

**Summary answer:** Human spermatozoa express uniformly ACE2 on the sperm head and the flagellum. Moreover, the short-ACE2 isoform is concentrated on the post-acrosomal region and midpiece.

**What is known already:** The Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2) infection is generating important concerns regarding not only the possible consequences on the respiratory system, but also on other organs, including the reproductive system. ACE2 is considered the main point of entry for the SARS-CoV-2 within the cells through the binding with the spike protein on the virus surface. Furthermore, ACE2 is expressed in human testes cells including Leydig cells, Sertoli cells and spermatogonia. However, to date, the expression and location of ACE2 in mature human spermatozoa has not been investigated yet.

**Study design, size, duration:** This was an *in vitro* study for the evaluation of the expression and immune-localization of full-length ACE2 and its isoform, short-ACE2, in human spermatozoa. Thirtyfour non-immunized healthy normozoospermic volunteers were enrolled in the study. The study was conducted from May to December 2021.

**Participants/materials, setting, methods:** Semen samples were collected by masturbation from non-immunized healthy normozoospermic volunteers. Motile sperm suspensions were obtained by swim-up procedure. The expression of ACE2 was assessed by Western-blot analysis, while the immune-localization of ACE2 was evaluated by immune-cytochemical analysis under confocal microscopy. Flow-cytometry experiments were also performed to assess the surface protein expression on a large number of cells.

**Main results and the role of chance:** The Western-blot analysis of sperm extracts demonstrated two specific bands, one of approximately 120 KDa, corresponding to the glycosylated full-length ACE2, and a second one of approximately 52 KDa, the molecular weight of the protein recently termed short-ACE2. The immune-cytochemical analysis showed a uniform localization of full-length ACE2 along both the sperm head and the flagellum, whereas the short isoform was preferentially located in the post-acrosomal region of the sperm head and the midpiece. At the flow cytometer, semen samples displayed a wide between-subject variability both in the percentage of ACE2-positive spermatozoa and the density of protein surface expression.

**Limitations, reasons for caution:** Further studies are needed to determine whether short-ACE2 is a cleavage product from the full-length protein or if it is originated during spermatogenesis. Moreover, the role and the interaction of ACE2 with SARS-CoV-2 in human spermatozoa should be clarified to evaluate the possible impact of the virus on sperm biology.

**Wider implications of the findings:** Since mature spermatozoa are transcriptionally silent and SARS-CoV-2 is an RNA virus, it is unlikely that the virus could affect sperm biology by replicating itself. Nevertheless, the potential effects related to modifications of the sperm membrane or interaction with other receptors or specific proteins cannot be ruled out.

**Trial registration number:** not applicable

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**P-030 ACE2 receptor and its isoform short-ACE2 are expressed on human spermatozoa**

**M. Ramal-Sanchez<sup>1</sup>, C. Castellini<sup>2</sup>, C. Cimini<sup>1</sup>, A. Taraschi<sup>1</sup>, L. Valbonetti<sup>1</sup>, A. Barbonetti<sup>2</sup>, N. Bernabò<sup>1</sup>, B. Barboni<sup>1</sup>**

<sup>1</sup>University of Teramo, Faculty of Biosciences and Technology for Food- Agriculture and Environment, Teramo, Italy

<sup>2</sup>University of L'Aquila, Department of Clinical Medicine- Life- Health and Environmental Sciences- University of L'Aquila- L'Aquila- Italy, L'Aquila, Italy

**Study question:** Do human spermatozoa express angiotensin-converting enzyme 2 (ACE2) receptor? What would be its localization?