B_4 virus in particular seems implicated at the onset of juvenile diabetes (Gamble *et al.*, 1969). Though a viral aetiology is unproven these three clinical cases with a myocardial lesion are interesting and lend support to sudies suggesting that viral infection may predispose to juvenile diabetes.

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PRELIMINARY COMMUNICATIONS

Cervical Plasma Cell Population in Infertile Patients

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Summary

Cervical biopsy specimens were obtained from 50 controls and 50 women attending an infertility clinic. Sections were cut and stained, by a direct immunofluorescent technique, with fluorescein-labelled sheep antihuman IgA, antihuman IgM, and antihuman IgG in an attempt to show a difference between the two groups. A significant increase in the number of plasma cells containing IgA was found in many of those infertile women in whom no other abnormality could be detected.

Introduction

Much of the recent literature pertaining to immunological infertility revolves around the study of the male reproductive system, in particular, the origin and nature of antigens associated with spermatozoa and seminal plasma (Shulman, 1972). Others have concentrated on the problem of the significance of antibodies found in the cervical mucus (Parish and Ward, 1968).

The decision to examine the cervical tissue itself in an attempt to show an alteration in cell population or structure in immunologically infertile patients stemmed from two important observations. Firstly, it has been well established that the secretory IgA system provides a first line of defence for the mucosal linings of the body. This has been best illustrated in the bowel (fig. 1) and the respiratory systems but it has also been shown that plasma cells containing IgA are to be found in many other organs of the body (Drayton *et al.*, 1971). Secondly, it has been clearly shown that there is an increase in the number of plasma cells containing IgA in the cervices of women suffering from local infections such as candidiasis, gonorrhoea, and trichomonal vaginitis (Chipperfield and Evans, 1972).

These findings led to the postulation that spermatozoa in certain cases of infertility might produce a similar plasma cell reaction, to the detriment of the sperm.

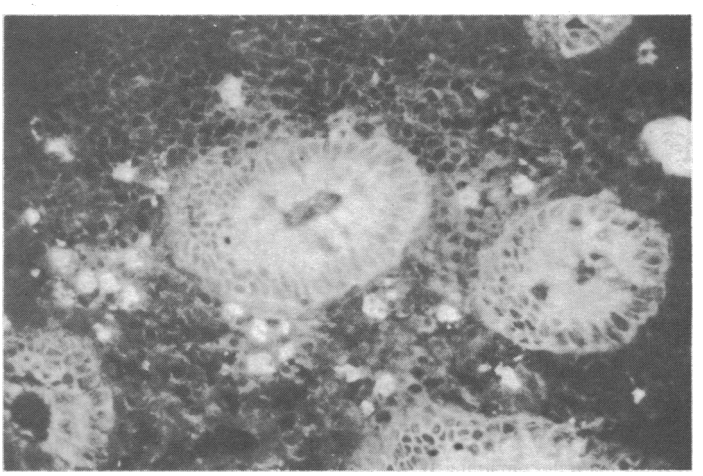

FIG. 1—High-power view of appendix stained with sheep antihuman fluorescein-labelled IgA showing plasma cells containing IgA near columnar epithelium.

Method

Using a hollow steel tube 6-cm long and just over 3 mm in diameter with a sharp cutting edge at one end small but undamaged biopsy specimens were obtained from both endocervix and ectocervix. In the first instance the technique was carried out on specimens obtained at hysterectomy (fig. 2). This group subsequently provided the controls which were incorporated into the series. In the strictest sense these were not ideal as controls as, though there was no history of infertility recorded in the group, at least one of these patients might have developed a late secondary immunological infertility which had gone undiagnosed. A true control series would have been made up of a group of

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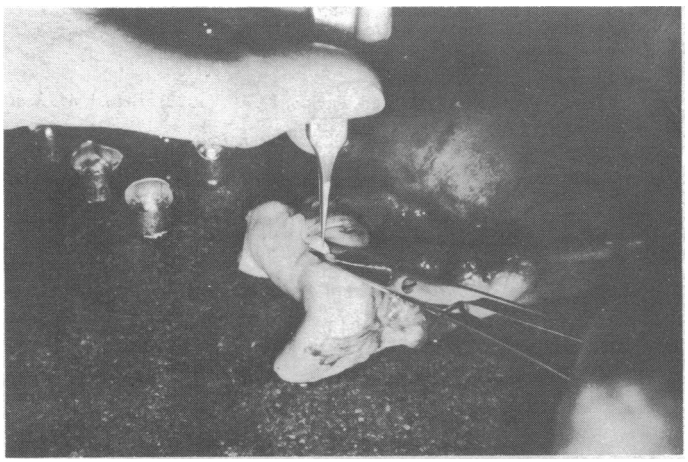

FIG. 2—Biopsy specimen of cervix being cut at its base before snap-freezing.

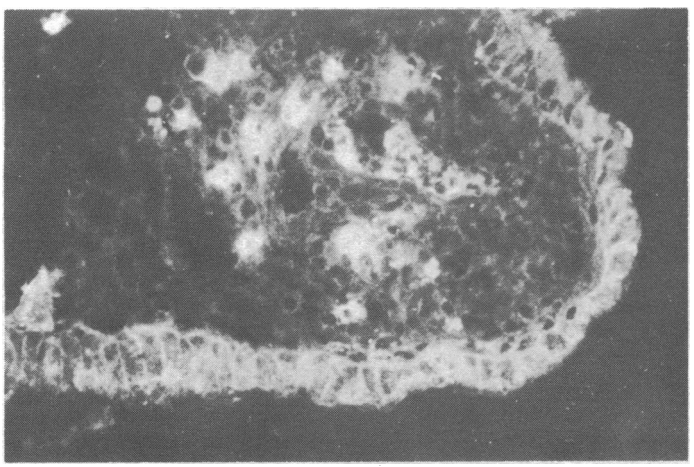

FIG.3—High-power view of endocervical biopsy specimen showing numerous plasma cells containing IgA.

women whose cervices had not been subjected to any possible antigenic stimulus from semen. Such a group would obviously be very difficult to collect. Biopsy specimens were then taken from a group of 50 infertile women.

Having obtained a specimen it was immediately "snap-frozen" using isopentane and liquid nitrogen. Sections were cut and stained, by a direct immunofluorescent technique, with fluorescein-labelled sheep antihuman IgA, antihuman IgM, and antihuman IgG. In each case up to 20 individual sections were examined (figs. 3 and 4).

Results

One of the problems which arises when using an immunofluorescent technique is the need to develop a quantitative method for assessing the material studied. We made a direct count of the number of plasma cells present. We noted that in any one section not only were individual plasma cells bisected but often portions of varying sizes of other cells were found. This complicated the counting technique, but with experience it was not difficult to distinguish between a portion of a plasma cell and an artefact. Most patients were clearly negative or positive for plasma cells. In those cases where there was any doubt the technique was repeated a further five times.

Out of the 50 controls only one woman had a significant increase in the number of plasma cells containing IgA. In contrast, in the infertile group 12 women showed an increase. The most important finding, however, came when we split the infertile patients into groups. These groups were assessed before the histories were appraised to eliminate a subjective bias as much as possible. Eleven out of the 12 cases with an increased number of IgA-containing plasma cells were found to be associated with those couples who had a clinically normal essential triad (see table)—that is, a normal ovulation pattern, tubal patency and function, and normal semen. Five couples had two conditions and therefore were recorded in two of the categories. In none of the many slides stained with antihuman IgM or IgG were there any significant findings.

Breakdown of results in 50 infertile couples

Patient Group	Positive for Plasma Cells	Negative for Plasma Cells
No. not ovulating	1	4
No. with tubal obstruction	0	8
No. with subfertile semenanalysis	0	8
No. with unknown cause	11	13
No. with inconclusive results	0	4
No. with incomplete investigations	0	6
Total	12	43

Discussion

These findings show that there is an increase in the number of plasma cells containing IgA in the cervix of those women in infertile couples previously labelled "cause unknown" and suggest the possibility that an immunological factor may be responsible. Though it is possible or even probable that the antigen(s) responsible for this plasma cell reaction may be related directly to spermatozoa or semen it has, as yet, not been scientifically established.

Our results certainly indicate possible avenues for further research into the nature and function of these plasma cells. Our method is a new approach to tests for immunological infertility and may perhaps add to those already used.

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