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(MP44-15) Efficient selection method for human round spermatid in TESE negative men

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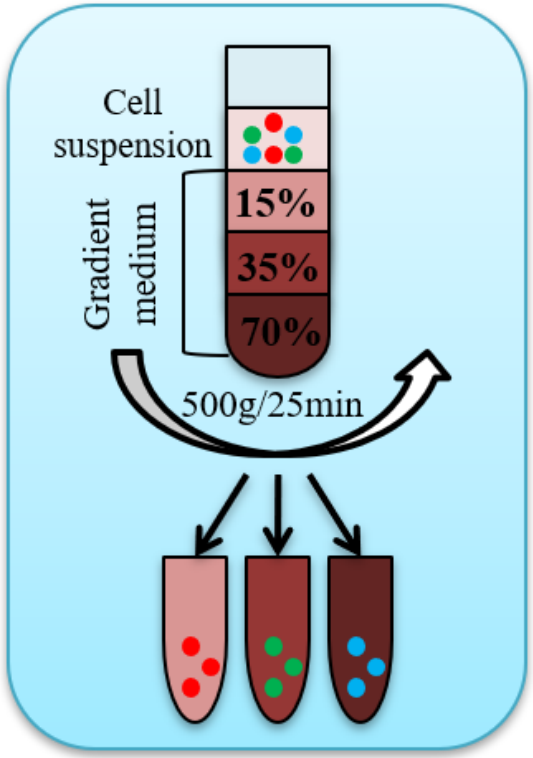
Background

- In more than 50% of non-obstructive Azoospermia patients no sperm is found in testis tissue.
- Round Spermatid Injection (ROSI) has been proposed recently to give these kind of patients their own biologic child.
- The most significant challenge of utilizing ROSI is effectively separating spermatids (haploid cells) from other testicular cells (diploid cells).
- The main objective in this study is establishing a practical method to isolate round spermatids from the other cells.

Method

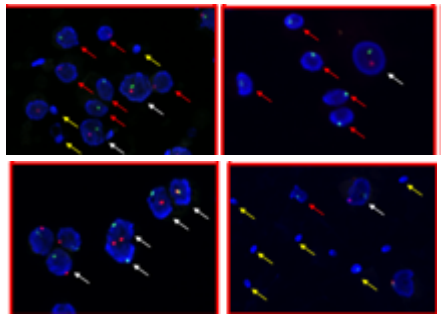
- Enzymatic testis tissue digestion.
- Preparing a gradient medium with different concentrations of 15%, 35% and 70 (in a base of sperm gradient medium that is routinely used at IVF clinics).
- Forced cells to put in different layers based on their size and density.
- Analyzing cell ploidy by FISH and flow-cytometry.

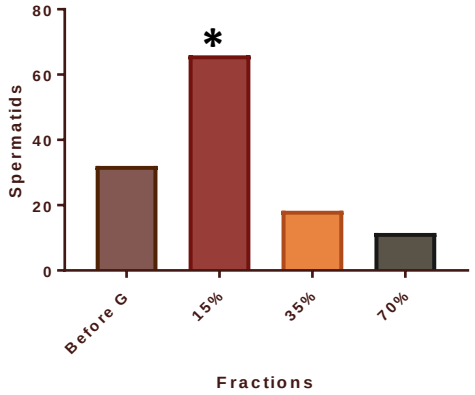


Results

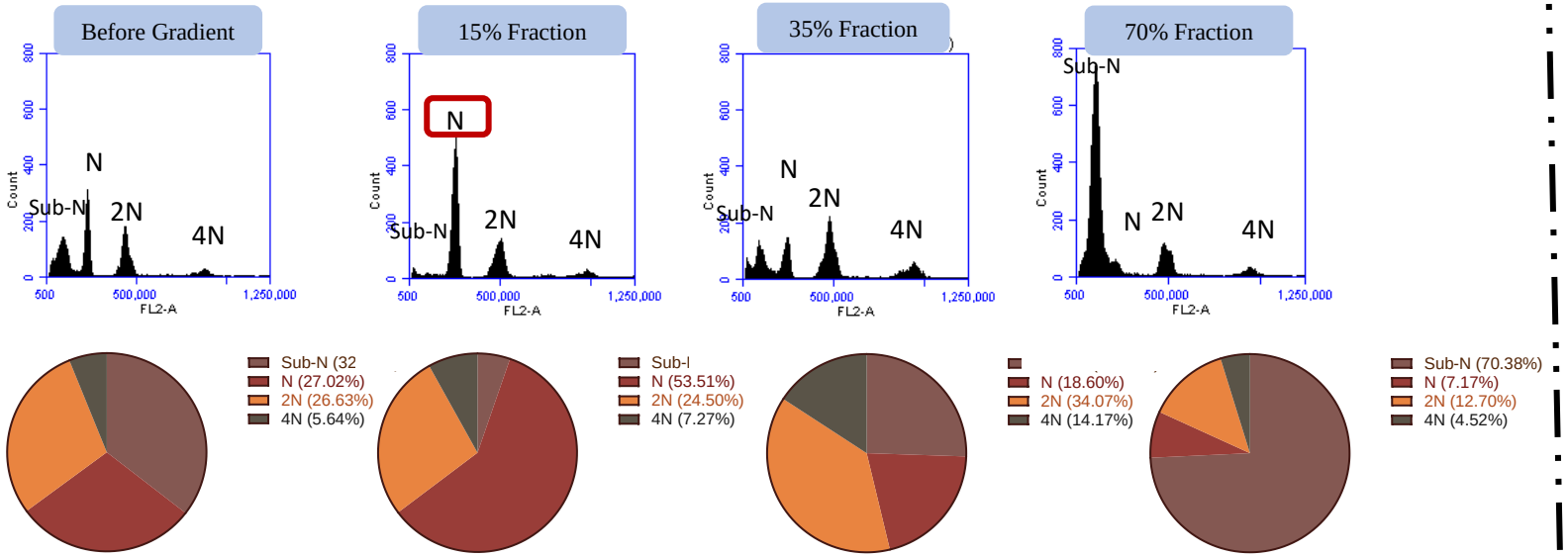
1. FISH (Fluorescence In Situ Hybridization): Distinguish between haploid and diploid cells via X/Y probs.

X: Red
Y: green





2. Cell cycle flow-cytometry: to detect how much N cells (haploid) are exist in the different fractions.



Conclusion

- We were able to establish an efficient method of human round spermatid enrichment in order to use in ROSI.

ROSI clinical trial

ClinicalTrials.gov Identifier: NCT04298255

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