Title and authors
Human sperm lipid profiling in men with asthenospermia
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Study question
Is sperm lipid profile in asthenospermia different from normozoospermia?

Summary answer
Asthenospermic semen samples showed an alteration in cholesterol sulphate/seminolipid ratio.

What is already known
Lipid composition of spermatozoa is important in determining the functional characteristics of the spermatozoa, in particular on motility, acrosomal exocytosis or fusogenic properties of the sperm.

Study design, size, duration
Sperm samples from 33 men undergoing IVF/ICSI treatment were collected of which 9 with normozoospermia (group A; control) and 11 with asthenospermia (group B) were included in this study. The total progressively motile sperm cells/ejaculate were 166.4 ± 44.3 millions and 6.4 ± 5.9 millions in group A and B respectively. Semen samples were collected, frozen, and stored at -80 °C until processed.

Participants/materials, setting, methods
We used Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) in the negative ion mode combined with Thin Layer Chromatography (TLC) to elucidate the lipid composition.

The signals of the MALDI mass spectra (3 replicates/sample) were exported in a final matrix in a compatible format for the multivariate analysis with ClinProTools 3.0 software and t-test was used to confirm significant differences between the two lipid patterns. Peaks with P <0.05 were considered statistically significant.

Main results and the role of chance
Our data on the lipid composition of whole ejaculate give novel information on lipid molecular species and components. In contrast to previous literature reports, we have found only traces of cardiolipin; the only cardiolipin species found in sperm has four palmitic acid chains (corresponding to the MS peak at m/z 1352.3). The mass spectrometry and chromatographic (TLC) comparative analyses of control and asthenospermic samples suggest an alteration in cholesterol sulphate/seminolipid ratio. As regards sulfolipids, TLC and MALDI-TOF mass spectra replicates
showed that the content of the cholesterol sulphate (corresponding to the MS peak at m/z 465.4) and of the sulfoquinovosyl acylalkyl glycerol (namely S-QDG or seminolipid corresponding to the MS peak at 795.8) were significantly different between the two groups (P<0.05). The mean intensity of the cholesterol sulphate MS peak and of the seminolipid MS peak were 40.0±6.8 and 278.9±70.6 in group A and 156.4±53.9 and 59.7±25.5 in group B respectively. The specific lipid composition of seminal plasma and of spermatozoa is under investigation.

**Limitations, reasons for caution:**
The seminolipid is exclusively present in the spermatozoa whereas the cholesterol sulphate is a component of the seminal fluid and of blood, too. In future studies, it may be interesting to measure the cholesterol levels as well as its derivative cholesterol sulphate in the blood in order to verify patient specific differences.

**Wider implications of the findings:**
Our results suggest that the MALDI-TOF/MS lipid profile of sperm may represent a diagnostic tool for the assessment of asthenospermic conditions in the clinical practice.