Antibodies to various components of sperm and seminal plasma are present in the sera of certain individuals, particularly vasectomised men and infertile couples, and there has been much speculation over their pathogenetic role. Recently, using a variety of methods, anti-sperm antibodies have also been detected in the sera of healthy homosexual men, or those who presented with HIV infection, or (other) sexually transmitted diseases. Together with data from experimental animal modes, therefore, it has been suggested that the introduction of spermatozoa into the rectum may lead to the development of a humoral immune response. However, many of these earlier studies either lacked information on the subjects' HIV status or used methods for measuring sperm antibodies that were only poorly reproducible, and with the exception of one study did not attempt to correlate the findings with specific sexual practices. A recent editorial stated that the most useful assay for anti-sperm antibodies is the immunobead method and this prompted us to use this technique to investigate sperm antibody titre and prevalence in a group of well documented homosexual men, to see to what extent it is correlated with recent sexual behaviour, HIV status, and indices of immune function.

Patients
Between January and March 1988, sixty four homosexuals (range 28–60 yr, mean 40.7) attending the Royal Melbourne Hospital HIV clinic as part of an ongoing epidemiological study completed questionnaires on their sexual practices over the previous six months. Based on their responses men were categorised into the following groups: Group 1—unprotected oral intercourse only (n = 13), Group 2—unprotected oral and anal intercourse (n = 39), Group 3—unprotected anal intercourse only (n = 2), and Group 4—celibate (n = 6).

All individuals in the first three groups were recipients of sperm; six men who reported always using condoms during the previous six months were excluded from the study. On the basis of informed consent, blood was obtained for anti-sperm and anti-HIV antibody testing as well as for quantitation of lymphocyte subsets. In addition sera were obtained from two homosexual men who had attended the Melbourne Sexually Transmissible Diseases Centre for treatment of rectal gonorrhoea; these were placed in Group 2.

Methods
Fresh donor sperm was obtained from volunteers. These were HIV antibody negative heterosexual men who were regular sperm donors in an in-vitro fertilisation program. Test sera were screened for anti-sperm antibodies using a minor modification of the indirect immunobead assay previously described.

Burnet Clinical Research Unit, Walter and Eliza Hall Institute of Medical Research, Melbourne
B P Mulhall, S Fieldhouse, S Clark, L Carter, L Harrison, B Donovan, R V Short

HIV Service, Royal Melbourne Hospital, Parkville, Victoria
L Carter
Department of Physiology, Monash University, Melbourne
S Fieldhouse, R V Short
Department of Public Health, University of Sydney, Australia
B Donovan

Abstract
The sera of 60 homosexual males were examined for the presence of antibodies to sperm using an indirect immunobead test (IBT). Six of 60 (10%) had antibodies of IgG isotype; in addition two of the six had antibodies of IgA isotype. The presence of antibodies was associated with the practice of unprotected receptive anal intercourse in the previous six months. Antibodies were not found in homosexual men who were celibate, or who practised only oral intercourse during the same period. There was no correlation between the presence of anti-sperm antibodies and antibodies to human immunodeficiency virus (HIV), or numbers of T lymphocytes. These preliminary results lend support to the hypothesis that antigen presentation in the lower gut may be a source of sensitisation against sperm. The possibility that anti-sperm antibodies may be a marker of receptive anal intercourse merits further investigation.

This is essentially a rosetting technique: briefly, serum at a dilution of 1:10 was incubated for 30 min
at 37°C with live sperm (count > 60 × 10⁶/ml and
motility > 50%) from donors who were anti-sperm antibody negative (determined by incubation of
sperm with IgG or IgA coated immunobeads in the
absence of serum). After washing to remove free
immunoglobulin, the sperm were added to
immunobeads coated with rabbit anti-human IgG or
IgA (Biorad, Ca), and under magnification (× 25),
100 motile sperm were counted. The result was
considered positive if > 10% of motile sperm had
two or more beads attached. In each assay positive
and negative control sera were included. Positive
control sera were obtained from heterosexual men
attending an infertility clinic. To minimise interassay
variation results were expressed relative to a positive
control value of 100%. In addition, all positive
samples were precipitated with PEG, ammonium
sulphate, and more specifically, anti IgG or IgA
coated beads, to check that anti-sperm activity
resided in the immunoglobulin fraction. All positive
sera were also serially diluted to determine anti-
sperm antibody titre. Sera were screened for
antibodies to HIV using an ELISA assay and
confirmed by Western Blot. At the same time absolute
numbers of CD4+ and CD8+ T lymphocytes were
determined using the monoclonal antibodies Leu 3a
and Leu 2 (Becton Dickinson, Ca) and flow
cytometry (Coulter Epics IV, Ca).

**Results**

Six of 60 (10%) sera tested positive for antibodies to
sperm. All six had antibodies of IgG isotype; two of
the six also had antibodies of IgA isotype. For IgG,
three sera were positive at a dilution of 1:100 and the
remaining three at a dilution of 1:50. The positive
control sera were positive at a dilution of 1:500. For
IgA, titres were lower in controls (1:200) and in
patients (1:50). Anti-sperm activity was completely
removed in all cases by precipitation with coated
beads. Three different sperm donors were used and
the inter-donor variation was less than 5%.

All six subjects positive for anti-sperm antibodies
were from Groups 2 and 3 (table 1), viz. they
practised receptive anal intercourse; none of the
thirteen recipients of oral intercourse alone had

<table>
<thead>
<tr>
<th>Anti-sperm antibodies and sexual practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptive anal intercourse</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Anti-sperm antibody status</td>
</tr>
<tr>
<td>+ve</td>
</tr>
<tr>
<td>-ve</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>35</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>19</td>
</tr>
</tbody>
</table>

Anti-sperm antibodies detected in the immunobead test compared with sexual practices as determined by questionnaire.

**Table 2 Anti-sperm antibodies and HIV status**

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Anti-sperm antibody status</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td>2</td>
</tr>
<tr>
<td>-ve</td>
<td>28</td>
</tr>
</tbody>
</table>

Anti-sperm antibodies compared with the presence of antibodies to HIV.

**Discussion**

The prevalence of antibodies to sperm in this group of
homosexual men was 10%, and was confined to
17% of those who had practised unprotected anal
receptive intercourse in the previous six months.
Other investigations using different methods have
found prevalence rates of 20–50% in similar
groups, although their studies were performed
earlier in the HIV epidemic, before the availability of
HIV testing, and when subjects were more likely
to have practised unprotected anal receptive
intercourse more frequently. In addition "naturally
occurring" antibodies have been detected in the
general male and female population. Previous
methods for detecting anti-sperm antibodies have
included sperm immobilisation, agglutination,
indirect immunofluorescence, enzyme-linked
immunosorbent assays (ELISA) and Western blotting,
and have been striking in their lack of consistency and
correlation, particularly when different target sperm
preparations were used. In the present study,
employing the immunobead test, the variation be-
tween three different sperm donors was less than 5%.
However there is every likelihood that the antibodies
recognised in the immunobead test are different from
those detected in previous assays. In addition, the
prevalence of these antibodies in the heterosexual
population has not been fully investigated.

Experimental studies in rabbits have demon-
Antisperm antibodies in homosexual men: prevalence and correlation with sexual behaviour

This study was supported by a grant from Family Health International, North Carolina 27709, USA. S.C. (C J Martin Fellow) and L.H. are supported by the National Health and Medical Research Council of Australia. The authors thank P Hayes of Melbourne Communicable Diseases Centre for obtaining sera, L Collins from Prince Henry’s Hospital for help with the method and in recruiting sperm donors, and Ms Iras Collins for expert secretarial assistance.

Address for reprints: Dr B P Mulhall, Department of Public Health, University of Sydney, NSW 2006, Australia.