

# GDA-J/F3 Monoclonal Antibody: The Ideal Probe for Sperm Detection in Forensic Science

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## INTRODUCTION

One essential procedure in the scientific investigation of sexual assaults is the identification of semen. The potential of using an immunological approach has been investigated by the study of three monoclonal antibodies for their suitability for the identification of sperm.

## MATERIALS & METHODS

### Sperm Donors

Seminal samples and peripheral blood leucocytes (PBL) were obtained from 20 medical students. The semen was handled within 1h of donation.

### Preparation of Demembrated Sperm

The sperm membranes were disrupted by repeated freezing and thawing using liquid nitrogen (Jassim & Festenstein 1987a).

### Monoclonal Antibodies (MoAbs)

Details of GDA-J/F3 MoAb production have been published elsewhere (Jassim & Festenstein 1987a). Mice were immunised with sperm and their precursors (spermatids and spermatocytes) obtained from seminal samples of an oligospermic donor. Anti- $\alpha$  and  $\beta$  tubulin MoAbs were purchased from Amersham (Code N° 356, 357). The hybridoma cell lines for W6/32 anti-HLA Class I (HB95) and anti-leucocyte (HB12) MoAbs were obtained from the American Type Culture Collection (ATCC).

### Indirect Immunofluorescence (IIF) and Peroxidase (IIP) Tests of Frozen Sections and Cell Suspension

These standard techniques were performed as previously described (Jassim & Festenstein 1987a).

### Immunoperoxidase Electron Microscopy (IEM)

Sperm cell precursors obtained from an oligospermic donor were dried onto a slide. These were tested with GDA-J/F3 MoAb using immunoperoxidase test (Jassim & Festenstein 1987a). The tested cells were prepared for electron microscopy using conventional techniques (Jassim & Festenstein 1987b).

### Human Fibroblast Cultures

The fibroblasts were a kind gift from T Purkis (Dept. Dermatology). For IIF screening, the fibroblasts which were cultured onto slides were tested either viable (the cells were not allowed to dry and the washing was with PBS), or after air drying.

### RESULTS

#### Reaction of GDA-J/F3 and Anti-Tubulin MoAbs With Ejaculated Sperm

Using the IIF test, the three MoAbs stained the tails of all demembrated sperm. However, the reaction of the anti- $\alpha$  and - $\beta$  tubulin antibodies was with the whole tail while that of GDA-J/F3 MoAb was with the principal piece only (Fig. 1).

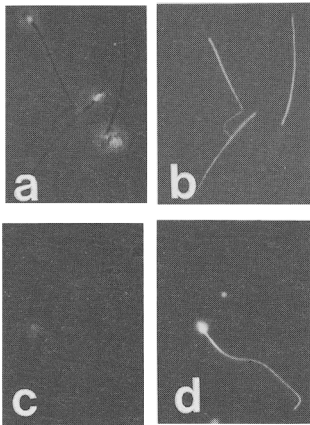


Fig. 1. IIF test of demembrated sperm with GDA-J/F3 (a,b) and anti- $\alpha$  or - $\beta$  tubulin MoAbs (c,d). GDA-J/F3 stains the principal piece only while the other 2 antibodies react with the whole tail. (a,c: phase contrast; b,d: fluorescence microscopy).



Fig. 2. IEM of dried germ cells with GDA-J/F3 MoAb. The reaction (electron dense deposits) is seen on the fibrous sheath just beneath the cytoplasmic membrane of a spermatid (arrows).

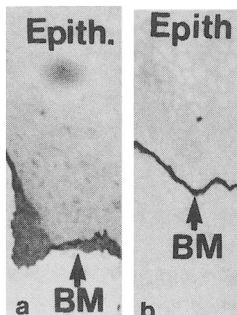


Fig. 3. IIF test of GDA-J/F3 MoAb with frozen section of (a) lip, (b) cervix. The antibody stains the basement membrane (BM), but not the lining epithelium (Epith).

### Ultrastructural Localisation of GDA-J/F3

For ultralocalisation, dried germ cells were tested with GDA-J/F3 MoAb using IEM. GDA-J/F3 was found localised to the fibrous sheath (Fig. 2).

### Reaction of GDA-J/F3 MoAb with Lining Epithelium

To exclude the reactivity of GDA-J/F3 MoAb with the lining epithelium, frozen sections from lip, tongue, buccal cavity and tonsillar area as well as from vagina and cervix were tested with the antibody using IIF and IIP tests. In both tests, GDA-J/F3 MoAb did not react with the lining epithelium of all these tissues. Instead, the antibody stained the basement membranes (Fig. 3).

### Reaction of GDA-J/F3 MoAb with PBL

Leucocytes were screened with GDA-J/F3, anti-Class I HLA and anti-leucocyte MoAbs using IIF test. In contrast to the anti-Class I HLA and anti-leucocyte MoAbs which stained the PBL, GDA-J/F3 did not react with the leucocytes (data not shown).

### Reaction of GDA-J/F3 and Anti-tubulin MoAbs with Fibroblasts

Using IIF test the three antibodies failed to stain the surface of the viable cells but as might be expected the microtubules of the dried fibroblasts were stained by the anti- $\alpha$  and  $\beta$  tubulin antibodies but not by the GDA-J/F3 MoAb.

## DISCUSSION

Three MoAbs (GDA-J/F3 and anti- $\alpha$  and - $\beta$  tubulin) were used to assess their suitability for sperm detection. The 3 antibodies recognise intracellular antigens inside the sperm tails. The anti-tubulin MoAbs react with the axonemal microtubules while the GDA-J/F3 with the fibrous sheath. This was based on the following:- (1) the reaction of GDA-J/F3 MoAb was limited to the principal piece, (2) non reactivity with cilia of respiratory epithelium (unpublished data) which lack the fibrous sheath and (3) immunoelectron microscopy.

GDA-J/F3 MoAb which lacks the reactivity with epithelial cells, leucocytes or fibroblasts seems to be the most specific probe for sperm; and thus the most suitable for their identification. The other 2 antibodies recognise tubulin which shows a ubiquitous tissue distribution as it forms the microtubules in all tissues (Schliwa, 1986). Currently, GDA-J/F3 MoAb is being investigated for its use in the detection of sperm in sexual assault cases.

## ACKNOWLEDGEMENT

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