CASE REPORT

Successful treatment with ICSI of infertility caused by azoospermia associated with adrenal rests in the testes

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Congenital adrenal hyperplasia (CAH) is a well-recognized, but uncommon, cause of azoospermia and infertility in men. Commonly this is due to undertreatment of excessive adrenal androgen secretion which suppresses gonadotrophin stimulation of the testes. A less common complication of CAH is development of adrenal tissue within the testes; this is important to recognize because it may be confused with malignancy leading to unnecessary surgery. In this case report, a man is described with simple virilizing CAH due to 21-hydroxylase deficiency who presented with azoospermia and was found to have adrenal rests. Investigations concluded that there was adequate adrenal suppression with glucocorticoids and that azoospermia was due to obstruction by adrenal rest tissue, strategically situated at the hilum of the testes. Spermatozoa were able to be retrieved by testicular aspiration from the man and these were used to successfully establish a pregnancy using intracytoplasmic sperm injection of his wife’s oocytes.

Key words: adrenal rests/azoospermia/congenital adrenal hyperplasia/intracytoplasmic sperm injection

Introduction

Congenital adrenal hyperplasia (CAH) results from a genetic mutation which produces a deficiency of one of the steroidogenic enzymes in the adrenal glands. The commonest abnormality is 21-hydroxylase deficiency occurring in between 1 in 10 000 and 1 in 20 000 births (Pang and Clark, 1993; Thil’ en et al., 1998). This results in reduced cortisol synthesis, increased adrenocorticotrophic hormone (ACTH) secretion and excessive adrenal androgen production. In a survey of women with this condition, only 20% achieved a pregnancy (Mulaikal et al., 1987); this was attributed to a number of factors including reduced heterosexual activity, inadequate vaginal reconstruction and sub-optimal hormone replacement. Infertility in men with CAH is less common, though azoospermia resulting from gonadotrophin suppression by excessive adrenal androgens is well described (Wischusen et al., 1981; Augarten et al., 1991; Mirsky and Hines, 1989; Valentino et al., 1997) and is important to recognize because it is a reversible form of infertility.

CAH in males may also be associated with bilateral testicular tumours, due to growth of ACTH-dependent adrenal tissue (Cutfield et al., 1983; Cunnah et al., 1989). Clinically, these tumours may present as painful, enlarged, irregular testes which may initially be confused with Leydig cell tumours (Kovacs and Asa, 1998). However, histologically adrenal rests lack Reinke crystalloids, they are bilateral and not autonomous and adequate suppression of ACTH by exogenous glucocorticoids may lead to a dramatic reduction in tumour size (Cutfield et al., 1983; Oberman et al., 1993). In this report, we describe persistence of adrenal tumours in the testes and azoospermia, despite more than adequate glucocorticoid treatment. This was confirmed by estimation of steroid hormones from gonadal vein samples and by testicular biopsy. Since normal spermatogenesis could be demonstrated in some areas of the testes on biopsy, it was concluded that obstruction was the most likely cause of azoospermia. It was possible to retrieve mature spermatozoa from the testes by aspiration and these were used to fertilize his wife’s oocytes to achieve a successful pregnancy.

Case report

A 29 year old man was referred for investigation and management of azoospermia. His wife had been unable to conceive over 12 months despite having regular ovulatory cycles and regular intercourse during the peri-ovulatory period. At the age of 6 months, he was diagnosed with a non-salt losing form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. During childhood and adolescence his management was supervised by paediatric endocrinologists in a specialized clinic, but his control was variable because of poor compliance with medication. By the age of 6 years, his bone age was advanced by three years and it remained so throughout child-
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Table I. Baseline hormone concentrations whilst taking prednisolone

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Results</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-OHP (nmol/l)</td>
<td>8</td>
<td>&lt;6</td>
</tr>
<tr>
<td>ACTH (ng/l)</td>
<td>10.4</td>
<td>10–60</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>11</td>
<td>10–25</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>26</td>
<td>7–40</td>
</tr>
<tr>
<td>DHEA-S (µmol/l)</td>
<td>0.2</td>
<td>2.6–14.0</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>&lt;20</td>
<td>190–690</td>
</tr>
<tr>
<td>Prolactin (mIU/l)</td>
<td>149</td>
<td>30–450</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>7</td>
<td>1–6</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>8</td>
<td>1–10</td>
</tr>
</tbody>
</table>

The reference ranges are those quoted by the kit manufacturers as follows: cortisol, TDX FLX System, Abbott Laboratories, North Ryde, NSW, Australia; ACTH, Immolite analgen, Diagnostic Products Corp., Los Angeles, CA, USA; DHEA-S, DHEA-S-7-RIA, Diagnostic System Lab., Parramatta, NSW, Australia; aldosterone, Coat-a-Count, Diagnostic Products Corp.; 17-OHP, Coat-a-Count, Diagnostic Products Corp. LH, FSH and prolactin are Biodclone IRMA kits, Marrickville, NSW, Australia. We have established a local reference range for males between 20 and 40 years. 17-OHP = 17-hydroxyprogesterone; ACTH = adrenocorticotropic hormone; SHBG = sex hormone binding globulin; DHEA-S = dehydroepiandrosterone sulphate.

hood. Puberty began spontaneously at age 11 years and his growth ceased at 14 years of age. During his adolescent years, his compliance was quite irregular, but following his marriage at the age of 22 years, his medication was supervised by his wife. He had taken prednisolone, 10 mg twice a day, regularly since that time.

On examination, he was mildly obese and Cushingoid. His height was 167 cm (10th centile) and weight was 85 kg. His mid-parental height was 177 cm, though he has a brother who is 185 cm (90th centile). His blood pressure was 130/80 without a postural drop and he had normal secondary sexual characteristics without gynaecomastia. Both testes were abnormal to palpation, being nodular and hard with a volume in excess of 25 ml. The vas deferens and epididymis were palpable on both sides and were normal.

A clinical diagnosis of congenital adrenal rests in the testes was made and this was supported by typical changes on a testicular ultrasound showing bilateral hypoechoic intratesticular masses which were heterogeneous and of increased vascularization (Vanzulli et al., 1992; Avila et al., 1996). These measured 2 cm on the right and 0.5 cm on the left. The remaining testicular tissue showed irregular hyperechoic regions consistent with fibrosis. Initial biochemical investigations were performed whilst this patient was receiving his usual dose of prednisolone and these results confirmed near-complete suppression of the hypothalamic-pituitary-adrenal axis, with normal concentrations of gonadotrophins and serum testosterone (Table I).

Although our patient had a serum concentration of 17-hydroxyprogesterone which was above the normal reference range (<6 nmol/l), the range in untreated patients with congenital adrenal hyperplasia is between 350 and 500 nmol/l (Young et al., 1994). It is generally accepted that suppression of 17-hydroxyprogesterone into the normal range can only be achieved in patients with congenital adrenal hyperplasia by giving supraphysiological doses of glucocorticoids (Lippe et al., 1974; Hughes et al., 1976). This patient’s low serum concentra-

tion of 17-hydroxyprogesterone is consistent with his low serum cortisol, DHEA and ACTH (Table I) and indicate major suppression of the hypothalamic-pituitary-adrenal axis.

In order to confirm the adrenal nature of the testicular masses, venous samples were collected, with the patient’s consent, from the inferior vena cava, the left testicular and the left adrenal veins before and 30 min after i.v. injection of synthetic ACTH (Tetraconsactrin 250 µg; Novartis Pharmaceuticals, North Ryde, NSW, Australia); at the time of study the patient had been without his normal medication for 18 h. Samples were assayed for hormones by established radioimmunoassays (Table II). In addition, immunoreactive inhibin B was assayed using an inhibin B specific enzyme-linked immunosorbent assay (Groome et al., 1996) and also by a radioimmunoassay which measures both inhibin and free α subunit products (Robertson et al., 1989). The results showed low concentrations of serum ACTH, cortisol and dehydroepiandrosterone sulphate (DHEA), even in adrenal vein samples, which is consistent with long-term suppression of endogenous ACTH. The poor response of cortisol to ACTH is in keeping with this and with deficiency of 21-hydroxylase. Plasma aldosterone concentrations were well maintained, consistent with absence of clinical symptoms of salt deficiency and in keeping with normal activity within the zona glomerulosa of 21-hydroxylase. Following ACTH, there was a small increase in adrenal vein testosterone, but its contribution to the overall peripheral concentration, compared to the gonad, was small. Gonadal vein testosterone concentrations were normal, in keeping with non-suppressed gonadotrophin concentrations.

It was clear that the testes contained functioning adrenal tissue since gonadal vein samples showed substantial amounts of cortisol and aldosterone and these concentrations increased further after ACTH stimulation (Table II). It is of interest that this tissue, like the normally placed adrenal gland, also lacked the 21-hydroxylase enzyme because basal and stimulated concentrations of 17-hydroxyprogesterone are comparable to those found in the adrenal vein. This is consistent with direct measurement of 21-hydroxylase activity reported in the in-vitro study of a similar tumour (Clark et al., 1990).

Basal samples from gonadal and peripheral veins showed similar concentrations of inhibin B and ACTH caused no significant change. However, when measured in the assay which includes α subunits, gonadal vein concentrations were higher in the basal state than in peripheral samples and there was a significant increase after ACTH (Table II). A few days after recovery from this investigation, a 5000 IU human chorionionic gonadotrophin (HCG) stimulation test was performed which showed a normal testosterone response, consistent with normal leydig cell function (Table III) (Padron et al., 1980). Inhibin B concentrations in the peripheral vein sample did not change after HCG, but the less specific assay showed a definite increase in inhibin.

Biopsy of the major nodule, with the patient’s informed consent, showed that it was composed of tissue principally resembling adrenocortical cells. However in some areas, these cells were interspersed with hyalinized outlines of seminiferous tubules that lack an epithelium and in other areas with identifiable seminiferous tubules showing a severely reduced
compartment of germ cells. In a biopsy taken away from the site of the nodules, the seminiferous tubules were of normal diameter with all stages of germ cell development present, including plentiful elongated spermatids. The numbers of germ cells varied from being normal to a moderate degree of hypospermatogenesis. Normal Leydig cells were identified in the intertubular tissue.

The man’s glucocorticoid dosage was reduced to prednisolone 10 mg taken at 2200 h and 2.5 mg at 0800 h. After 6 months, his semen analysis remained unchanged. Samples of stored spermatozoa did not thaw successfully, so a needle aspiration of the testes was performed and motile spermatozoa obtained from this sample were used for intracytoplasmic sperm injection into his wife’s oocytes (Van Steirteghem et al., 1993). A successful singleton pregnancy was achieved in the first cycle of treatment and a normal male child with a birthweight of 2640 g and Apgar scores of 9 and 10 was delivered at 33 weeks gestation. The neonatal course was uneventful.

Discussion
Although the finding of enlarged, irregular testes in a man with infertility usually raises concern about malignancy, a history of congenital adrenal hyperplasia is more suggestive of the presence of benign adrenal rests. Palpable testicular nodules have been described in up to 24% of male patients with CAH (Avila et al., 1996) and these are detected even more commonly by the use of magnetic resonance imaging or ultrasound (Avila et al., 1999). In this patient, the adrenal nature of the nodules was demonstrated by the finding of cortisol and aldosterone secretion in the gonadal veins which increased after ACTH stimulation.

Aberrant adrenal tissue has been described in up to 50% of newborn infants but it usually atrophies within a few years (Schechter, 1968). Adrenal rest tumours are a complication of conditions with uncontrolled ACTH secretion such as Addison’s disease (Cohen, 1946), Cushing’s disease (Hamwi et al., 1963), Nelson’s syndrome (Verdonk et al., 1982) and glucocorticoid resistance (Chrousos et al., 1993). They are a well-recognized complication of both the salt-losing and simple virilizing forms of 21-hydroxylase deficiency (Willi et al., 1991), as well as other forms of congenital adrenal hyperplasia (Srikanth et al., 1992).

The origin of these tumours is still debated but it is generally accepted that they are derived from ectopic adrenal cells which migrate with primitive gonadal cells from the urogenital ridge along the path of migration in the kidney, supradiaphragmatic
region, the spermatic cord and the testes (Ventura et al., 1998),
as well as in various parts of the female genital tract. Adrenal
rest cells retain ACTH receptors, and, under continued stimula-
tion by ACTH, they may become adenomatous although this
is usually a late finding. More commonly, adequate suppression
of nocturnal hypersecretion of ACTH by a late evening dose
of synthetic glucocorticoid will cause regression in tumour
size and restoration of spermatogenesis (Cunnah et al., 1989).
In the patient in this report, however, large nodules persisted
in both testes despite compliance with treatment and he
remained azoospermic and infertile, a situation in keeping with
previous reports (Bonaccorsi et al., 1987; Keely et al., 1993).
This is a recognized complication of previous poor control of
CAH and is an indication of adenomatous transformation of
the adrenal rests (Rutgers et al., 1988; Clark et al., 1990;
Blumberg-Tick et al., 1991).

In the patient, the testicular biopsy showed areas of normal
spermatogenesis in parts of the testes distant from the adrenal
rests, although there was a reduction in germ cells and hy-
pospermatogenesis was a feature in areas closer to the hilum.
The degree of normal spermatogenesis, confirmed later by the
ease of obtaining viable spermatozoa by testicular biopsy,
combined with the total absence of spermatozoa from the
ejaculate on a number of occasions, suggested that obstruction
was the most likely cause of azoospermia and infertility in
this man. Given the propensity of adrenal rests to develop
close to the hilum of the testes, it was postulated that this
strategic placement caused obstruction to small effenter ducts
carrying spermatozoa, either as a result of the relatively
inelastic structure of the tunica albuginea or by reduced local
blood supply in this area. An alternative theoretical cause for
azoospermia is that local adrenal steroids or metabolites derived
from the adrenal rests are toxic to Sertoli or germ cells, as has
been proposed to explain spermatogenic abnormalities in
some men with varicoceles (Comhaire and Vermeulen, 1974;
Takihara et al., 1991). Although there was evidence of hypos-
permatogenesis in some areas of the biopsy, this was not
widespread and Sertoli cell function was normal, as judged by
the measurement of peripheral and gonadal vein inhibin B
concentrations.

It is of interest that concentrations of inhibin B did not alter
significantly in either gonadal or adrenal vein samples after
injection of ACTH or HCG. However, there was a significant
increase when the samples were assayed for both dimeric
inhibin and the free α subunit. This is interpreted this to mean
that it is the free α subunit which is increased in these samples
and that it is produced by both Leydig cells and the adrenal
cortex. This conclusion is in keeping with the studies demon-
strating that the adrenal cortex can secrete free α subunits
(Crawford et al., 1987; Nishi et al., 1995) and with the
observation that free α subunit increases after HCG in a
normal man (McLachlan et al., 1988).

Although rare, a history of congenital adrenal hyperplasia
should be sought in all men with azoospermia or with an
incidentally discovered testicular mass and the relevant inves-
tigations should be performed when this possibility is raised.
There is a tendency to concentrate medical attention on children
with CAH, particularly those with salt-losing forms of the
condition. This case, however, emphasizes the need for adoles-
cent patients to be carefully handled during the transition to
adult care. There is clearly a need for continued control of
CAH throughout adult life, even if salt wasting is not a clinical
problem. Finally, in men with CAH whose azoospermia persists
despite adequate glucocorticoid treatment, it might still be
possible to recover testicular spermatozoa, and, as this case
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References
male infertility in late onset congenital adrenal hyperplasia. J. Endocrinol.
Invest., 14, 237–240.
rest tissue in congenital adrenal hyperplasia: findings at Gray-scale and
color Doppler US. Radiology, 198, 99–104.
tissue in congenital adrenal hyperplasia: comparison of MR imaging and
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Azoospermia and adrenal rests


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