Subcellular localization of antisperm-directed monoclonal antibodies

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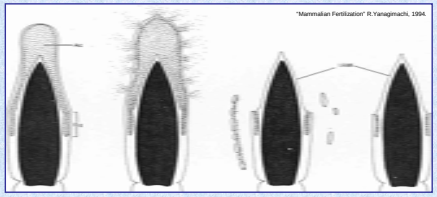
INTRODUCTION

Human antisperm antibodies (ASA) are detectable in fertile and infertile men and women and are closely linked to immune infertility. These proteins may hinder fertilization by inhibiting sperm motility, penetration of the cervical mucus, capacitation, acrosome reaction, or triggering a cascade leading to sperm lysis. It has been shown that couples with ASA (anti-sperm antibodies) in their serum, semen, or follicular fluid have lower pregnancy rates than couples without ASA. It is suspected that an inverse relationship exists between the number of sperm bound by ASA and their fertilizing capability. Preliminary data from our lab suggests that sperm surface antigens affect capacitation and acrosome reaction. Monoclonal antibodies 37 and 77 had consistently reproducible results indicating that these antibodies induced release of the acrosome compartment. The specific aim of this project is to determine the subcellular binding sites of human antisperm monoclonal antibodies in washed, capacitated, and acrosome reacted sperm. Characterization of binding sites of ASA could contribute to the understanding of infertility in couples.

Figure 1: Diagram of human sperm acrosome reaction.



METHODS

HUMAN SPERM WASHING

- Semen specimens obtained following approved human subjects protocols
- pH, volume, and cell count of each sample recorded
- 1.5 mL of each sample washed using Percoll gradient (*Suarez et al.*)
- Sample centrifuged 25 minutes, 500g
- Top and interface layers removed and HSM added to bottom layer to give final volume of 10 mL
- Sample centrifuged again 10 minutes, 500g
- Supernatant removed and pellet resuspended in HSM to final cell count of 20- 40 million per mL

CAPACITATION

- 500 µL of washed sample transferred to 35 mm Petri dish
- 500 µL HSM with 7% BSA added to sample (final concentration 3.5%)
- Incubated 4 hours, 37°C, 5% CO_2
- After 4 hours, sample removed from Petri dish and placed into conical vial
- HSM added to bring total volume to 4 mL
- Sample centrifuged 5 minutes, 500 g
- Pellet resuspended to 500 µL in HSM

ACROSOME REACTION

- 200 µL of capacitated cells placed into conical vial
- 2 µL of 1mM Bromo ionophore A23187 (Molecular Probes) added to sample
- Sample incubated 30 minutes, 37°C, 5% CO_2
- After 30 minutes, 1000 µL HSM added and centrifuged 5 minutes, 500 g
- Pellet resuspended in 200 µL HSM

SLIDE PREPARATION

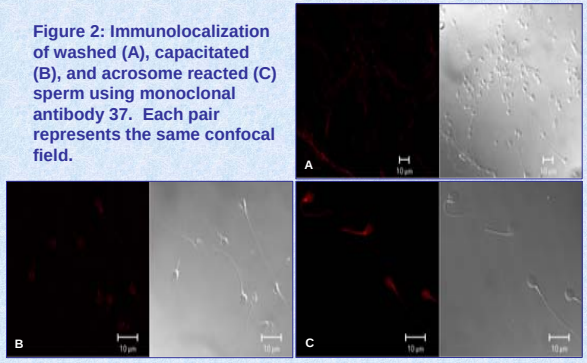
- 8 µL of each sample (washed, capacitated, or acrosome reacted) dried onto Esco Fluoro slide
- Slides fixed 10 minutes in 100% methanol
- Slides washed 3 times with 1X PBS

IMMUNOLOCALIZATION

- 20 ul of monoclonal mouse–anti-human sperm antibodies or SP20 added to each slide and incubated for 30 minutes at room temperature in moisture box
- Slides are washed three times with 1X PBS
- 20 ul of secondary goat-anti-mouse IgG (KPL) (1:80,000) added to each slide and incubated for 15 minutes in a moisture box
- Control slides incubated with 20 ul of goat-anti-mouse IgG antibody (KPL) (1:80,000), 15 minutes in moisture box
- Slides are washed three times with 1X PBS and once with distilled H_20
- Slides are mounted with 8 ul of Mowiol and a No. 1 coverslip
- Staining pattern scored using Zeiss LSM 510 Meta Confocal Microscope

RESULTS

Figure 2: Immunolocalization of washed (A), capacitated (B), and acrosome reacted (C) sperm using monoclonal antibody 37. Each pair represents the same confocal field.



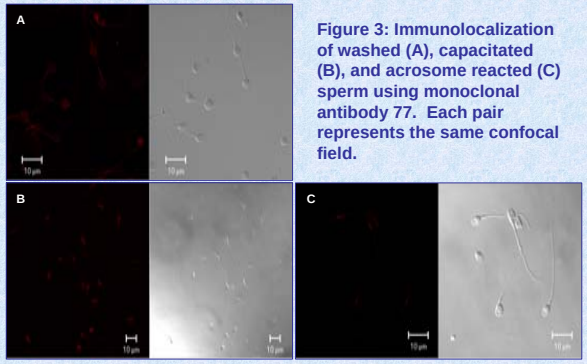
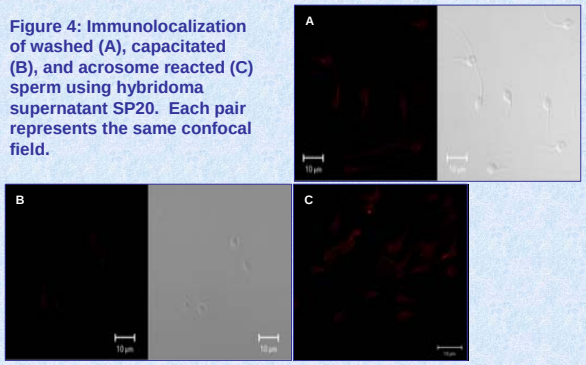


Figure 3: Immunolocalization of washed (A), capacitated (B), and acrosome reacted (C) sperm using monoclonal antibody 77. Each pair represents the same confocal field.

Figure 4: Immunolocalization of washed (A), capacitated (B), and acrosome reacted (C) sperm using hybridoma supernatant SP20. Each pair represents the same confocal field.



CONCLUSIONS

Immunolocalization with monoclonal antibody 37

- Washed cells exhibited faint staining in the midpiece region, consistent with the control
- Capacitated cells exhibited diffuse head staining
- Acrosome reacted cells displayed bright fluorescence in the posterior head region

Immunolocalization with monoclonal antibody 77

- Washed cells showed staining in the posterior head region
- Capacitated cells had diffuse head staining
- Acrosome reacted cells exhibited faint fluorescence in the midpiece, consistent with the control

Immunolocalization with hybridoma supernatant SP20

- Washed, capacitated, and acrosome reacted cells exhibited very faint staining in the midpiece, consistent with the control

FUTURE DIRECTIONS

- Double label cells with anti-sperm antibodies and FITC-PSA to determine ASA binding site and acrosome status simultaneously
- Repeat experiments with different donors

REFERENCES

Chui, W., Chamley, L.Fertility and Sterility. Vol. 83, No. 3 (2004).
Dogmala, A., Kurpisz, Z. Reproductive Biology and Endocrinology. Vol. 2 No. 11 (2004).
Hardy, C., Clydesdale G, Mobbs KJ. Reproduction. 2004; 128: 395-407.
Nass, S., National Academics Press, Washington D.C. c2004. 25-39.
Shetty, J. Naaby-Hansen, S., Shibahara, H., Bronson, R, Flickinger, C. and Herr, J Biology of Reproduction. Vol. 61 (1999): 61- 69.

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