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Testicular sampling in unilateral steroidogenesis

Testicular vein sampling can reveal gonadotropin-independent unilateral steroidogenesis supporting spermatogenesis

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Suppressed gonadotropins combined with high-normal serum testosterone concentrations in oligozoospermic men suggest either use of exogenous testosterone or presence of a testosterone-producing tumour. We describe the case of a 31-year-old man referred for primary infertility. Gonadotropins were undetectably low, however with testosterone and estradiol in the high-normal range. Semen analysis showed oligoasthenospermia. He denied using exogenous testosterone. Scrotal ultrasound only showed microlithiasis and millimetric hypolucent lesions in the left testis, but no intratesticular mass. HCG was low. To investigate unilateral hormone secretion, selective testicular venous sampling was performed. Testosterone and estradiol were clearly higher on the left side than on the right (130 vs 26 nmol/L and 1388 vs 62 pmol/L respectively), with a left spermatic vein-to-periphery gradient of 4.3 for testosterone and 13 for estradiol; while there were no similar gradients on the right side. This confirms that all sex steroid secretion came from the left testis. The patient was therefore referred for left orchidectomy. Histopathology only revealed multifocal seminoma, germ cell neoplasia in situ and Leydig cell hyperplasia, but no choriocarcinoma. However, gonadotrophin levels increased after orchidectomy, indicating that the source of gonadotropin-independent sex steroid secretion was removed. Testosterone and estradiol decreased to the mid-normal range. Sperm concentration improved. This report thus shows that endogenous testosterone secretion in one testicle supports spermatogenesis without measurable levels of gonadotropins. Selective testicular venous sampling is useful to identify the site of unilateral secretion when the clinical picture is inconclusive. However, histopathology could not reveal the factor that stimulated Leydig cell steroidogenesis.

**Introduction**

Gonadotropins can be suppressed in men using exogenous testosterone preparations or in the presence of a testosterone-producing testicular tumour. However, both LH and FSH are needed to support quantitatively and qualitatively normal spermatogenesis (1). Here, we describe an unusual case of a man with oligozoospermia, suppressed gonadotropins and high-normal testosterone, a small unilateral seminoma, but no choriocarcinoma or measurable HCG.

**Case description**

A 31-year-old Caucasian male was referred to our tertiary care andrology unit because of primary involuntary infertility during a 1.5 years period with a biochemical picture of high-normal testosterone despite suppressed gonadotropins. He denied using exogenous testosterone. He had a history of bilateral cryptorchidism that was surgically treated at a young age. On clinical examination, he had small testes (approximately 12 ml) with normal consistency and no palpable testicular mass. He had a normal physical appearance and did not have gynaecomastia.
Three semen samples assessed over a two-month period all showed oligoasthenospermia (Table 1). Hormone analysis showed suppressed gonadotropins (LH and FSH both <0.1 U/L), however with total and free testosterone levels in the high normal range (29.5 nmol/L and 412 pmol/L respectively). Estradiol and inhibin B were normal (Table 1). Sex hormone-binding globulin was slightly increased. Prolactin and other pituitary hormones were normal. Alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) were also within the normal range (2.3 µg/L and 1.8 U/L respectively). To exclude a potential technical error with respect to the HCG measurement, the sample was remeasured with three different methods (Roche ECLIA "hCG + beta", Brahms Kryptor free beta hCG, Siemens Immulite hCG). All three methods confirmed that HCG was very low (between 0.6 and 1.8 IU/L).

The observation of undetectable gonadotrophins thus suggested an endogenous source of sex steroid hypersecretion. However, on scrotal ultrasound he only had diffuse microlithiasis and three small millimetric hypolucent testicular lesions in the left testis, but no testicular mass (Image 1). There were no suspicious focal areas nor microlithiasis visible in the right testis.

Since no intratesticular mass could be detected clinically or sonographically, a selective testicular venous sampling was performed to further investigate possible unilateral gonadotropin-independent sex steroid production in the testes. Both testosterone and estradiol levels were higher in the left spermatic vein, with a testis-to-periphery gradient of 4.3 and 13 respectively (Table 2), confirming that all sex steroid secretion came from the left testis. There was no gradient in the right spermatic vein, indicating absent sex steroid secretion in the right testis (Table 2).

Based on these results orchidectomy of the left testis was performed. Histopathology showed a multifocal seminoma, with a diameter of the largest focus of 3 mm and with associated diffuse germ cell neoplasia in situ (GCNIS) in the adjacent seminiferous tubules (Image 2). There was focal spermatogenesis with mature spermatids in a few seminiferous tubules. However, there were no isolated syncytiotrophoblastic cells, nor choriocarcinoma. Discrete Leydig cell hyperplasia was observed, which was confirmed by immunohistochemical staining for inhibin (Image 2D). Despite extensive examination of the whole testis, no Leydig cell tumour could be found. Additional analysis of the spermatic cord could also not reveal an extratesticular Leydig cell tumour. To our surprise, the hormonal profile could thus not be explained by the pathology findings.

Three weeks after orchidectomy, his gonadotrophins increased, indicating recovery of the hypothalamic-pituitary-testis axis, hereby confirming that the source of gonadotropin-independent sex steroid secretion was removed. Testosterone and estradiol decreased to the mid-normal range. Sperm concentration also increased, but asthenospermia remained. Four months postoperatively testosterone and LH were normal, whereas FSH remained slightly elevated. Sperm concentration further increased and motility improved (Table 1).

Seventeen months after orchidectomy, his wife gave birth to a healthy child, conceived via intracytoplasmic sperm injection (ICSI) with a fresh semen sample.

Discussion

We report the case of a man with oligoasthenospermia despite suppressed gonadotropins. His testosterone levels were in the high-normal range due to gonadotropin-independent testosterone secretion in the left testis.

The most obvious explanation of the observed hormonal profile with repeatedly high-normal testosterone concentrations combined with undetectable gonadotropins and impaired semen quality would have been use of testosterone. Notably, this usually results in extreme oligozoospermia or even azoospermia (2). Our patient consistently denied using steroid-containing preparations.
Alternatively, the suppressed gonadotropins could be the consequence of a sex steroid-producing testicular tumour. Autonomous hormone production (most frequently HCG or estradiol) may occur in certain types of testicular tumours, such as choriocarcinomas, seminomas or Leydig cell tumours. The supraphysiological hormonal production often results in clinical manifestations such as gynaecomastia, combined with suppressed gonadotropins and high testosterone levels. However, our patient did not have a palpable testicular mass nor evidence of a testicular tumour on ultrasound. Furthermore, both HCG and estradiol were in the normal range. As technical errors with HCG measurements have been reported (3), it was remeasured with three different methods, all showing consistently low values.

However, not only gonadotropins regulate sex steroid production by the Leydig cells, but also paracrine factors can stimulate Leydig cell steroidogenesis (4). An alternative hypothesis could thus be that enhanced paracrine stimulation resulted in the observed Leydig cell hyperplasia and increased testosterone production. Additionally, it has been shown that aromatase can be expressed in seminoma cells, inducing local conversion of testosterone to estradiol (5). The T/E2 ratio was much lower in the left spermatic vein than in the right (99 vs 434), and is lower than values reported in literature (6,7). The testosterone concentration measured in the left spermatic vein was also lower than expected (6–8). These observations indicate that estradiol secretion in the left testis was increased and this could induce suppression of gonadotropin secretion.

Recently, a case report of a patient with azoospermia, who also had normal testosterone and suppressed gonadotropins was reported. This patient however had high androstenedione levels, and pathology showed a Leydig cell tumour (9). Another case report described a patient with a malignant Leydig cell tumour who presented with normal testosterone together with low LH and FSH. After orchidectomy testosterone decreased and gonadotropins increased, suggesting suppression of the gonadal axis by autonomous hormone production in the tumour despite normal testosterone. No estradiol or androstenedione levels nor semen parameters were however reported in this case (10).

It is generally accepted that both LH and FSH are needed to support quantitatively and qualitatively normal spermatogenesis. LH is believed to be the dominant gonadotrophin in humans, as LH stimulated testosterone production is the key factor in spermatogenesis (11). However, this dogma has been questioned recently, as data from murine models suggest that strong FSH stimulation can maintain spermatogenesis in mice treated with anti-androgens (1). Furthermore, spermatogenesis is preserved in FSH receptor knockout mice and in men with inactivating mutations in the FSH receptor (1). In contrast, men with an inactivating mutation in FSH-B all have azoospermia, whereas spermatogenesis is normal in FSH-B knockout mice. This suggests that at least in mice, spermatogenesis can occur without FSH stimulation. The findings from our patient indicate that also in humans, spermatogenesis is possible without FSH stimulation as long as the intratesticular testosterone levels remain normal.

Due to the unusual hormonal profile and the absence of a testicular mass on ultrasound, an exceptional diagnostic procedure was needed to solve this intriguing case. Selective testicular venous sampling was crucial for both diagnosis and decision to perform orchidectomy. It has mainly been used for research purposes, but there are case reports indicating its relevance for the diagnosis of patients with unusual hormonal profiles (12). In our case, this procedure confirmed gonadotropin-independent sex-steroid secretion in the left testis, which was responsible for the suppressed gonadotropins.

It is remarkable that the pathological images in our patient only showed a multifocal seminoma with GCNIS, as well as discrete Leydig cell hyperplasia, without Leydig cell tumour or choriocarcinoma. Although seminomas can produce HCG, leading to gonadotropin suppression and high testosterone levels, HCG was always in the normal range in our patient.
The pathology findings could thus not reveal the factor that stimulated gonadotropin-independent Leydig cell steroidogenesis. However, the rise in gonadotropin levels above the upper limit of normal and the decrease in sex steroids after orchidectomy confirm the presence of supraphysiological hormone secretion. Sperm concentration increased in the months after orchidectomy as well.

**Conclusion and novel insights for clinical practice**

Endogenous gonadotropin-independent testosterone secretion in one testis may support spermatogenesis even without gonadotropins. It is mandatory to measure both LH and FSH in men with oligozoospermia. In patients with suppressed gonadotropins, normal sex steroid levels and no testicular mass, selective testicular venous sampling can be crucial in identifying the site of hormonal overproduction. Finally, it is remarkable that histopathology could not reveal the focus of gonadotropin-independent sex steroid secretion.

**Consent**

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

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**Disclosure summary:**

None of the co-authors has a conflict of interest that is relevant to the subject matter or materials included in this Work.

**Data Availability**

Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

**References**


**Image 1:** Ultrasound of the left testis showing diffuse microlithiasis and three millimetric hypolucent lesions in the left testis.

**Image 2:** Histology. A: seminoma (H&E staining, 5x). B: seminoma (Immunostaining for PLAP, 5x). C: germ cell neoplasia in situ (GCNIS) (Immunostaining for PLAP, 5x). D: Leydig cell hyperplasia (Immunostaining for inhibin, 5x)

**Table 1:** Semen and hormonal parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>At time of diagnosis</th>
<th>4 weeks after orchidectomy</th>
<th>4 months after orchidectomy</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Semen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>3.7</td>
<td>3.0</td>
<td>2.5</td>
<td>≥1.5 mL</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>2.4</td>
<td>7.1</td>
<td>4.1</td>
<td>≥15 million/mL</td>
</tr>
<tr>
<td>% progressive</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>≥32%</td>
</tr>
<tr>
<td>% non-progressive</td>
<td>4</td>
<td>11</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>% immotile</td>
<td>95</td>
<td>85</td>
<td>92</td>
<td>75</td>
</tr>
<tr>
<td>% normal morphology</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>&gt;4%</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>≤0.1</td>
<td>24</td>
<td>7.8</td>
<td>1.7-8.6 U/L</td>
</tr>
<tr>
<td>FSH</td>
<td>≤0.1</td>
<td>10</td>
<td>9.8</td>
<td>1.2-7.7 U/L</td>
</tr>
<tr>
<td>Prolactin</td>
<td>17.7</td>
<td></td>
<td></td>
<td>2.0-18.0 µg/L</td>
</tr>
<tr>
<td>Testosterone</td>
<td>29.5 (851)</td>
<td>17.8 (512)</td>
<td>19.1 (551)</td>
<td>10.4-34.7 nmol/L (300-1000 ng/dL)</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>412 (12)</td>
<td>281 (8)</td>
<td>232 (7)</td>
<td>174-694 pmol/L (5-20 ng/dL)</td>
</tr>
<tr>
<td>SHBG</td>
<td>66</td>
<td>51</td>
<td>73</td>
<td>24-55 nmol/L</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>3.7 (106)</td>
<td></td>
<td></td>
<td>1.4-5.2 nmol/L (40-150 ng/dL)</td>
</tr>
<tr>
<td>DHEAS</td>
<td>383</td>
<td>160-449 µg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol</td>
<td>142 (39)</td>
<td>64 (17)</td>
<td>59 (16)</td>
<td>37-147 pmol/L (10-40 ng/L)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.5</td>
<td></td>
<td></td>
<td>≤0.1 µg/L</td>
</tr>
<tr>
<td>Inhibin B</td>
<td>185</td>
<td>186</td>
<td>172</td>
<td>105-439 ng/L</td>
</tr>
<tr>
<td>HCG</td>
<td>1.8</td>
<td>0.6</td>
<td>0.6</td>
<td>≤2 IU/L</td>
</tr>
<tr>
<td>Alpha fetoprotein</td>
<td>2.3</td>
<td>2.4</td>
<td>2.6</td>
<td>≤18.6 µg/L</td>
</tr>
</tbody>
</table>

Table 2: Results of the selective testicular venous sampling

<table>
<thead>
<tr>
<th></th>
<th>Left VS</th>
<th>Left VS-P gradient</th>
<th>Right VS</th>
<th>Right VS-P gradient</th>
<th>Left VI</th>
<th>Right VI</th>
<th>VCI</th>
<th>Peripheral</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T (nmol/L)</strong> (ng/dL)</td>
<td>130 (3744)</td>
<td>4.3</td>
<td>26 (739)</td>
<td>0.9</td>
<td>26 (752)</td>
<td>29 (834)</td>
<td>34 (968)</td>
<td>30 (859)</td>
</tr>
<tr>
<td><strong>Free T (pmol/L)</strong> (ng/dL)</td>
<td>2925 (84.3)</td>
<td>330 (9.5)</td>
<td>33 (9.6)</td>
<td>375 (10.8)</td>
<td>458 (13.2)</td>
<td>326 (9.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SHBG (nmol/L)</strong></td>
<td>82</td>
<td>72</td>
<td>73</td>
<td>74</td>
<td>73</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Estradiol (pmol/L)</strong> (ng/L)</td>
<td>1388 (378)*</td>
<td>13</td>
<td>62 (17)*</td>
<td>0.6</td>
<td>84 (23)*</td>
<td>77 (21)*</td>
<td>95 (26)*</td>
<td>106 (29)*</td>
</tr>
<tr>
<td><strong>HCG (U/L)</strong></td>
<td>1.1</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VS: spermatic vein. VI: iliac vein. VCI: inferior vena cava
T: testosterone, SHBG: sex hormone binding globulin, HCG: human chorionic gonadotrophin
*a Measured by immunoassay, b Measured by liquid chromatography-tandem mass spectrometry