Magnetic Bead Capture of Sperm Cells by Sperm-Specific Antibodies

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ABSTRACT

Intimate swabs from sexual assault kit evidence contain cell mixtures composed of predominantly female epithelial cells and relatively few sperm cells. The currently accepted methodology used to separate these cells is time-consuming and labor-intensive. This study was performed to examine an alternate method using antibodies for sperm-specific proteins coupled with paramagnetic beads to isolate sperm cells from these mixtures for downstream DNA analysis.

INTRODUCTION

Differential lysis or preferential lysis extraction is the currently established method of separating epithelial and sperm cells in mixtures from sexual assault evidence. This technique exploits the presence of disulfide bonds found in sperm head membrane proteins. These disulfide bonds allow the sperm cells to resist detergent-based lysis that disrupts the cell membranes of epithelial cells. The remaining whole sperm cells are lysed with reducing agents such as dithiothreitol. Differential extraction has been shown to yield varying success in extraction efficiency ranging from 50-90% loss of male DNA compared to direct DNA extraction.[1,2] Alternate cell separation techniques such as laser microdissection, flow cytometry, and acoustic trapping have been reported. However, these methods also present various challenges to forensic lab implementation including cost, training, and specialized equipment.

Instead of cell membrane structural differences, the methodology used in this study targeted proteins specifically expressed on sperm cell membranes as the basis for differential extraction. Hyaluronidase PH-20 (SPAM1), sperm acrosome membrane-associated protein 1 (SPACA1), and zona pellucida binding protein 1 (ZPBP1) were chosen based on previous characterizations of sperm-specific expression. Antibodies for these molecules were conjugated to paramagnetic beads for a magnetic bead-based cell separation.

MATERIALS AND METHODS

Sample Preparation

- Seminal fluid and buccal swabs were collected from volunteers (SHSU Institutional Review Board Protocol IRB-2020-248).
- Semen samples were washed with PBS (2 mM EDTA, 0.1% BSA) to remove debris and plasma.
- Sperm cell suspensions were counted and prepared for isolation.

DNA Extraction, Quantification, and STR Analysis

- DNA was extracted from bead-isolated cells using the QIAamp® DNA Investigator kit (QIAGEN, Hilden, Germany).
- DNA extracts were quantified with Investigator® Quantiplex® Pro Kit (QIAGEN) and amplified with Investigator® 24plex QS kit (QIAGEN).
- Amplified products were separated and detected on a 3500 Genetic Analyzer (Thermo Fisher). Data were analyzed with GeneMapper® ID-X v1.4.

Bead Preparation and Cell Isolation

- Performed following manufacturer recommendations with Dynabeads® M-270 Carboxylic Acid (Thermo Fisher Scientific, Waltham, MA) (Fig. 1).
- Antibodies used were anti-rabbit IgG polyclonal anti-SPAM1, anti-SPACA1, and anti-ZPBP (Thermo Fisher Scientific).

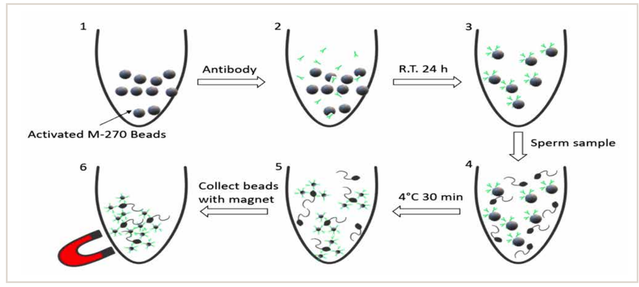

Figure 1: Antibody-bead coupling and cell isolation workflow

RESULTS AND DISCUSSION

Antibody Concentration Optimization

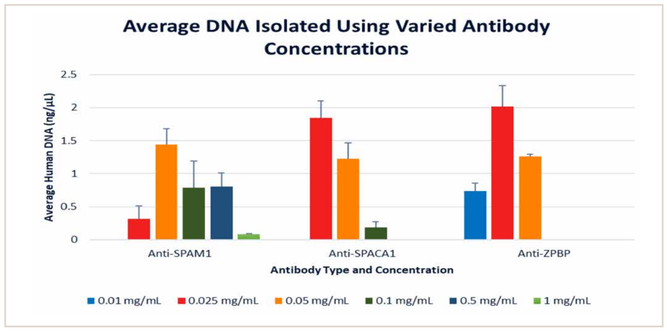

Figure 2: **Optimization of concentrations for anti-SPAM1, anti-SPACA1, and anti-ZPBP antibodies.** DNA extracted from cells isolated with anti-ZPBP beads had the highest concentration compared to DNA extracts from anti-SPAM1 and anti-SPACA1 beads. Reported as µ+σ.

Single Antibody vs. Antibody Cocktail

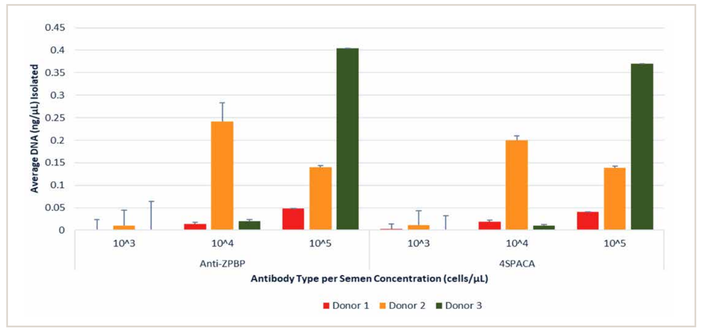

Figure 3: **DNA extracted from anti-ZPBP beads or beads conjugated with a cocktail of all three antibodies.** Cells isolated with anti-ZPBP beads yielded similar amounts of DNA to bead cocktail of all three antibodies. Reported as µ+σ.

Successful STR Profile Generation

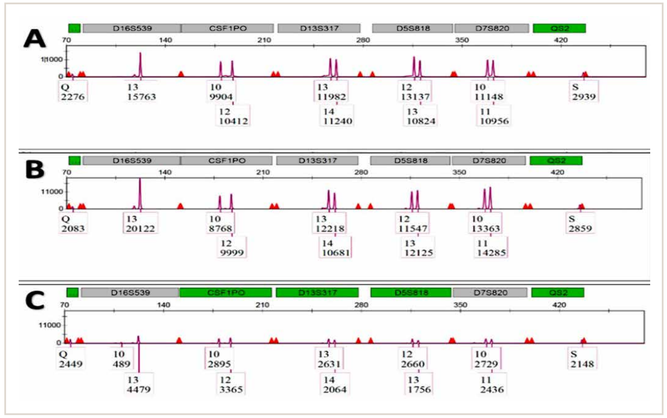

Figure 4: **Representative STR profiles from three different sperm cell concentration reaction.** STR profiles obtained from isolation reactions with (A) 106, (B) 105, and (C) 103 cells/mL isolated with anti-ZPBP beads showed no indication of degradation or inhibition.

Findings

- Optimization of antibody concentration showed anti-ZPBP at 0.025 mg/mL to isolate cells that yielded the highest amount of DNA (Fig. 2).
- Anti-ZPBP beads and cocktail beads (conjugated to anti-SPAM1, anti-SPACA1, and anti-ZPBP beads) yielded comparable DNA extract amounts (Fig. 3).
- DNA extracts from cells isolated with anti-ZPBP conjugated beads were successfully amplified and electrophoresed.
- Even at concentrations as low as 103 cells/mL, extracted DNA was sufficient to generate STR profiles without indications of degradation or inhibition (Fig. 4).

Future Directions

- Still need to demonstrate efficacy of antibody conjugated magnetic bead-based cell isolation in cell mixtures, ideally with vaginal epithelial cells to simulate sexual assault kit evidence.
- Determine if there are non-specific interactions between the beads, cells, and antibodies using epithelial cells or other non-sperm cells.
- Use blocking agents (0.1% BSA) to prevent and reduce non-specific interactions

CONCLUSIONS

- Magnetic beads conjugated with sperm-specific antibodies successfully isolated cells for DNA extraction and STR profile generation from single source semen samples.
- Of the antibodies used, anti-ZPBP conjugated to magnetic beads isolated cells that yielded the most DNA.
- Beads conjugated with a combination of the three antibodies used in this study (anti-SPAM1, anti-SPACA1, and anti-ZPBP) performed similarly to the anti-ZPBP conjugated beads.

REFERENCES

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