P-021 An accurate and reliable screening for SARS-CoV-2 in human sperm samples by RT-PCR: A requirement to evaluate the viral contamination risk during SARS-CoV-2 pandemic

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Study question: How to ensure a reliable and accurate detection of SARS-CoV-2 in seminal plasma and spermatozoa fractions of human sperm samples?
Summary answer: This RT-PCR assay showed high sensibility, repeatability and reproducibility for SARS-CoV-2 detection in seminal plasma and spermatozoa fractions, with a detection limit of 17 genomes/reaction.
What is known already: SARS-CoV-2 pandemic brings numerous concerns, such as the safety of gametes for patients undergoing assisted reproductive technologies, fertility preservation or sperm donation. Transient viremia and expression of SARS-CoV-2 receptors in testis and accessory glands bring the question of the presence of the virus in sperm samples. Moreover, the contamination during sperm collection may be possible. The few available studies about this issue mostly showed the absence of SARS-CoV-2 detection in semen of COVID-19 patients, except one reported study. All these studies performed SARS-CoV-2 detection with RT-PCR assays approved for naso-pharyngeal swabs, without a process specifically validated for semen fractions.
Study design, size, duration: Method validation was conducted between July 2020 and January 2021. SARS-CoV-2 direct detection was performed according to the French Society of Microbiology guidelines (SFM). Repeatability (n=6), reproducibility (n=3), limit of quantification (n=2) and of detection (n=6) were evaluated in seminal plasma (SP) and spermatozoa samples isolated after density gradient centrifugation and cryopreserved. In addition, variability of the whole analytical method efficiency was evaluated in samples of men with normal (n=6) or altered sperm parameters (n=6).
Participants/materials, setting, methods: Samples were surplus semen obtained from men undergoing routine semen analysis after granting informed consent. Assays were performed on SP and frozen spermatozoa fractions. After automated RNA extraction (MGISP-960, MGI-Tech®), real-time RT-PCR was performed using the one-step multiplex TaqPath COVID-19 kit (ThermoFisher®) targeting three viral regions (ORF1, nucleocapsid-N and spike-S proteins). An exogenous internal control was added before RNA extraction. Positive samples and dilution ranges were prepared with a standard (SARS-CoV-2 inactivated virus, QnosticTM Randox®).
Main results and the role of chance: RT-PCR assay applied for human sperm samples has been previously validated and is routinely used for SARS-CoV-2 detection in naso-pharyngeal swabs. We evaluated the efficiency of RNA extraction and RT-PCR for SARS-CoV-2 detection in semen fractions. The qualitative and quantitative performance of the whole analytical method was validated with an accuracy profile for SP and spermatozoa fractions. Overall, for repeatability, the standard deviation (SD) of the cycle threshold (Ct) was lower than 0.40 for the strong positive sample and 0.50 for the low positive one. An exception was observed for the S target of the low positive SP samples (SD=3) which was consistent with S being the less sensitive target of the assay. For reproducibility, SD of the Ct was lower than 0.30 for the strong positive sample and 0.80 for the low positive, except for the S target of the low positive (SD=1.5). The linearity range was determined for N target, the most sensitive target of the RT-PCR assay. It layed between 5200 and 52 SARS-CoV-2 genomes/reaction. The limit of detection of the RT-PCR assay was 17 viral genomes/reaction. Equal efficiency of the assay was observed for SP and spermatozoa independently of semen parameters (normal and altered sperm parameters). Limitations, reasons for caution: Our detection method was validated for the whole process: RNA extraction (reagents and system), RT-PCR (reagents and thermocycler QuantStudio 5™) and for both SP and frozen spermatozoa fractions. Variability might be observed with a different extraction system or a different type of biological sample.
Wider implications of the findings: This validated RT-PCR assay enables accurate and reliable screening of SARS-CoV-2 in SP and spermatozoa fractions, mandatory to investigate the presence of the virus in semen samples of patients undergoing assisted reproductive techniques, fertility preservation or sperm donation, and to ensure viral safety in the cryobanking process during covid-19 pandemic.
Trial registration number: EudraCT 2020-A01409-30