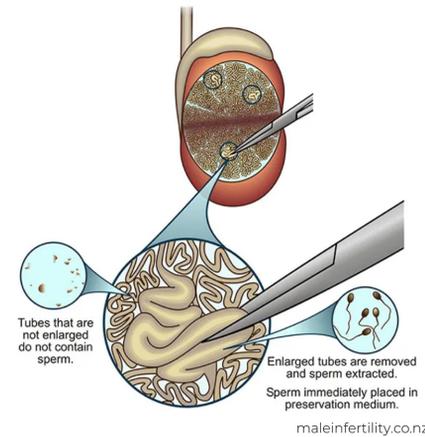


Seminal Plasma cfDNA Predicts Sperm Retrieval in Non-Obstructive Azoospermia

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Background

- NOA is the most severe form of male infertility
- mTESE is invasive and unpredictable
- Current markers are limited in their ability to predict retrieval success
- A noninvasive molecular predictor is needed

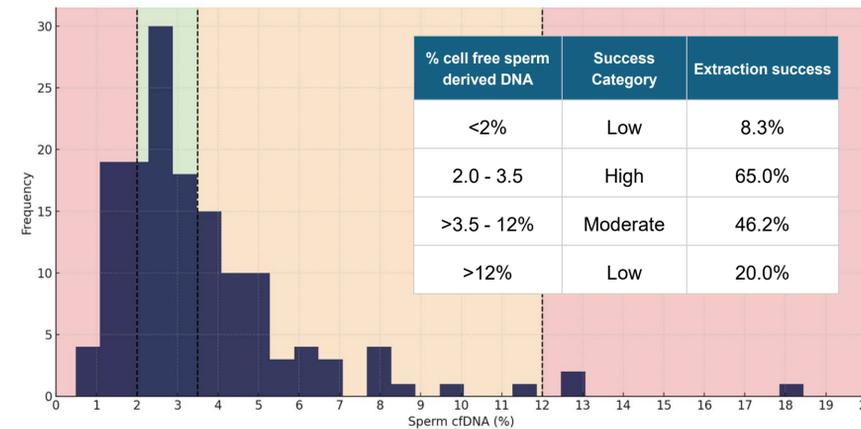


Methods

- 160 men with NOA were sequenced using native methylation (Oxford Nanopore)
- 68 had known retrieval outcomes
- Post-vasectomy, cryptospermic, and normospermic controls were included
- Successful extraction defined as ≥ 1 sperm retrieved for IVF-ICSI

Results

Figure 1
 Sliding-window analysis across the continuous cfDNA spectrum identified regions with consistent divergence in retrieval probability, which were confirmed as stable success categories using likelihood-based performance metrics including PPV, NPV, and likelihood ratios.



Results, cont'd

Figure 3
 Men in the High category had nearly **12-fold higher odds** of retrieval compared with the Low category, while the Moderate group had more than 5-fold higher odds than Low. The Low category demonstrated a strong negative predictive signal with **PPV ~13.8%**, while the High category showed an **NPV of ~68.8%**, supporting meaningful clinical stratification.

Comparison	Odds Ratio	p-value
High vs Low	11.76	0.00116
Moderate vs Low	5.43	0.02734
High vs Moderate	2.167	0.2436

Success Category	PPV	NPV
High	65%	68.8%
Moderate	46.2%	61.9%
Low	13.6%	45.7%

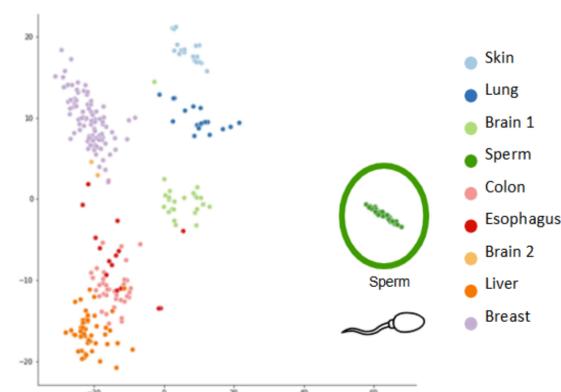
Objective

Determine whether sperm-derived cfDNA in semen can **stratify likelihood of successful sperm retrieval**.

Biological Basis

- Seminal plasma contains cell-free DNA
- Sperm have a unique methylation signature
- Quantifying sperm-derived cfDNA provides a direct molecular measure of spermatogenesis

PCA Plot DNA Methylation from Nine Tissue Types



Success rate by defined category

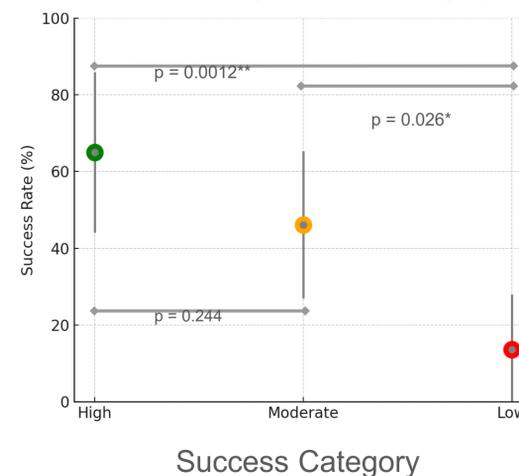


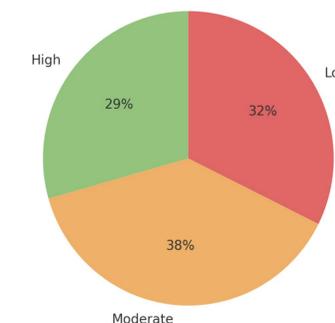
Figure 2

Three distinct probability groups emerged, with retrieval success of 65% in the High category, 46% in the Moderate category, and 14% in the Low category, demonstrating clear stratification of surgical outcomes.

Success Category	% Success	95% CI
High	65.0%	44.1% - 85.9%
Moderate	46.2%	27% - 65.3%
Low	13.6%	0% - 28%

Clinical Implications

- Reflects active spermatogenesis
- Identifies strong negative prognostic group
- Enables individualized counseling prior to surgery
 May reduce unnecessary invasive procedures



Sperm-derived cfDNA in seminal plasma provides a noninvasive molecular stratification tool for surgical sperm retrieval in men with NOA.

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