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 tumor. We describe the case of a 31-year-old man referred for primary infertility. Gonadotropins were undetectably low, but testosterone and estradiol were in the high-normal range. Semen analysis showed oligoasthenospermia. He denied using exogenous testosterone. Scrotal ultrasound showed microlithiasis and millimetric hypolucent lesions in the left testis but no intratesticular mass. Human chorionic gonadotropin 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; there were no similar gradients on the right side. This finding confirms that all sex steroid secretion came from the left testis. The patient was therefore referred for left orchidectomy. Histopathology 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.

Abbreviations: GCNIS, germ cell neoplasia *in situ*; HCG, human chorionic gonadotropin.

Gonadotropins can be suppressed in men by the use of exogenous testosterone preparations or in the presence of a testosterone-producing testicular tumor. 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, high-normal testosterone, and a small unilateral seminoma but no choriocarcinoma or measurable human chorionic gonadotropin (HCG).

1. Case Description

A 31-year-old Caucasian man was referred to our tertiary care andrology unit because of primary involuntary infertility during a 1.5-year 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 gynecomastia.

Three semen samples assessed over a 2-month period all showed oligoasthenospermia (Table 1). Hormone analysis showed suppressed gonadotropins (LH and FSH both <0.1 U/L), but total and free testosterone levels were in the high normal range (29.5 nmol/L and 412 pmol/L, respectively). Estradiol and inhibin B were normal (Table 1). SHBG was slightly elevated. Prolactin and other pituitary hormones were normal. α-Fetoprotein and HCG were also within the normal range (2.3 µg/L and 1.8 U/L, respectively). To exclude a potential technical error

Table 1. Semen and Hormonal Parameters

	At Time of Diagnosis			4 Wk After Orchidectomy	4 Mo After Orchidectomy	Reference Range
	Sample 1	Sample 2	Sample 3			
Semen						
Volume, mL	3.7	3.0	2.5	3.3		≥1.5
Sperm concentration, million cells/mL	2.4	7.1	4.1	10	27	≥15
% Progressive	1	4	2	0	3	≥32
% Nonprogressive	4	11	6	1	22	
% Immotile	95	85	92	99	75	
% Normal morphology	0	NA	NA	0	NA	>4
Hormones						
LH, U/L	<0.1			24	7.8	1.7–8.6
FSH, U/L	<0.1			10	9.8	1.2–7.7
Prolactin, µg/L	17.7					2.0–18.0
Testosterone, nmol/L (ng/dL)	29.5 (851)			17.8 (512)	19.1 (551)	10.4–34.7 (300–1000)
Free testosterone, pmol/L (ng/dL)	412 (12)			281 (8)	232 (7)	174–694 (5–20)
SHBG, nmol/L	66			51	73	24–55
Androstenedione,[a] nmol/L (ng/dL)	3.7 (106)					1.4–5.2 (40–150)
Dehydroepiandrosterone sulfate, µg/dL	383					160–449
Estradiol,[a] pmol/L (ng/L)	142 (39)			64 (17)	59 (16)	37–147 (10–40)
Progesterone, µg/L	0.5					≤0.1
Inhibin B, ng/L	185			186	172	105–439
HCG, IU/L	1.8			0.6	0.6	≤2
α-Fetoprotein, µg/L	2.3			2.4	2.6	≤13.6

Abbreviation: NA, not available.
[a]Measured by liquid chromatography–tandem mass spectrometry.

with respect to the HCG measurement, the sample was remeasured with three different methods (Roche ECLIA "hCG + beta," Brahms Kryptor free beta hCG, and 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 had diffuse microlithiasis and three small millimetric hypolucent testicular lesions in the left testis but no testicular mass (Fig. 1). No suspicious focal areas or microlithiasis was visible in the right testis.

Because no intratesticular mass could be detected clinically or sonographically, 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 testis-to-periphery gradients 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 profuse germ cell neoplasia *in situ* (GCNIS) in the adjacent seminiferous tubules (Fig. 2). There was focal spermatogenesis with mature spermatids in a few seminiferous tubules. However, there were no isolated syncytiotrophoblastic cells or choriocarcinoma. Discrete Leydig cell hyperplasia was observed, which was confirmed by immunohistochemical staining for inhibin (Fig. 2D). Despite extensive examination of the whole testis, no Leydig cell tumor could be found. Additional analysis of the spermatic cord also did not reveal an extratesticular Leydig cell tumor. 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 and thereby 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 with a fresh semen sample.

2. Discussion

We report the case of a man with oligoasthenospermia despite suppressed gonadotropins. His testosterone levels were in the high-normal range because of gonadotropin-independent testosterone secretion in the left testis.
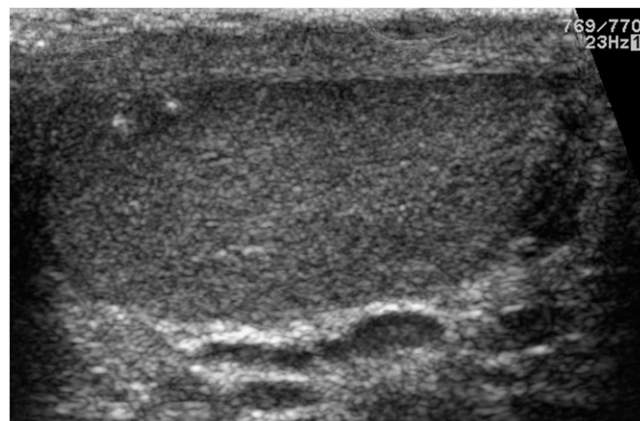
Figure 1. Ultrasound of the left testis showing diffuse microlithiasis and three millimetric hypolucent lesions in the left testis.

Table 2.   Results of Selective Testicular Venous Sampling

	Left VS	Left VS-P Gradient	Right VS	Right VS-P Gradient	Left VI	Right VI	Inferior Vena Cava	Peripheral
Total T, nmol/L (ng/dL)	130 (3744)	4.3	26 (739)	0.9	26 (752)	29 (834)	34 (968)	30 (859)
Free T, pmol/L (ng/dL)	2925 (84.3)		330 (9.5)		33 (9.6)	375 (10.8)	458 (13.2)	326 (9.4)
SHBG, nmol/L	82		72		73	74	73	90
Estradiol, pmol/L (ng/L)	1388 (378)[a]	13	62 (17)[b]	0.6	84 (23)[b]	77 (21)[b]	95 (26)[b]	106 (29)[b]
HCG, U/L	1.1		0.8		0.9	0.9	0.9	0.9

Abbreviations: P, periphery; T, testosterone; VI, iliac vein; VS, spermatic vein.
[a]Measured by immunoassay.
[b]Measured by liquid chromatography-tandem mass spectrometry.

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 tumor. Autonomous hormone production (usually HCG or estradiol) may occur in certain types of testicular tumors, such as choriocarcinomas, seminomas, or Leydig cell tumors. The supraphysiological hormonal production often results in clinical manifestations such as gynecomastia, combined with suppressed gonadotropins and high testosterone levels. However, our patient did not have a palpable testicular mass or evidence of a testicular tumor on ultrasound. Furthermore, both HCG and estradiol were in the normal range. Because technical errors with HCG measurements have been reported [3], it was remeasured with three different methods, all showing consistently low values.

However, not only do 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 increased paracrine stimulation resulted in the observed Leydig cell hyperplasia and elevated testosterone production. Additionally, it has been shown that aromatase can be expressed in seminoma cells, inducing local conversion of testosterone to estradiol [5]. The testosterone/estradiol 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 elevated, which 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. However, this patient had high androstenedione levels, and pathology showed a Leydig cell tumor [9]. Another case report described a patient with a malignant Leydig cell tumor 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 tumor despite normal testosterone. No estradiol or androstenedione levels or semen parameters were reported in this case, however [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, because LH-stimulated testosterone production is the key factor in spermatogenesis [11]. However, this dogma has been questioned recently, because data from murine models suggest that strong FSH stimulation can maintain spermatogenesis in mice treated with
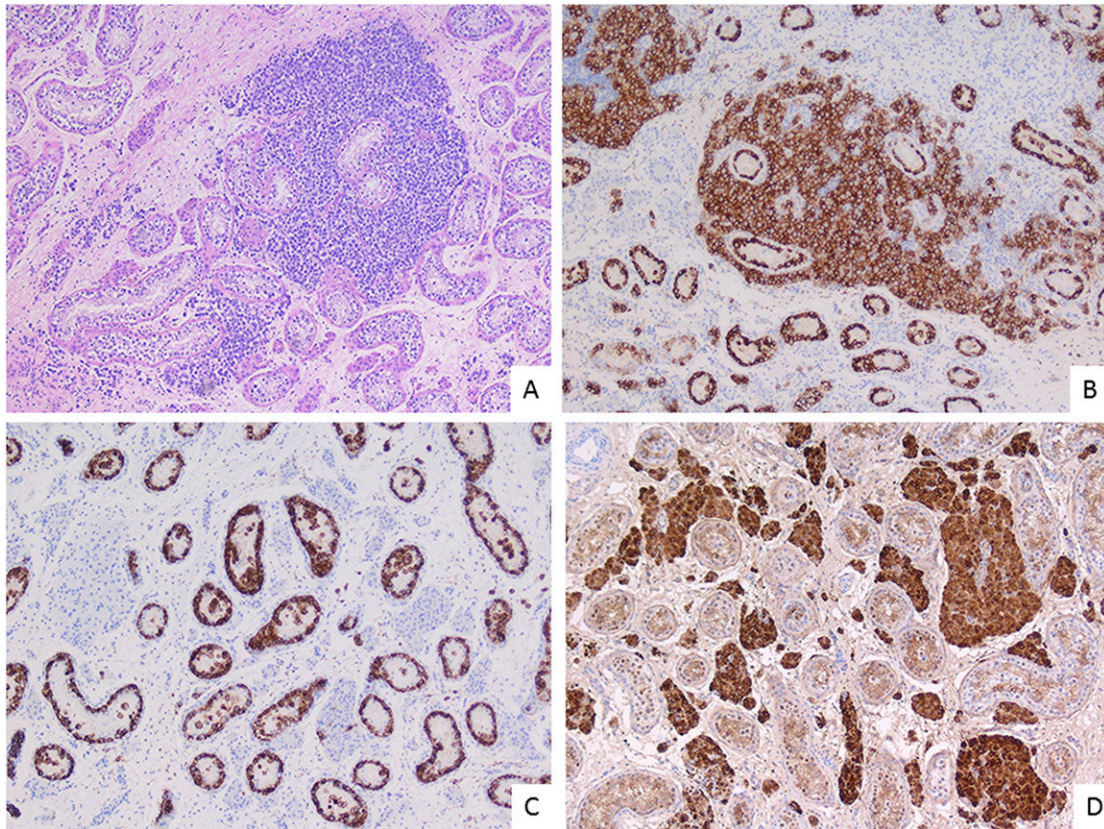
Figure 2. Histology. (A) Seminoma (hematoxylin and eosin staining, ×5). (B) Seminoma (immunostaining for placental alkaline phosphatase, ×5). (C) GCNIS (immunostaining for placental alkaline phosphatase, ×5). (D) Leydig cell hyperplasia (immunostaining for inhibin, ×5).

antiandrogens [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 in humans, spermatogenesis is possible without FSH stimulation as long as the intratesticular testosterone levels remain normal.

Because of 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 the decision to perform orchidectomy. It has been used mainly for research purposes, but there are case reports indicating its relevance for the diagnosis of conditions leading to 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 of our patient showed only a multifocal seminoma with GCNIS, and discrete Leydig cell hyperplasia, without Leydig cell tumor or choriocarcinoma. Although seminomas can produce HCG, leading to gonadotropin suppression and high testosterone levels, HCG was always in the normal range for our patient. The pathology findings thus could 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.

3. Conclusion and Insights for Clinical Practice

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

Acknowledgments

Additional Information

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