number 10.670±4.830, mean number of abnormal oocytes is 3.580±5.470, mean germinal vesicles is 2.190±1.380, mean MI is 1.860±0.950, and mean MII is 6.520±3.810, maturation rate maturation rate is poor in 21 (38.900%) and normal in 9 (16.700%) cases (Table 1 & 2).

For patients with positive serum SARS-CoV-2 IgG: Mean oocyte number 10.960±5.890, mean number of abnormal oocytes is 1.640±0.920, mean germinal vesicles is 2.550±0.930, mean MI is 2.130±1.250, mean MII is 6.880±4.540, maturation rate is poor in 16 (29.600%) and normal in 8 (14.800%) cases. There are no significant differences between groups when the above parameters as considered as shown in Table 1 & 2.

On other hand assessment of embryo quality as follow: patients with negative SARS-CoV-2 IgG display the following: mean grade I embryo 2.450±1.390, and mean number of embryo transfer 2.630±1.000. On other hand, cases with positive serum SARS-CoV-2 IgG: mean grade I embryo 2.540±1.650, and mean number of embryo transfer 2.580±1.210. There are no significant differences between cases with negative and positive SARS-CoV-2 IgG when preceded parameters considered: for grade I embryo (P=0.82), and for number of embryo transfer (P=0.86) (Table

1 & 2).

In contrary, fertilization rate in those cases with negative SARS-COVID-19 IgG as follow: low fertilization rate in 8 (14.800%) and high one in 22 (40.700%). On other hand, in cases with positive serum SARS-COVID-19 IgG: low fertilization rate in 5 (9.300%) and high one in 19 (35.200%). These indicate that SARS-CoV-2 IgG level not affect fertilization (p=0.61) (Table 1 and 2).

Table 2: Oocytes and embryo quality in the studied groups

parameters	serum SARS-COVID-19 IG		p-value
	negative(<1)(N=30)	positive (≥1) (N=24)	
	mean ±SD	mean ±SD	
No. of oocytes	10.670±4.830	10.960±5.890	0.84
AB oocytes	3.580±5.470	1.640±0.920	0.25
G.V.	2.190±1.380	2.550±0.930	0.46
M I	1.860±0.950	2.130±1.250	0.52
MII	6.520±3.810	6.880±4.540	0.75
G I embryo	2.450±1.390	2.540±1.650	0.82
NO. ET	2.630±1.000	2.580±1.210	0.86

\*P-value <0.05 represent denote significant differences. N: total number, AB: Abnormal. G.V.: germinal vesicles, M I: metaphase I, MII: metaphase II, GI: grade I, ET: embryo transfer.

## DISCUSSION

SARS-COVID -19 pandemic is a major global problem impinging on all life aspects and disturbing health systems. Assisted reproductive techniques not an exception. Data regarding long term effect of the virus on human infertility are still limited if not lacking. SARS-COVID -19 pandemic demand urgent actions. On 27<sup>th</sup> of February, ESHRE launched a statement in which it advise to avoid any trial for ART pregnancies in those who met the diagnosis of SARS-COVID -19 infection. Later on they expand the statement to include patient not met the diagnostic criteria for the disease. Further expansion of the statement not to start ART procedure in order to avoid pregnancy to avoid possible complication due to SARS-COVID -19 infection and to redistribute the resources to health care systems. The exclusive exception is cryopreservation of gametes, embryos and germinal tissues for those patients otherwise could become infertile and this is considered as an emergency procedure [15]. There is limited data on effects of SARS-COVID-19 on human fertility [16].

This study designed to declare the long term effect on SARS-CoV-2 infection on oocytes and embryo quality in patients undergoing ICSI procedure.

The following parameters considered to asses oocytes quality: “Mean oocyte numbers, mean number of abnormal oocytes, mean germinal vesicles, mean MI oocytes, mean MII oocytes. There are no significant differences in patients with serum SARS-CoV-2  $<1$  &  $\geq 1$  (p value are 0.8420, 0.2580, 0.4610, 0.5100 and 0.7560 respectively).

Meanwhile, embryo qualities assessed by the following:” mean grade I embryos, mean numbers of embryo transferred, and fertilization rates. There are no significant differences between those with negative and positive serum SARS-CoV-2 IgG groups (p value is 0.827, 0.869, & 0.618 respectively).

In a study done on 3 groups of infertile women attend IVF centers for egg retrieval: nine patients recovered from COVID-19, nine patients are vaccinated and fourteen are non-vaccinated. The focus on following parameters” number of retrieved oocytes, oocytes yield (proportion of retrieved oocytes obtained from mature follicles as detected at the ultrasound on the trigger day), oocytes quality markers, and mature oocytes number”. They stated that there is no significant differences regarding these parameters in the studied groups [17].

Another study by (Wang et al., 2021) [18] performed in IVF center in Wuhan. They studied women had IVF procedure and are negative for SARS-CoV-2 RNA with positive serum SARS-CoV-2 antibodies. They compared them with patients unaffected by SARS-CoV-2. They compared mature oocytes rates, fertilization rates and numbers of retrieved oocytes. They stated that there are no significant differences between groups for these parameters.

In contrary, other results found that women with raised SARS-CoV-2 IgG levels had decreased number of mature and retrieved oocytes [19]. Another study done enrolled nine

couples. They evaluate IVF outcomes before and after SARS-CoV-2 infection. They found no significant differences in number of picked up oocytes and fertilization rate but number of top quality embryo (embryo having more than 7 blastomeres of day 3, fragmentation  $\leq 10\%$ , and blastomeres of equal size) is significantly less after COVID-19 infection [20]. Our results is in consistence with results of others denoting absence of detrimental effect of SARS-CoV-2 infection on embryo and oocytes qualities and hence we advised doctor safely perform IVF procedure in women having anteceded SARS-CoV-2.

It is stated that SARS-CoV-2 infection raise oxidative stress [21]. Oxidative stress might impinge oocytes and embryo quality and thereby altering female fertility [22]. Our study stated that parameters responsible for embryo and oocytes quality not altered in patients recovered from SARS-COVID-2 infection and this is the same by other study [18] but blastocyst formation is altered in spite of similarity of oocytes number, fertilization rate, implantation, miscarriage and clinical pregnancy rate.

Limitation of our study is we did not include pregnancy outcome whether biochemical or clinical.

The strength of our study is that it is novel study that designed to investigate the potential effect of SARS-COVID-19 infection on ART protocols in spite of the limited data regarding such subject in order to participate with others to design a guidelines regarding ART protocol in the era of SARS-COVID-19 pandemic. We study the potential effect SARS-CoV-2 on ICSI parameters as oocytes and embryo quality and this is another strength point.

## CONCLUSION

We conclude that SARS-COVID-19 infection after recovery does not affect embryo and oocytes quality in patients undergoing ICSI.

**Conflict of Interest:** “The authors declare that they have no potential conflicts of interest to disclose.”

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## AUTHOR’S CONTRIBUTION

- Conceptualization: HSH, HALM
- Data curation: HALM
- Formal analysis: HSH, AAM
- Methodology: HSH, AAM
- Project administration: HAS, MMS
- Resources: HSH, HALM
- Software: HSH, HALM
- Supervision: HSH, HALM
- Validation: AAM, HALM
- Visualization: AAM, HSH.
- Writing—original draft: AAM, HALM, HSH
- writing— review & editing: HSH, HALM, AAM

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