Testicular needle aspiration as an alternative to biopsy for the assessment of spermatogenesis

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The technique of fine needle aspiration (FNA) may have a role as a reliable, quick and easy method of obtaining testicular tissue. Recent advances in the management of male subfertility and, in particular, the finding that spermatozoa recovered from the epididymis and testis can result in embryo generation after intracytoplasmic sperm injection (ICSI), question the traditional role of open testicular biopsy for the assessment of spermatogenesis. FNA of the testis was performed on 19 cases of male subfertility and histological and cytological preparations obtained were assessed by light microscopy. FNA provided intact testicular tubules adequate for the histological assessment of spermatogenesis in all cases. There was good correlation with the cytological preparations which gave an indication of the number of mature spermatozoa present. FNA should be considered as a simple alternative to open testicular biopsy in the current investigation of male subfertility and as a method of retrieving spermatozoa for assisted conception using ICSI.

Key words: azoospermia/cytology/histology/needle aspiration/ testes

Introduction

Open scrotal exploration remains a necessity to establish the cause of intrascrotal pathology, e.g. testicular tumours. However, the situation is less clear when it comes to establishing the underlying cause of oligo- or azoospermia in subfertile males.

The assessment of these patients encompasses a detailed history, physical examination and the performance of various investigations. The latter includes blood tests [chromosomal analysis, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin and testosterone], ultrasound, vasography and usually open testicular biopsy.

In the past, the finding of a raised FSH concentration often predetermined that infertile couples were recommended to use donor spermatozoa without further investigation. However, focal areas of spermatogenesis may exist in men with small testes and/or raised FSH values, and even in men previously considered to be devoid of spermatogenesis such as in Sertoli cell only syndrome (Craft and Tsirigotis, 1995).

In such circumstances, it is now possible to recover spermatozoa for intracytoplasmic sperm injection (ICSI) enabling fertilization of oocytes and fatherhood following embryo transfer and implantation (Silber, 1995). The completed work-up of these patients therefore has usually included open surgical biopsy for histopathological assessment of spermatogenesis. Alternative methods of testicular biopsy have been explored for cytological purposes, including fine needle aspiration (FNA). The latter technique was first described in 1930 by Martin and by Stewart in 1933. Today it is a widespread method of cytological diagnosis. Obrant and Persson (1965) and Persson et al. (1971) described the use of testicular FNA in men with fertility problems.

Despite the simplicity of the technique and its accuracy, its use has not become routine. More recently, Gottschalk-Sabag et al. (1993) have correlated the cytological findings of testicular FNA with the histology deduced by open biopsy. Mallidis and Baker (1994) have described an FNA biopsy technique for histological assessment but reported technical problems with a 45% incidence of histological artefacts.

We present our early experiences with testicular FNA to obtain specimens for both histological and cytological examination.

Materials and methods

The material consisted of testicular aspirates from 19 men with oligo- or azoospermia aged from 34–53 years (Table I). This procedure was part of a diagnostic work-up and also included percutaneous epididymal sperm aspiration (PESA) in obstructive cases to establish whether spermatozoa could be retrieved from the epididymis prior to subsequent in-vitro fertilization (IVF) and ICSI treatment. Concurrent bilateral open biopsy was performed in two cases.

A 19, or 21, gauge Butterfly needle (Venisystems, Abbott Ltd, Sligo, Republic of Ireland) was passed directly into the testis under general anaesthesia with Propofol (Diprivan) or local anaesthesia using Bupivacaine (Marcaine) injected around the spermatic cord at the superficial inguinal foramen are practical alternatives, although not used in this study. Once the needle was in the testicular substance, strong negative pressure was exerted using a 20 ml attached syringe (B.Braun Melsungen AG, Melsungen, Germany). The microtubing set was then occluded by Spencer Wells forceps to maintain constant suction into the aspiration needle. The needle itself was moved within the substance of the testis until small aliquots of aspirated testicular tissue could be observed to appear within the microtubing set. The needle was then slowly withdrawn from the testis through the scrotal skin and a core of attached testicular tissue was cut off, on withdrawal, from the skin surface. The procedure was undertaken at different
Table 1. Clinical data on 19 patients with oligo- or azoospermia

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of patients</th>
<th>Elevated FSH present</th>
<th>History of trauma</th>
<th>Previous biopsy</th>
<th>Indeterminate FNA cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretory</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Obstructive</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Severe oligospermia</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>&lt;1 \times 10^6</td>
<td></td>
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</tr>
</tbody>
</table>

FSH = follicle-stimulating hormone.
FNA = fine needle aspiration.

**Histology**

In this instance, Bouin’s fluid was injected through the microtubing set and the contained tissue was concentrated by centrifugation using the Shandon Cytospin 2, and the cytoblock prepared using the Shandon cytoblock method. The specimen was then processed in the conventional way: 3 μm sections were cut and stained with haematoxylin and eosin, Masson trichrome and Ziehl Neelsen stains.

**Results**

**Cytology**

Smears adequate for assessment were obtained in all cases, although three of the 19 were unsuitable because of background blood staining which obscured cellular detail.

Sertoli cells and spermatogenic cells from spermatogonia to mature spermatozoa, were readily identified (Figure 1). An approximate assessment of the number of mature spermatozoa gave reasonable correlation with the differential Johnsen score (Johnsen, 1970) made on the histological aspirates, i.e. when mature spermatozoa were present in the aspirate, the Johnsen score was high and when spermatozoa were not observed, e.g. in Sertoli cell only syndrome, the score was low. However, it was not possible to assess the viability of spermatozoa in these preparations.

Figure 2 shows a scanty smear obtained from a case of severe hypospermatogenesis showing a few spermatogenic precursors and no mature spermatozoa. The smears from a case of Sertoli cell only syndrome were extremely scanty, showing only a few Sertoli cells and no spermatogenic precursors or spermatozoa. This was confirmed by concurrent FNA histology and open biopsy.

In the second case in which concurrent FNA and open biopsy were performed, the smears contained groups of Leydig cells with no spermatogenic cells identified and the biopsies all showed Leydig cell hyperplasia.

**Histology**

In 17 cases, bilateral testicular aspirates were performed and between 20–25 intact tubules were seen in each cytoblock preparation (average 100). In two cases, tissue was obtained from only one testis and assessment was therefore unilateral. The germinal epithelium was well preserved and adequate for precise assessment of all stages of spermatogenesis (Figures 3 and 4). The needle biopsy material easily allowed a differential Johnsen score (Johnsen, 1970) to be calculated.
In some biopsies there was evidence of tubular rupture with spermatogenic cells and streaked nuclear material in the interstitium. These were not included in the overall assessment. Evidence of haemorrhage was present in some specimens but did not appear to obscure cellular detail of the tubules. Thickened tubular basement membranes and hyalinized tubules indicating scarring were readily identified in the Masson trichome preparation.

The appearances of the biopsies were categorized as follows:

normal; mild, moderate or severe hypospermatogenesis, and absent spermatogenesis (Sertoli cell only). The presence of scarring was noted and an approximate percentage of scarred tubules was also made. No case of germinal cell arrest, or in-situ malignancy, was identified in this series.

In one of the two cases in which bilateral concurrent open biopsies were performed, the diagnosis of Sertoli cell only syndrome (absent spermatogenesis) was made on both aspirates (Figure 5) and open biopsy (Figure 6). In the second, the open biopsy showed marked Leydig cell hyperplasia with scanty
tubules containing Sertoli cells only (Figure 7) suggesting using up to three separate punctures may provide a more representative sample than the single piece of tissue obtained by open biopsy. As with FNA procedures at other sites it is a quick and inexpensive method. Follow up of patients who had undergone testicular sperm aspiration (TESA) to identify spermatozoa for fertility treatment using ICSI has not shown long-term post-operative complications (Tsirigotis and Craft, 1995).

The role of histological assessment in the treatment of non-obstructive oligozoospermia and azoospermia has become critical following recent advantages in the management of the subfertile male aimed at promoting biological fatherhood and reducing donor sperm consideration. One can now predict that men having poor quality spermatozoa, even with minimal progressive motility, are as likely to achieve a clinical pregnancy following IVF and ICSI as other infertile couples do with conventional IVF and normal spermatozoa, whether the sample be obtained from the testis, epididymis or ejaculate (Silber et al., 1993; Madgar et al., 1996). More recently it has been shown that, even in the Sertoli cell only syndrome, the defect may not be total and testicular exploration may yield small numbers of spermatozoa in focal areas (Silber, 1995).

Now that almost all categories of oligo- or azoospermia may be potentially treatable, histological assessment may be considered no longer relevant. This is not our view since testicular aspiration biopsy is a simple, reliable, rapid and inexpensive method of differentiating between obstructive and non-obstructive oligo- and azoospermia and in determining the severity of the condition. This could also be concluded from the fact that only three out of the 19 patients studied showed indeterminate results on FNA testicular cytology, but not on aspiration biopsy, where a firm histological diagnosis was made. The three patients with inconclusive cytology showed on the histology aspirates (i) moderate to marked hypospermatogenesis (post-vasectomy), (ii) moderate hypospermatogenesis with scarring (absent vas) and (iii) advanced scarring (secretory azoospermia with small testes and raised FSH values) respectively. Although it was not in the scope of this study to report data on the clinical management of this group of patients, it is worth mentioning that nine out of the 16 patients with conclusive FNA cytology and biopsy, and one patient with indeterminate cytology but conclusive histology, have already undergone an equal number of ICSI cycles with successful epididymal (#3) and testicular (#7) sperm retrieval.

Since the completion of this series of patients in which both aspiration cytology and histology were carried out, eight more patients have undergone FNA testicular aspiration and a firm histological diagnosis was made, in all cases from the testicular aspirates. However, further work (i.e. a larger series) is needed before conclusions about the reliability of the technique, or whether FNA aspiration histology could replace conventional open biopsy, can be reached. On present evidence, it can be stated that FNA testicular cytology/biopsy can be a useful initial screening for the presence of mature spermatozoa and subsequent suitability for ICSI.

It is, therefore, our opinion that testicular aspiration ensures...
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that couples experiencing infertility due to a profound male problem can embark on IVF and ICSI treatment with some confidence provided that spermatozoa are identified in the aspirate. The technique is equally of value as a diagnostic method for determining germ cell in-situ malignancy, Leydig cell hyperplasia, and the Sertoli cell only syndrome.

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References


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