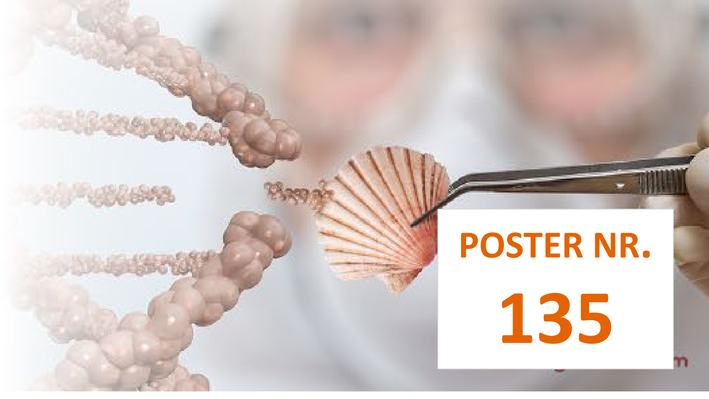

Exploring Immunomagnetic Isolation of Spermatozoa

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INTRODUCTION

Forensic DNA labs often process sexual assault kits, where separating female epithelial cells from male spermatozoa is critical but time-consuming using conventional methods. These methods involve preferential lysis with reducing agents, which can lead to DNA loss[1]. Biomolecule-targeting cell isolation methods, commonly used in clinical research, offer a potential solution. This study focuses on sperm-specific proteins—SPAM1, SPACA1, and ZPBP1—shown to improve sperm cell enrichment[2]. The objective was to assess whether magnetic beads conjugated to antibodies targeting these proteins can effectively isolate sperm cells from mixtures.

MATERIALS AND METHODS

Immunocytochemistry

- 4% Formaldehyde fixation, R.T. 15 minutes
- 5% Normal goat serum blocking, R.T. 2 hours
- 1° Antibody incubation, 4°C overnight
- 2° Antibody & DAPI incubation, R.T. 1 hour
- Leica FS CB microscope

Bead Preparation & Cell Isolation

- Performed following manufacturer recommendations with Dynabeads® M-450 Epoxy (Thermo Fisher Scientific, Waltham, MA).
- Antibodies used were anti-rabbit IgG polyclonal anti-SPAM1, anti-SPACA1, and anti ZPBP (Thermo Fisher Scientific)

DNA Extraction, Quantification, & Isolation

- Bead-isolated sperm or vaginal epithelial cells
- QIAamp® DNA Investigator Kit
- Investigator® Quantiplex® Pro Kit

RESULTS

Antibody Binding Confirmation

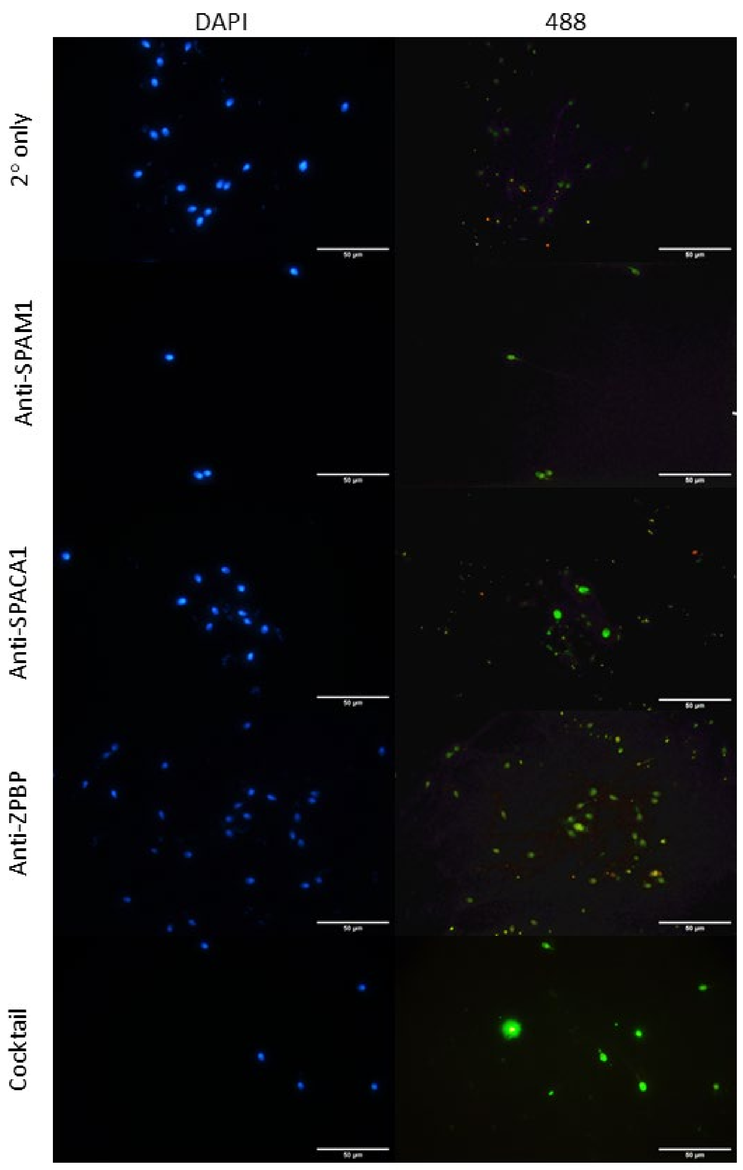

Figure 1. Target protein binding confirmation by immunocytochemistry in spermatozoa. Each target protein was detected by anti-SPAM1, anti-SPACA1, anti-ZPBP, or combined in an antibody cocktail. Scale bars represents 50 µm.

Antibody Cocktail-Bead Specificity

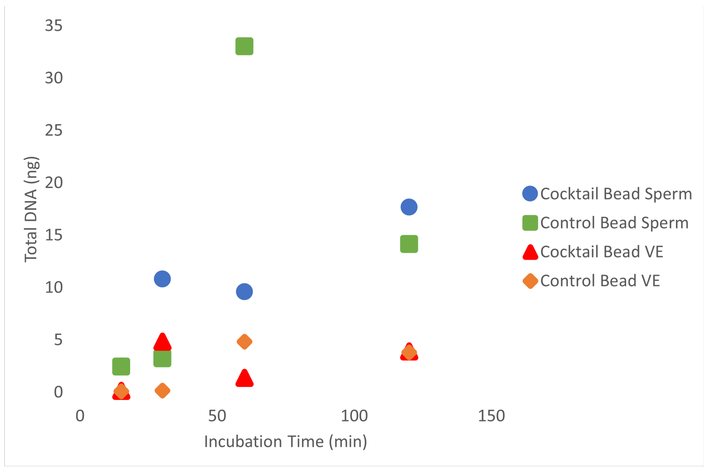

Figure 2. Non-specific interaction between BSA-blocked magnetic beads and cells. Increasing incubation time increased non-specific interactions between the magnetic bead complexes and both spermatozoa and vaginal epithelial (VE) cells.

Antibody Cocktail-Bead Sensitivity

Figure 3. Minimal differences observed between control beads and antibody cocktail-conjugated beads. Majority of cells were found in supernatant fraction rather than the bead-complex fraction.

CONCLUSIONS

- Immunocytochemistry confirmed antigen-binding capability of all three antibodies (**Figure 1**).
- Vaginal epithelial cells showed cross-reactivity with the magnetic beads (**Figure 2**).
- Similar results between antibody-coated and uncoated beads indicated non-specific cell-bead interactions (**Figure 3**).

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