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RESEARCH

Semen quality in patients with pituitary disease and adult-onset hypogonadotropic hypogonadism

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Abstract

Objective: Gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)) are released from the pituitary gland and stimulate Leydig cells to produce testosterone and initiates spermatogenesis. Little is known about how and when the deterioration of semen quality occurs in patients with adult-onset gonadotropin insufficiency.

Design and methods: A retrospective study comprising 20 testosterone-deficient men (median age, 29 years) with acquired pituitary disease who delivered semen for cryopreservation before initiation of testosterone therapy. Semen variables and hormone concentrations were compared to those of young healthy men ($n = 340$).

Results: Thirteen of 20 patients (65%) and 82% of controls had total sperm counts above 39 million and progressive motile spermatozoa above 32% ($P = 0.05$). For the individual semen variables, there were no significant differences in semen volume (median (intraquartile range) 3.0 (1.3–6.8) vs 3.2 (2.3–4.3) mL, $P = 0.47$), sperm concentration 41 (11–71) vs 43 (22–73) mill/mL ($P = 0.56$) or total sperm counts ($P = 0.66$). One patient had azoospermia. Patients vs controls had lower serum testosterone 5.4 (2.2–7.6) vs 19.7 (15.5–24.5) nmol/L ($P = 0.001$), calculated free testosterone (cfT) 145 (56–183) vs 464 (359–574) pmol/L ($P < 0.001$), LH 1.5 (1.1–2.1) vs 3.1 (2.3–4.0) U/L ($P = 0.002$) and inhibin b ($P < 0.001$). Levels of FSH were similar ($P = 0.63$). Testosterone/LH ratio and cfT/LH ratio were reduced in patients (both $P < 0.001$).

Conclusions: Despite Leydig cell insufficiency in patients with acquired pituitary insufficiency, the majority presented with normal semen quality based on the determination of the number of progressively motile spermatozoa. In addition, the data suggest reduced LH bioactivity in patients with pituitary insufficiency.

Key Words

- ▶ semen quality
- ▶ bioactivity of gonadotropins
- ▶ hypogonadotropic hypogonadism
- ▶ male reproduction

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Introduction

The gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are released from the gonadotropic cells in the anterior pituitary gland. In men, the main target cells for LH are Leydig cells with subsequent testosterone secretion. FSH primarily stimulates Sertoli cells adjacent to germ cells within the seminiferous

tubules. Intratesticular testosterone stimulated by LH, and stimulation of Sertoli cells by FSH seems both important for induction of spermatogenesis in humans, although some controversy exists (1, 2). Through negative feedback, testosterone is the predominant regulator of LH, whereas inhibin b is the predominant regulator of

FSH (2). Congenital hypogonadotropic hypogonadism (CHH) is associated with an infantile phenotype with lack of testicular growth and absent testosterone and sperm production. In contrast to CHH, normal testicular volume can be observed in patients with acquired adult-onset hypogonadotropic hypogonadism (3, 4).

The vast majority of patients with adult-onset pituitary insufficiency, independent of reason, have partial insufficiency with detectable but inappropriately low levels of pituitary hormones including gonadotropins (5), and the condition will usually result in testosterone deficiency at some point (6, 7). However, little is known about how and when deterioration of semen quality occurs in these patients. Previous studies have primarily examined semen quality in prolactinoma patients with reversible hypogonadism, and the effect of dopamine agonist treatment (8, 9, 10, 11, 12). There are *in vitro*, as well as clinical data, suggesting that prolactin *per se* affects male reproductive tissue in addition to the well-known suppressive effect on release of gonadotropins (13, 14, 15). Therefore, results obtained in patients with hyperprolactinemia may not be extrapolated to patients with hypogonadotropic hypogonadism due to other diseases.

In the present study, we examined semen quality in a group of adult men with documented pituitary disease, at the time when secondary testosterone deficiency requiring testosterone treatment became apparent. The aim was to evaluate, if the two components of the gonadal axis, spermatogenesis and steroidogenesis, was influenced differently by pituitary insufficiency.

Subjects and methods

Participants

It was a single-center retrospective study comprising patients from Department of Endocrinology, Rigshospitalet University of Copenhagen. Patients with pituitary diseases were offered cryopreservation of semen for future fertility treatment before testosterone therapy was initiated, or in selected younger cases before pituitary surgery or radiation therapy. All patients who started testosterone therapy between 2002 and 2016 due to pituitary diseases were evaluated ($n=409$). Forty-seven patients (11.5%) desired cryopreservation of semen. A diagnosis of hypogonadotropic hypogonadism was based on at least two measurements of low testosterone (non-fasting) with inappropriately low levels of gonadotropins

and symptoms of testosterone deficiency. This approach is in agreement with current guidelines and reflects that there is no consensus on strict criteria to make a diagnosis of hypogonadotropic hypogonadism (16). Exclusion criteria were previous or ongoing treatment with testosterone or gonadotropins, onset of testosterone deficiency before puberty and testicular disease including cryptorchidism. Twenty patients were included in the study and 27 were excluded due to: Treatment with gonadotropins ($n=5$), treatment with testosterone ($n=11$), normal plasma testosterone on the day of cryopreservation ($n=3$), childhood-onset hypogonadotropic hypogonadism ($n=3$), bilateral cryptorchidism ($n=2$), unable to ejaculate ($n=2$) and lack of clinical data ($n=1$).

Four of the included patients had fathered children (2–8 years from conception to cryopreservation of semen).

Data on patient diagnoses are given in Table 1.

The control subjects were part of an ongoing cross-sectional study of young Danish men who were included in collaboration with the Danish military health board (17). In Denmark all young men, except those with chronic severe diseases, are required to attend a compulsory medical examination before they may be considered for military service. The overall study population was recruited from 1996 to 2010 and consisted of 4867 individuals (average participation rate 24%) (17). The present study consisted of all participants from the year 2005 without a history of pituitary or testicular disease including cryptorchidism ($n=340$).

Levels of reproductive hormones in patients were also compared to age-specific reference ranges.

Physical examination

On the day the first semen sample was delivered to the semen laboratory, patients and controls underwent a physical examination at the Department of Growth and Reproduction, including evaluation of gynecomastia and assessment of testicular size by a wooden orchidometer (Pharmacia and Upjohn, Denmark) and by ultrasonographic evaluation. Furthermore, blood samples were collected for measurement of reproductive hormones.

Semen samples

All semen samples were analyzed at Department of Growth and Reproduction at Rigshospitalet. Patients and controls had been instructed to abstain from ejaculation

Table 1 Patients characteristics.

	Numbers
Primary diagnosis	
Acromegaly	8
Prolactinoma	3
Mixed GH and prolactin secreting adenoma	2
Empty sella	2
Non-secreting adenoma	2
Craniopharyngioma	1
Medulla blastoma	1
Hypophysitis	1
Hormone substitution	
Thyroxine	5
Hydrocortisone	5
Growth hormone	1
Desmopressin	1
Other treatments	
Somatostatin analogs	2
Dopamine agonists	5
Radiation therapy before cryopreservation of semen	2
Pituitary surgery before cryopreservation of semen	7
Time since initial diagnosis of pituitary disease, months	5 (range 1–108)

Diagnoses and substitution therapy in the 20 patients with secondary testosterone deficiency.

for at least 48 h before attendance at the clinic. The semen samples were obtained by masturbation into a clean tube at a room adjacent to the laboratory. Samples were maintained at 37°C during liquefaction analysis. Semen analyses were performed based on the WHO guidelines, 1992, with modifications as previously described (17). Semen volume was estimated through weighing of the collecting tubes. For the assessment of sperm motility phase contrast microscopy ($\times 400$ magnification) was done on fresh semen samples. Samples were classified as either progressive motile (WHO motility classes A+B), non-progressive motile (WHO C) or immotile (WHO D). Sperm concentration was assessed using a haemocytometer (Bürker-Türk) on semen samples diluted in a solution of NaHCO_3 , formaldehyde and distilled water. Total sperm count was calculated as semen volume \times sperm concentration, and total number of progressively motile spermatozoa as total sperm count \times percentage of progressively motile spermatozoa. Normal values for semen quality was based on the WHO 2010 guidelines (sperm counts above 39 million and progressive motile spermatozoa above 32%) (18).

In controls, semen characteristics were based on a single semen sample, whereas in the patients, it was

based on the average of available samples (median 2 (range 1–5)).

Blood samples

Blood samples were drawn from an antecubital vein in the non-fasting state. Measurements of all reproductive hormone levels in patients and controls as well as the age-specific reference ranges were done at the same laboratory at Department of Growth and Reproduction.

Serum levels of FSH, LH, sex hormone-binding globulin (SHBG), testosterone and estradiol were measured by time-resolved immunofluorometric assays (Delfia, PerkinElmer). Intra-assay and interassay CVs for FSH and LH were 3 and 5%. Intra-assay and interassay CVs for both SHBG and testosterone were <8 and $<5\%$. For estradiol intra-assay and interassay CVs were <4 and 8%. Serum inhibin b was measured by a specific two-sided enzyme immunometric assay (Serotec, UK), intra-assay and interassay CVs 15 and 18%. Free testosterone (cFT) was calculated assuming a fixed albumin value of 43.0 g/L using the calculation suggested by Vermeulen *et al.* (19).

Statistics

Descriptive statistics were used to summarize the subject characteristics. Results are presented as median and interquartile range. Continuous variables were compared by *T*-test, whereas categorical data were compared by χ^2 -test. Associations between variables were performed by regression analyses, examined for interaction between explanatory variables. To approximate normal distribution of the residuals reproductive hormone concentrations and percentage of motile spermatozoa were natural logarithm (ln) transformed, and other semen variables were cubic root transformed.

The relationship in patients between total-testosterone and cFT vs the corresponding LH and between inhibin b vs the corresponding FSH were compared to controls from the general population using two-dimensional bivariate reference charts (20). The bivariate reference curves (97.5 percentile) separate individuals with an abnormal LH-testosterone (or LH-cFT or FSH-inhibin b) combination from individuals with a normal combination.

The statistical analyses were performed by SPSS, version 22. Two-sided *P* values <0.05 were considered significant.

Ethics

The study was approved by The Danish Data Protection Agency (no:2012-58-0004) and conducted in accordance with the Helsinki Declaration. It was a retrospective study not requiring informed consent.

Results

Characteristics of the 20 patients are provided in Tables 1 and 2. The 20 included patients were significantly younger than the patients who did not desire cryopreservation of semen ($n=362$), (29 (25–37) vs 53 (38–65) years, $P<0.001$). Seven of the included patients had pituitary surgery before cryopreservation of semen. Patients with a history of acromegaly ($n=8$), prolactinomas ($n=3$)

and mixed growth hormone and prolactin secreting adenomas ($n=2$) comprised the largest groups of patients (Table 1). Five were treated with dopamine agonist and two with somatostatin analogs (Table 1). Five patients had slightly elevated s-prolactin (less than 5-times upper normal range). Among the ten patients diagnosed with acromegaly eight had elevated serum insulin-like growth factor 1 (IGF-I) concentrations (z-score between 4 and 12). Details on other hormone replacement therapies are given in Table 1.

The majority of the included patients started testosterone therapy soon after cryopreservation of semen. In three patients, testosterone treatment was postponed to preserve fertility.

Compared to healthy controls, patients were older and presented more frequently with gynecomastia (Table 2). Serum estradiol was lower in the patients, whereas

Table 2 Hormone levels and semen quality.

	Patients ($n=20$)	Controls ($n=340$)	P value	Z scores (patients)
Age	29 (25–37)	19±1	<0.001	
BMI (patients, $n=9$)	26 (21–30)	22 (20–24)	0.14	
Abstinence (days)	4 (3–7)	2 (2–3)	0.11	
Average testicular volume (orc) (mL)	20 (17–25)	20 (19–25)	0.61	
Average testicular volume (US) (mL)	14 (10–18)	14 (11–17)	0.62	
Gynecomastia (patients, $n=17$), numbers	4/17	5/340	<0.001	
Biochemical markers				
Total testosterone (nmol/L)	5.4 (2.2–7.6)	19.7 (15.5–24.5)	<0.001	–2.5 (–3.4 to –1.8)
SHBG (nmol/L)	17 (10–34)	26 (20–34)	0.03	–1.9 (–2.2 to 0)
Calculated free testosterone (cFT) (pmol/L)	145 (56–183)	464 (359–574)	<0.001	–2.0 (–2.9 to –1.6)
LH (U/L)	1.5 (1.1–2.1)	3.1 (2.3–4.0)	0.002	–1.8 (–2.0 to –1.2)
FSH (U/L)	2.4 (1.4–4.5)	2.2 (1.5–3.3)	0.63	–1.4 (–2.0 to 0.2)
Estradiol (pmol/L)	40 (20–70)	60 (50–80)	0.001	–1.9 (–2.2 to 0)
Inhibin B (ng/L)	142 (129–184)	227 (176–285)	<0.001	–0.2 (–0.5 to 0.1)
Estradiol/total testosterone ratio	10.1 (5.0–15.5)	3.3 (2.7–4.1)	0.013	
Total testosterone/LH ratio	3.2 (1.5–4.8)	6.5 (4.6–8.7)	<0.001	
Calculated free testosterone/LH ratio	77 (37–118)	152 (111–206)	<0.001	
Inhibin b/FSH ratio	63 (35–116)	105 (57–171)	0.01	
IGF-I (µg/L)	628 (253–1161)	279 (233–328)	0.03	4.5 (0.2–10)
Prolactin (U/L)	250 (128–478)			
Semen characteristics				
Semen volume (mL)	3.0 (1.3–6.8)	3.2 (2.3–4.3)	0.47	
Sperm concentration (mill./mL)	41 (11–71)	43 (22–73)	0.56	
Total sperm counts (mill.)	153 (15–288)	136 (65–235)	0.66	
Progressive motile (%)	49 (13–63)	57 (47–67)	0.04	
Progressive motile (mill.)	77 (17–177)	82 (34–140)	0.51	
Non-progressive motile (%)	7 (5–11)	5 (4–7)	0.13	
Non-progressive motile (mill.)	11 (3–22)	6 (3–12)	0.44	
Immotile (%)	41 (32–67)	37 (27–46)	0.14	
Immotile (mil)	56 (19–109)	45 (21–79)	0.8	
Normal semen quality, WHO criteria (%*)	65	82	0.05	

Comparisons between patients ($n=20$) and controls ($n=340$). Descriptive values are shown as medians and interquartile ranges. Hormone levels in patients are also compared to an age matched reference population and the results are expressed in standard deviation (s.d.) scores (z-scores).

*Normal semen quality was defined as total sperm counts above 39 million and progressive motile spermatozoa above 32%.

estradiol/testosterone ratio was significantly higher in the patients compared to controls (Table 2). Testicular size was similar in patients and controls.

Leydig cell function

Time interval from 1st documentation of serum testosterone <10nmol/L to cryopreservation was in median 105 (IQR) 30–210 days. Median time interval from initial diagnosis of pituitary disease to cryopreservation was 5 months (range 1–108). On the day semen was cryopreserved, patients had lower serum levels of total testosterone ($P=0.001$), SHBG ($P=0.03$), cT ($P<0.001$), LH ($P=0.002$) and estradiol ($P=0.001$) compared to controls (Fig. 1 and Table 2). Hormone levels in patients, compared to age-specific reference ranges presented as SD scores (z-scores) are also given in Table 2. The median z-score for testosterone and cT were minus 2.5 and minus 1.95, respectively. Fifteen patients had serum total testosterone below the lower limit for age- and assay-specific reference range.

Total testosterone/LH ratio as well as cT/LH ratio was significantly lower in patients compared to controls (Table 2). Adjusted for age and concentrations of LH serum testosterone and cT were reduced by 12.4 (7.4–17.4) nmol/L and 283 (168–399) pmol/L, respectively in patients compared to controls (both $P<0.001$). There were no significant interactions between patients/controls and LH in the two regression analyses. Serum LH was significantly associated to both total testosterone and cT (both $P<0.001$), whereas age did not significantly influence testosterone or cT (both $P=0.82$). The relationship between serum LH vs total testosterone and cT in patients are presented in Fig. 2A and B using two-dimensional bivariate plots. The patients are compared to a reference chart based on simultaneous measurements of LH and testosterone (2A) and LH and cT (2B) ($n=706$, age 20–50 years) (21). The curved lines in the two figures

represent the 97.5 percentile reference curves. If the point is inside the curve, i.e. is in the upper left corner of the plot, the patient has a normal LH testosterone/cT combination. The patient is abnormal if the point is outside, i.e. to the right or below the curve. As seen in the two figures, the majority of patients had an abnormal LH testosterone combination and LH-cT combination. When establishing the reference curves testosterone was measured by a radioimmunoassay (RIA) (21). Compared to the RIA, testosterone is on average measured 17% higher by the immunofluorometric assay used in the patients (data not shown). For LH and SHBG the same assays were used.

Levels of insulin-like growth factor 1 was significantly higher in the patients ($P=0.03$).

Spermatogenesis

Data on semen quality are presented in Table 2. One of 20 patients had azoospermia compared to four of the 340 controls. For the patient with azoospermia data on semen volume was not available, and in another patient with very low semen volume (0.2mL), data on sperm concentration were not determined.

Levels of inhibin b were reduced in patients ($P<0.001$) compared to controls, whereas FSH ($P=0.62$) did not differ. The inhibin b/FSH ratio was also reduced in patients (Table 2). The FSH-inhibin b profile was also compared to a reference chart in a bivariate plot based on inhibin b and FSH measurement (Fig. 3). Despite reduced inhibin b/FSH ratio, all men had a normal FSH-inhibin combination when compared to men from the general population (full drawn line). Sixteen of 20 patients also had a normal combination when compared to proven fertile men, who had fathered children without use of assisted reproductive technologies (dotted line). The reference chart was based

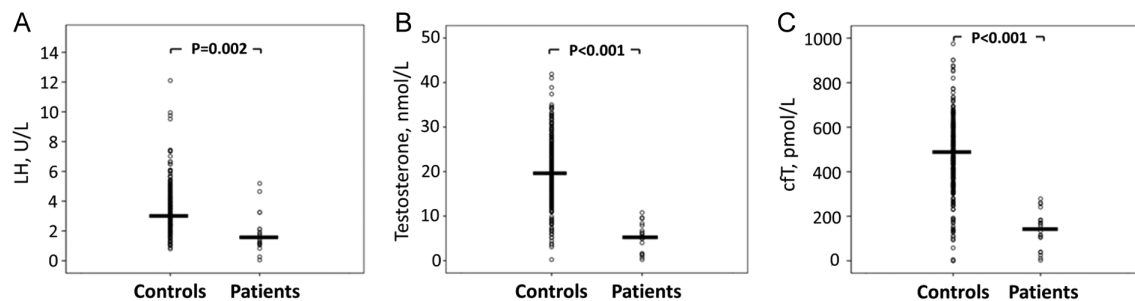


Figure 1 Levels of LH (A), testosterone (B) and calculated free testosterone (cT) (C) in controls and patients. All individual measurements are presented in the figure. Horizontal lines represent the median.

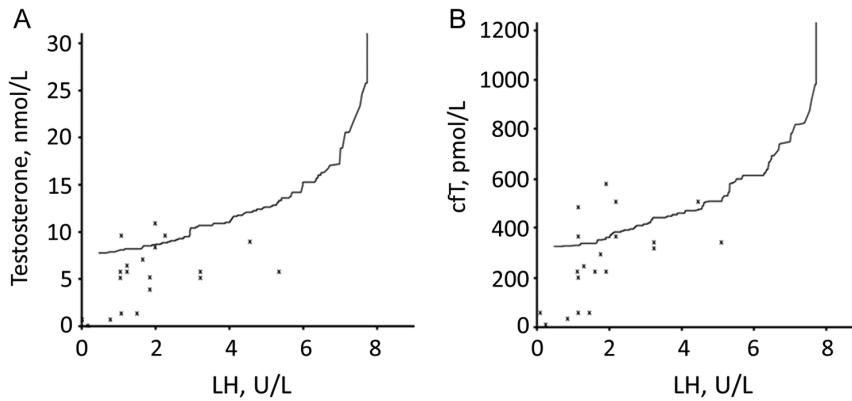


Figure 2 LH vs total testosterone (A) and LH vs calculated free testosterone (cFT) (B) in men with acquired hypogonadotropic hypogonadism plotted in a bivariate reference chart. The curves represent the 97.5 percentile for men from the general population ($n=706$, age 20–50 years) (19). Points below the 97.5 percentile represent patients with an abnormal LH-testosterone (and LH-cFT) combination, and points above and to the left of both 97.5 percentiles represent individuals that have a normal combination.

on measurements in men aged 20–45 years, $n=307$ (general population) and $n=303$ (proven fertile men). The reference curves were established using the same assays for FSH and inhibin b as used in the patients (22).

There was a significantly lower percentage of progressively motile spermatozoa in patients compared to controls 49 (13–63) vs 57 (47–67)% ($P=0.04$). Thirteen patients (65%) and 82% of controls had total sperm counts above 39 million and progressive motile spermatozoa above 32% ($P=0.05$), corresponding to the WHO 2010 reference levels for these variables (18). For the individual semen variables, there were no significant differences (patients vs controls) in semen volume 3.2 (1.4–5.6) vs 3.2 (2.3–4.3) mL ($P=0.47$), sperm concentration 41 (11–71) vs 43 (22–73) mill/mL ($P=0.56$) or total sperm counts 153 (15–288) vs 136 (65–235) mill ($P=0.66$) (Fig. 4).

In univariate regression analyses, FSH was inversely associated with total sperm count, sperm concentration and inhibin b in the controls (all $P<0.001$). By contrast, no significant association between FSH and total sperm counts ($P=0.77$), sperm concentration ($P=0.58$) or inhibin b ($P=0.87$) were observed in the patients.

In patients (univariate regression analyses), levels of testosterone were positively associated with semen volume ($P=0.01$) and total sperm counts ($P=0.03$) (Fig. 5), but not with the sperm concentration ($P=0.14$). In the controls, no significant associations were found between testosterone vs semen volume ($P=0.1$), total sperm counts ($P=0.20$) or sperm concentration ($P=0.06$).

A diagnosis of acromegaly or a diagnosis of prolactinoma was not significantly associated with total sperm count or sperm concentration. IGF-I, prolactin, duration of abstinence from latest ejaculation, time since initial diagnose of pituitary disease or duration of documented androgen deficiency also did not predict semen quality.

Discussion

We report that a large proportion of patients with adult-onset pituitary disease presented with normal semen quality at the time when testosterone deficiency became apparent. The only significant difference we could document was a lower percentage of progressively motile spermatozoa in patients compared to controls. Thus, despite severe and highly significant reduction of Leydig cell function with low total and calculated free

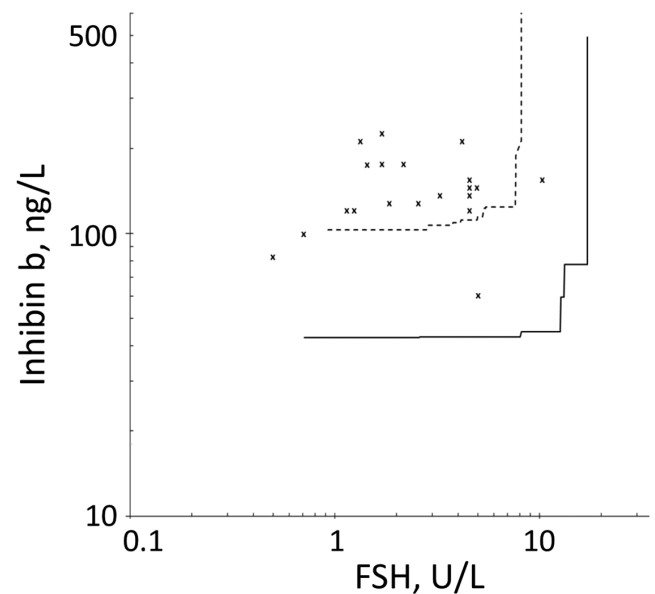


Figure 3 FSH vs inhibin b in men with acquired hypogonadotropic hypogonadism plotted in a bivariate reference chart. The curves represent the 97.5 percentile for proven fertile men (dotted line, $n=303$, age 20–45 years) and the 97.5 percentile for men from the general population (full drawn line, $n=307$, age 20–45 years) (20). Points above and to the left of both 97.5 percentiles represent patients that have a normal FSH-inhibin b combination. Points placed between the two 97.5 percentiles represent individuals who are identified as having normal hormone levels in relation to men from the general population but outside the normal range in relation to proven fertile men.

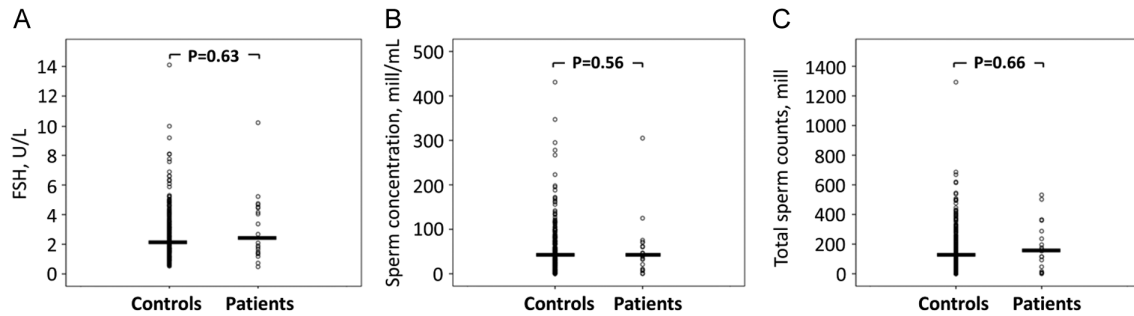


Figure 4

Levels of FSH (A), sperm concentration (B) and total sperm counts (C) in controls and patients. All individual measurements are presented in the figure. Horizontal lines represent the median.

testosterone in *all* patients, the majority actually had normal semen quality. Therefore, the data suggest that the hypogonadotropic state influences the two components of the gonadal axis, steroidogenesis and spermatogenesis, differently. In support of this hypothesis serum FSH, in contrast to LH, was completely unchanged between patients and controls. Levels of inhibin b were reduced in the patients, but still the majority had serum inhibin b and FSH-inhibin b combination (bivariate plot) within normal range for proven fertile men.

It is well known, that development of pituitary insufficiency often follows a well-defined pattern with the growth hormone axis as the most frequently affected axis, followed by the gonadal axis, the thyroid axis and the adrenal axis (5, 23). As a novel observation, our data point to the possibility that in case of partial pituitary insufficiency spermatogenesis seems more robust than Leydig cell function. In patients with primary gonadal failure, the complete opposite scenario is often observed with severe suppression of spermatogenesis with preserved Leydig cell function (24).

To our knowledge, there are five studies investigating semen quality in patients with hyperprolactinemia naïve to treatment with testosterone or gonadotropins. Serum testosterone at baseline was in all studies comparable to

the levels measured in our patients. Semen quality was reported to be reduced, but with very divergent results: In three studies, the sperm concentration at baseline was 6, 19 and 30 million/mL, respectively (8, 9, 10). The two remaining studies only reported total sperm counts (83 mill (11) and 44 mill, respectively (12). Two small studies ($n=6$ and $n=7$) found very poor semen quality with the majority having azoospermia in patients with acquired, and permanent secondary testosterone deficiency not caused by hyperprolactinemia (3, 25). A third study reported data from 10 infertile patients with adult-onset hypogonadotropic hypogonadism requiring gonadotropin treatment. Unfortunately, no specific data on baseline semen quality were provided in the paper, and it is unknown if the patients had been treated with testosterone prior to enrolment (4).

In agreement with the normal sperm counts our patients had testicular volume comparable with the healthy controls. By contrast, two previous studies found low sperm counts despite normal testicular size (3, 4). Thus, in patients with adult-onset hypogonadotropic hypogonadism, testicular size may not always be a predictor of semen quality. Compared to controls gynecomastia was more frequently observed in patients, despite reduced levels of estradiol. However, the estradiol/

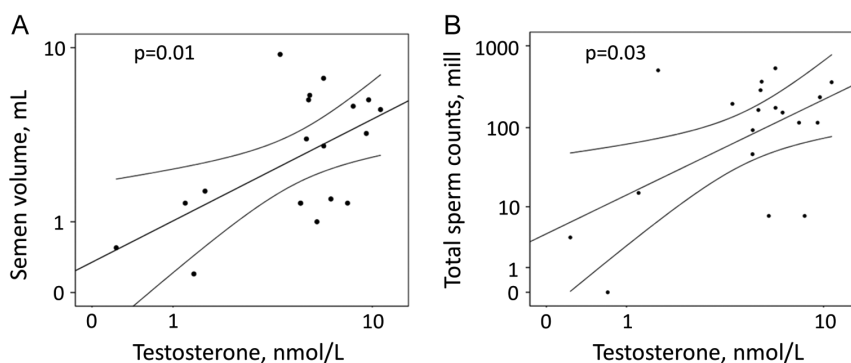


Figure 5

Relationship between testosterone vs semen volume (A) and total sperm counts (B) in patients with secondary hypogonadism.

testosterone ratio was highly increased in the patients, supporting that it is the balance between androgens and estrogens that are important for development of gynecomastia in hypogonadal men and not the level of estradiol (26).

An important factor that should be considered is the duration of testosterone deficiency. During treatment with supraphysiological doses of testosterone and fully suppressed levels of FSH and LH, oligospermia and in many cases azoospermia, develops after around 3–4 months (27). This is in agreement with the fact that formation of mature sperm takes approximately three months (28). In our study, testosterone deficiency was documented for a median of 3.5 months before evaluation of semen. Moreover, many of our patients already had low testosterone at the initial biochemical evaluation. Thus, the exact duration of low testosterone was unknown, but most likely significantly longer than the documented 3.5 months.

The relative importance of intratesticular testosterone vs FSH stimulation on spermatogenesis in humans is debated. Intratesticular testosterone is believed to play a pivotal role for spermatogenesis and semen volume (1), although data obtained in the so-called fertile eunuch syndrome (congenital testosterone deficiency caused by isolated LH deficiency) show that spermatogenesis can be present despite low testosterone (29). The role of FSH in relation to spermatogenesis is less clear. In experimental models spermatogenesis can be induced by human chorionic gonadotropin (stimulation of LH receptors), in the absence of FSH, but FSH stimulation seems necessary to achieve complete restoration of spermatogenesis (1, 2). Thus, a synergic effect between FSH and intratesticular testosterone has been suggested (1). Despite low circulating testosterone in our study, the combined effect of intratesticular testosterone and FSH was sufficient to induce normal spermatogenesis in most patients. However, within the patients both semen volume and total sperm counts were positively associated with testosterone, supporting the importance of intratesticular testosterone.

Taken together, divergent results on semen quality in male patients with secondary testosterone deficiency have been published. We only identified serum testosterone as a predictor of semen quality and volume, whereas for example, underlying disease, levels of gonadotropins or levels of prolactin did not influence semen quality. Based on the available data from the present study duration of testosterone deficiency could also not be identified as a risk factor for poor semen quality. One of twenty patients

had azoospermia, and therefore, we will still recommend early cryopreservation of semen in male patients with pituitary disease.

The diagnosis of hypogonadotropic testosterone deficiency was based on repeated measurements of low testosterone, reduced or normal serum concentration of LH and symptoms of testosterone deficiency. Moreover, there were structural changes in the pituitary on magnetic resonance imaging supporting a state of permanent pituitary insufficiency. Serum SHBG was reduced, which *per se* was unexpected in a testosterone deficient population (30). There could be several explanations including high levels of growth hormone/IGF-I in the acromegalic patients, insufficient replacement therapy with thyroxine and supraphysiological substitution therapy with hydrocortisone. Moreover, it cannot be excluded that higher BMI and higher age in patients compared to controls might have influenced SHBG. We did not have any valid information on tobacco smoking of the patients and can therefore not account for a possible effect on SHBG-levels. However, it should be emphasized that cT was substantially reduced compared to both the control subjects, and to an age matched reference material.

FSH, LH and thyroid-stimulating hormone (TSH) belong to the glycoprotein hormone family. These hormones consist of a common alpha subunit and a unique beta subunit (31). The hormones are glycosylated, and it has been shown that alterations in glycosylation change the ratio between bioactivity and immunoreactivity (31). This phenomenon has been quite extensively investigated in respect to TSH in patients with central hypothyroidism, and it is now well established that TSH bioactivity is often reduced in patients with pituitary disease (32, 33). Analogous to these observations, our data showed reduced testosterone/LH ratio, reduced cT/LH ratio, and using bivariate plots, we observed an abnormal relationship between total-testosterone and cT vs the corresponding LH in the majority of patients. Thus, we speculate that the bioactivity of LH might be reduced in male patients with central hypogonadism. This is to our knowledge a new observation. Alternatively, the findings should reflect that patients with pituitary disease in general also had a primary testicular failure, which we consider unlikely. Concerning FSH bioactivity our results are less clear. Reduced inhibin b/FSH ratio in patients point to the possibility of reduced bioactivity. On the other hand, the majority had serum inhibin b and FSH-inhibin b combination (bivariate plot) within normal range for proven fertile men, and they had

normal sperm counts supporting sufficient bioactivity. One previous study has investigated FSH bioactivity in male patients with non-functioning pituitary adenomas and found unchanged *in vitro* FSH bioactivity. By contrast clinically relevant changes in the bioactivity of gonadotropins have been documented in other clinical conditions such as anorexia nervosa (34), polycystic ovarian disease (35) and primary male hypogonadism (36). Thus, as for TSH, immunoreactive FSH and LH may not reflect FSH and LH bioactivity in patients with pituitary disease, and even a stimulation test probably does not add to the correct diagnosis, since secretion of the dysglycosylated molecules can be stimulated by hypothalamic hormones (32, 37). We acknowledge that the hypothesis of reduced bioactivity of gonadotropins is based on speculations and this topic merits further investigations.

There are several limitations to the presented study. The control subjects were not matched for age, but we do not believe that it is a major confounder, since there only seems to be minor changes in semen quality (38) and circulating testosterone levels (39) in individuals aged 20 vs 30 years. We studied a highly selected inhomogeneous group of patients comprising a small non-representative fraction of the entire group of patients diagnosed with hypogonadotropic hypogonadism at our department ($n=409$). The participants in the study were significantly younger and comprised a large fraction of patients with growth hormone excess, which might *per se* have a negative influence on semen quality (40). Finally, we did not have data on sperm morphology. There are data suggesting that morphology is affected in patients with secondary hypogonadism due to thalassemia (41).

In conclusion, despite severe testosterone deficiency, 13 of 20 patients had normal semen quality based on the 2010 WHO criteria for total sperm counts and motility. We will recommend, that all patients with secondary testosterone deficiency are offered cryopreservation of semen, before initiation of testosterone therapy. In many cases, cryopreserved semen seems to be of sufficient quality for fertility treatment. With this approach the patient and his partner have the opportunity to decide, if they prefer fertility treatment with cryopreserved semen or testosterone treatment withdrawal, and medical induction of spermatogenesis with gonadotropins, which would be the alternative treatment. Finally, our data point to the possibility that bioactivity of gonadotropins may be reduced in patients with pituitary insufficiency.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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