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P-460 SARS-CoV-2 infection of human ovarian cells: an *in vitro* model for the detection of the virus entry into the host cells

P. Piomboni[1], F.P. Luongo[1], F. Dragoni[2], M. Gentile[3], A. Boccuto[2], L. Boschi[1], G. Morgante[1], I. Vicenti[2], M. Zazzi[2], A. Luddi[1]

[1]*University of Siena, Molecular and Developmental Medicine, Siena, Italy*
[2]*University of Siena, Department of Medical Biotechnologies, Siena, Italy*
[3]*University of Siena, Department of Life Sciences, Siena, Italy*

Study question: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can enter and actively infect human follicular and granulosa cells?

Summary answer: Follicular Granulosa (GCs) and Cumulus cells (CCs) are susceptible to SARS-CoV-2 infection that is able to reproduce.

What is known already: To enter host cells, SARS-CoV-2 uses Spike S1 subunit to bind the receptor angiotensin-converting enzyme2 (ACE2), S2 subunit is cleaved by the host transmembrane serine protease 2 (TMPRSS2) or by cathepsin L (CSTL) to produce unlocked, fusion-catalyzing viral forms. CD147 (BSG) has been proposed as an additional host receptor for SARS-CoV-2. Female fertility is strictly dependent on oocyte quality and competence. ACE2 is highly expressed in the human ovaries and in the stromal endometrial cells, as well as in GCs and oocytes. The expression of ACE2 strongly suggests that it is potentially at a high risk of SARS-CoV-2 infection.

Study design, size, duration: In order to analyze the presence of host receptors ACE2 and co-receptors TMPRSS2-CSTL and BSG and consequently the susceptibility of GCs and CCs to SARS-CoV2 infection. GCs and CCs were collected from about 25 patients undergoing IVF/ICSI cycles at the UOSA of Assisted Reproductive techniques, from March 2020 to October 2021 at the Center of Couple Sterility, Siena University Hospital.

Participants/materials, setting, methods: GCs and CCs were collected from women undergoing IVF cycle. GCs were recovered from the follicular fluid according to the procedure previously published. After oocyte denuding, CCs were isolated and both GCs and CCs co-cultured with SARS-CoV-2 then the supernatant was used to infect VERO6 Cells. Host factors and SARS-CoV2 expression/localization were confirmed by RT-PCR, Western blot and Immunofluorescence. SARS-CoV2 infection and its effect on GCs and CCs were evaluated by Transmission and Immuno-electron microscopy.

Main results and the role of chance: qRT-PCR analysis and WB showed that ACE2, TMPRSS2, BSG and CTSL transcripts were expressed in both GCs and CCs cells, even if at different levels. ACE2 transcript was significantly increased in the CCs (0.43 vs 0.15; $p < 0.05$) with respect to GCs; this datum is very interesting since CCs are the follicular cells able to establish intimate connections with the developing oocyte (e.g. gap junctions). Therefore, contrary to what can be assumed, human CCs cannot act as a barrier against virus entry into the oocyte. Coreceptor BSG and CTSL were the most expressed in GCs with respect to TMPRSS2, (0.7 vs 0.3 and 0.8 vs 0.4 respectively; $p < 0.05$). Ultrastructural evaluation of human GCs and CCs infected with SARS-CoV-2 was carried out at different time points post-infection (24h, 48h and 72h). Infected human GCs showed cell-associated virus-like particles, virions were approximately spherical, with a diameter outside the lipid bilayer ranging from 50-150 nm and ultrastructural characteristics consistent with those described for other coronaviruses. Complete virions were also observed inside the cytoplasm as single or small groups of particles, either dispersed or within large vesicles. Immunoelectron microscopy confirmed these particles as SARS-CoV2.

Limitations, reasons for caution: This is a human *in vitro* study, and we cannot predict all the implications in female fertility and related to the oocyte

Wider implications of the findings: We provide evidence in favor of SARS-CoV-2 infection in GCs and CCs, the ovarian somatic cells that support oocyte development and competence acquisition. The close relationship between oocytes and follicular cells raises the hypothesis that these cells may represent a vehicle for the oocyte SARS-CoV-2 infection

Trial registration number: Not applicable