

P-I 13 α -tubulin and tyrosine phosphorylation immunolocalization on human sperm from a globozoospermic patient with proper embryo development after ICSI

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Study question: Can novel sperm biomarkers, such as α -tubulin and tyrosine phosphorylation, help to predict the fertilizing potential of a globozoospermic sample?

Summary answer: The characterization of α -tubulin and tyrosine phosphorylation provide additional information that could be useful to determine the fertilizing capacity of the globozoospermic samples.

What is known already: Globozoospermia is a severe disorder characterized by the acrosome absence and other sperm alterations such as tail structural disorders and chromatin condensation abnormalities. This set of defects makes globozoospermia correlates with primary infertility and low fertilisation rates. Therefore, additional studies are necessary to know how the globozoospermia affects the disposition of α -tubulin and tyrosine phosphorylation and the relationship between embryo development and pregnancy. In this context, previous studies have independently described different patterns of α -tubulin and tyrosine phosphorylation in normozoospermic samples. Specifically, the continuous α -tubulin distribution along the flagellum and tyrosine phosphorylation have been recently linked to proper sperm functionality.

Study design, size, duration: We conducted a prospective study. The sample was obtained from a globozoospermic patient in October 2019. This sample was divided into two fractions, one proceeded to ICSI and the another was fixed to characterize α -tubulin and tyrosine phosphorylation using confocal microscopy. A total of 200 sperm were analyzed for each biomarker.

Participants/materials, setting, methods: A 38-year-old man requested assisted reproduction in IVF Spain after a failed treatment with no fertilized oocytes. The clinical procedure was performed at IVF Spain and the characterization studies were made at the University of Alicante. The flagellar cytoskeleton was assessed using anti- α -tubulin antibody (Sigma-Aldrich) at a 1:600 dilution. Besides, tyrosine phosphorylation was detected using anti-phosphotyrosine primary antibody (PY20, Sigma-Aldrich) at a 1:500 dilution. Spermatozoa were evaluated using a confocal microscope (Zeiss LSM 800).

Main results and the role of chance: Regarding the α -tubulin characterization, only the 33% of spermatozoa showed continuous labelling in the tail and in the 67% the fluorescence appeared in the terminal piece of the flagellum. Otherwise, we only observed 0.5% of positive tyrosine phosphorylation in the studied cells, whereas the 99.5% of sperm analyzed did not show positive fluorescence. The clinical parameters showed a fertilization rate of 20% with only one embryo after the MII oocyte artificial activation by calcium ionophore. The embryo development was further adequate, and it acquired BtSAA status on day five. Unfortunately, the pregnancy did not ensue after the embryo transfer.

Limitations, reasons for caution: The main limitation of this study was that due to the very low frequency of globozoospermia (<0.01%) this research was conducted only in one patient. Thus, the present results are preliminary. We need to characterize the aforementioned biomarkers in additional globozoospermic samples to establish relationships with clinical parameters.

Wider implications of the findings: The study of potential candidate biomarkers like α -tubulin and tyrosine phosphorylation in globozoospermia and the linkage with clinical parameters would facilitate the diagnosis and improve the selection of more effective treatment techniques.

Trial registration number: not applicable