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★ Abstract title:

Differential sperm proteomic profile between sperm samples achieving pregnancy or not in intracytoplasmic sperm injection (ICSI) cycles in oocyte donation program

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Study question:

Are there any differences between sperm samples that failed vs. those achieving pregnancy regarding the sperm proteomic profile after ICSI cycles?

Summary answer:

This study reveals a differential protein profile between sperm samples that failed vs. those achieving pregnancy, able to be used as sperm fertility biomarkers.

What is known already:

There is a lack of sperm fertility markers, and several studies demonstrated that idiopathic male infertility is multifactorial. Although it is believed that sperm are cells translationally and transcriptionally silent, their functionality can be studied using proteomic approaches. Many proteins have been already identified having an important role in sperm motility, sperm-oocyte's zona pellucida interaction, metabolism, apoptosis, cellular cycle, meiosis, membrane transport, and ribonucleotide acid regulation.

Furthermore, some previous works from our group described differential molecular factors in sperm samples from which a pregnancy was obtained compared with those failing, defining molecular characteristics for physiologically competent sperm.

Study design, size, duration:

Descriptive, prospective and non-randomized biomedicine study evaluating the proteomic profile of spermatozoa from patients' ejaculates where pregnancies were (Group pregnant (P), n= 4) or were not (Group non-pregnant (NP), n=4) achieved after ICSI in an oocyte donation program aiming to standardize female factor. Aliquots from the same samples employed in ICSI procedures were collected, frozen until the reproductive results were known, and then assigned to their corresponding group and analysed.

Participants/materials, setting, methods:

Eight sperm samples from infertile males undergoing ICSI cycles in oocyte donation program with normal sperm parameters and total progressive motility >5mill were included in our study, 4 of them failed to achieve pregnancy and 4 who succeeded. Proteins were separated and analysed by means of SWATH-MS (Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra). Proteins are available via ProteomeXchange: identifier PXD006309. All proteins abundances were compared statistically between P and NP groups.

Main results and the role of chance:

We identified 2228 proteins, 53 of them were found in different abundance between sperm samples, being 37 significantly more abundant in sperm samples in P group, and 16 less abundant in this same group compared with NP. We applied Elastic-Net statistic method to identify the 27 proteins best performing to differentiate both groups. Also, we use PLS-DA analysis (Partial Least Squares Discriminant Analysis) verifying that the samples are classified accordingly to their group. Finally, we applied a *VIP-score* test over Elastic-Net and PLS-DA, in order to determine which proteins have more

importance in achieving pregnancy. We obtained 27 proteins with high *VIP-score* in Elastic-Net, and 300 in PLS-DA.

Then we applied functional analysis over the proteins obtained in the analysis PLS-DA+*vip-score*, obtaining 5 proteins more abundant in P group with reproduction functions (NME5, SPATA20, DNAI1, ARMC4 and AK7) and 10 less abundant in NP group (GNB2L1, PDIA4, YWHAZ, SYNGR1, FNTB, LCP1, NDUFS4, COG3, STOM and GIT1) with metabolic functions.

Finally, we identified 11 proteins present in PLS-DA+*VIP-score* analysis, located in membrane, susceptible to be selected by MACS (Magnetic-activated-cell-sorting) to develop a new system to select the best spermatozoon.

Limitations, reasons for caution:

Other molecular factors not included within this analysis could be involved in sperm fertility given that sperm function has been demonstrated multifactorial.

The low number of samples studied make us be cautious about our findings, but lead us to design a focused research on the main findings with bigger sample-sizes.

Wider implications of the findings:

The description of proteins linked to sperm fertility, open new possibilities regarding the development of male fertility diagnostic tools, culture media formulations, or, to design new sperm selection tools based on these molecular traits, using MACS of spermatozoa exhibiting specific proteins associated with the best reproductive results.

Trial registration number:

This study is not considered as a trial study

Keywords:

Sperm
SWATH-MS
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