## P-274 Undetectable viral RNA in follicular fluid (FF), cumulus cells (CC) and endometrial tissue in SARS-CoV-2 positive patients

**L. Boudry[1], W. Essahib[2], I. Mateizel[1], H. Va. d. Velde[1], D. D. Geyter[3], D. Piérard[3], V. Uvin[1], H. Tournaye[1], M. D. Brucker[1]**

*[1]Universitair Ziekenhuis Brussel, Centre for Reproductive Medicine, Brussels, Belgium ;*
*[2]Vrije Universiteit Brussel, Reproductive Immunology and Implantation REIM, Brussels, Belgium ;*
*[3]Universitair Ziekenhuis Brussel, Department of Microbiology, Brussels, Belgium*

**Study question:** Is there any indication for presence of viral RNA in FF, CC, immature oocytes or endometrial biopsy (EB) of SARS-CoV-2 patients undergoing ovarian stimulation?

**Summary answer:** Viral RNA is undetectable in FF, CC and EB with RT-PCR. However, S-protein expression on corona radiata cells suggests susceptibility to SARS-CoV-2 infection.

**What is known already:** The effects of a SARS-CoV-2 infection on the female reproductive system are still poorly understood. Theoretically, co-localisation of the angiotensin converting enzyme (ACE2) and transmembrane serine protease 2 (TMPRSS2) on human blastocysts implies susceptibility to viral infection, mediated by the coronavirus spike (S) protein. To date, SARS-CoV-2 RNA was undetectable in mature oocytes from COVID-19 patients, despite the expression of *ACE2* and *TMPRSS2*. The presence of viral RNA in endometrial tissue, immature oocytes, CC or FF has not yet been investigated in samples from patients with positive nasopharyngeal SARS-CoV-2 test.

**Study design, size, duration:** This is a prospective, single-centre, observational study including ten patients with a positive nasopharyngeal swab for SARS-CoV-2, performed 48 hours before oocyte retrieval (OR), from September 2020 to January 2021. A patient was eligible if she preferred to continue treatment following adequate counselling of the unknown but presumably low risk for vertical transmission. Since a freeze-all strategy was applied, an EB was performed.

**Participants/materials, setting, methods:** During OR, all protective measures were taken. Pooled FF, CC and EB from each patient were tested for viral RNA presence with RealStar® SARS-CoV-2 RT-PCR-Kit1.0 (Altona-Diagnostics). Ct values <40 were considered positive. EB was collected for pathological evaluation and cultured to obtain endometrial stromal cells (EnSC). Immature oocytes and EnSC were tested for S-protein expression by immunohistochemistry with anti-S antibody (MA5-35958, Thermo-Fisher Scientific) followed by Alexa Fluor™ 488-donkey-anti-mouse (Thermo-Fisher Scientific) and visualized with confocal microscopy.

**Main results and the role of chance:** SARS-CoV-2 RNA was undetectable in the pooled FF, CC and EB from all patients included in the study. Histological analysis of the EB showed no pathological modifications, including inflammatory reaction, as compared to biopsies collected from swab negative patients. After staining with anti-S antibody, cultured EnSC and immature oocytes tested negative for the S-protein. However, the binding of anti-S antibody was demonstrated on the corona radiata cells remaining on the zona pellucida after oocyte denudation for intra-cytoplasmatic sperm injection, indicating presence of SARS-CoV-2. In that case, the explanation for the undetectable viral RNA in CC could be that the viral RNA concentration remained under the detection limit of the currently used RT-PCR test.

**Limitations, reasons for caution:** This study was conducted in a small population (ten patients included) with different viral load, with mild or without symptoms of COVID-19. Another important limitation is the absence of validation of the RT-PCR protocol for the investigation of other types of samples than nasopharyngeal swabs.

**Wider implications of the findings:** The absence of SARS-CoV-2 RNA in all samples analysed represents a step further in reassuring a safe ART program for COVID-19 patients. However, the presence of S-protein on corona radiata cells warrants further investigation, since the theoretical possibility to infect human oocytes and/or embryos cannot be ruled out.

**Trial registration number:** NCT04425317