Clinical, genetic, biochemical, and testicular biopsy findings among 1,213 men evaluated for infertility

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Objective: To study the pathologic findings among men evaluated for infertility.
Design: A retrospective, single-center, cross-sectional study.
Setting: University hospital–based research center.
Participant(s): We included data from 1,213 medical records from infertile men referred for diagnostic work-up from 2005 to 2009.
Interventions(s): None.
Main Outcome Measure(s): Health history, clinical findings, chromosome/genetic aberrations, semen quality, reproductive hormones.
Result(s): In total, 64.4% of the infertile men had one or more reproductive disorders or factors influencing fertility, leaving 35.6% diagnosed as idiopathic infertile. In 244 patients (20%), including seven cases of testicular cancer and/or germ cell neoplasia in situ, a pathologic finding was first detected during diagnostic work-up. Two hundred four patients (16.8%) had a history of cryptorchidism and 154 (12.7%) of varicocele (grade 2 and 3). Thirty-three patients had chromosomal abnormalities, including 16 with sex chromosome abnormalities (11 with 47,XXY). Y-chromosome microdeletions were detected in 65 patients (5.4%). One hundred thirty-three had azoospermia, of which 58 had testicular biopsy findings (Sertoli cell–only syndrome: n = 23; spermatogenic arrest: n = 7; impaired spermatogenesis and atrophy: n = 28). Additionally, in idiopathic infertile men and infertile men with additional symptoms of testicular dysgenesis syndrome, 22.5% presented with a degree of Leydig cell insufficiency, with the highest frequency (33.1%) among patients with sperm concentration <5 million/mL.
Conclusion(s): We report pathologic findings that could explain the male-factor infertility in two-thirds of infertile men referred to our center. Thus, male infertility may be a sign of an underlying disease that warrants attention. (Fertil Steril® 2017;107:74–82. ©2016 by American Society for Reproductive Medicine.)
Key Words: Male infertility, testicular dysgenesis syndrome (TDS), chromosome abnormalities, Y-chromosome microdeletions, testicular cancer

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Infertility, defined as lack of pregnancy after 1 year of unprotected regular intercourse, affects ~15% of couples, and in ~50% of these couples it can be attributed to a male factor with or without a concomitant female problem (1). Infertile men most commonly present with oligozoospermia. However, some have normal sperm counts but abnormal sperm motility or morphology.

Some reproductive diseases of adult men, including reduced semen quality, cryptorchidism, hypospadias, and testicular cancer, may have their origin in a prenatal impairment of gonadal development and therefore may be classified within the testicular dysgenesis syndrome (TDS) (2). The clinical phenotype of the patient with TDS varies from the most common form where low sperm counts is the only symptom, to severe forms with poor semen quality, genital malformations, and/or testicular cancer in the

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same patient, bordering on disorders of sex differentiation (3). Severe cases of TDS are often a result of genetic or chromosomal aberrations (4, 5). However, in the majority of TDS cases no genetic factors have been identified, and environmental factors are suspected to be involved (6). We hypothesize that TDS is often a cause of low semen quality among infertile men.

The World Health Organization (WHO) has provided reference levels for the classically assessed semen quality variables (7). These reference levels may serve as clinical tools to identify men who may need fertility treatment. However, reduced fertility chances may occur even with semen quality above the lower WHO reference levels. This implies that changes of natural conception decline already at sperm concentrations <40–50 million/mL (8–10) and at morphologically normal spermatozoa <12%. Male infertility may result from primary testicular problems, some of which are easily identified from medical history and clinical investigations or from biochemical, chromosomal, and genetic analyses (11). Nevertheless, the underlying cause is often not found in men evaluated for infertility, and a high proportion of these men are categorized as idiopathic infertile. Many previous studies suffered from small sample sizes and selected patient populations. Therefore, we evaluated the pathologic findings that could contribute to fertility problems in a large series of 1,213 infertile men referred to our andrology clinic during a 5-year period.

PATIENTS AND METHODS

Patient Population

We included retrospectively infertile men examined from January 1, 2005, to December 31, 2009 at the andrology clinic at the Department of Growth and Reproduction, Rigshospitalet (Copenhagen, Denmark). The men were referred for diagnostic work-up prior to fertility treatment with their partner as either in vitro fertilization (IVF) or intra-cytoplasmatic sperm injection (ICSI). All men with the referral diagnosis "male infertility" (DN469) coded in the national registry according to the WHO International Classification of Diseases, Tenth Revision, were included regardless of ethnicity (~80% of Danish origin). After exclusion of 37 misclassified patients and 91 subjects who did not complete the examination program, 1,213 men were included. The study was approved by the Danish Data Protection Agency (no. 2010-41-4366).

Clinical Examination

At the patient's first visit to the andrology clinic, the medical history was recorded and a physical examination performed. Testicular location and the presence of gynecomastia, hypospadias, testicular tumor, varicocele, and abnormalities of vas deferens and the epididymis were evaluated. Testicular size was assessed with the use of a Prader wooden orchidometer and ultrasound.

Semen Analysis

The patients were advised to produce two semen samples by means of masturbation, ≥14 days apart. All complied except for 109 men who delivered only one sample. The men had been advised to keep an ejaculation abstinence period of ≥2 days. After ejaculation, the semen samples were kept at 37°C until liquefaction.

The analysis was performed based on the WHO guidelines of 1992 (12) as slightly modified according to our interobserver variation study (13). For sperm motility assessment, 10 μL well mixed semen was placed on a glass slide kept at 37°C, covered with a 22 × 22 mm coverslip, placed in the heated stage in the microscope, and examined at ×400 magnification. The spermatozoa were categorized as progressively motile, nonprogressively motile, or immotile. Subsequently, sperm concentration was determined with the use of a Bürker-Türk hemocytometer (Paul Marienfeld), after diluting 100–200 μL well mixed semen in a solution containing formaldehyde. Only spermatozoa with a tail were counted. For assessment of the morphology of the spermatozoa, smears were air dried at room temperature and fixed in 96% ethanol for 5 minutes before Papanicolaou staining. Sperm morphology was assessed according to strict criteria (14).

Reproductive Hormone Analyses

Nonfasting blood samples were drawn from the antecubital vein for the majority of the patients between 8 a.m. and 2 p.m. Serum was separated from centrifuged blood samples and the concentrations of FSH, LH, and SHBG were measured by means of time-resolved immunofluorometric assays (Delfia, Perkin Elmer). Intra- and interassay coefficients of variation (CVs) were 3% and 5%, respectively, for the FSH and LH assays, and the detection limits (LODs) were 0.06 IU/L and 0.05 IU/L, respectively. Intra- and interassay CVs for SHBG were 5.8% and 6.4%, with an LOD of 0.2 nmol/L. Serum T and E_2 was measured by radioimmunoassay (respectively, Siemens Coat-A-Count total testosterone assay and Pantex direct estradiol assay). LOD were 0.23 nmol/L for T, and 18 pmol/L for E_2. Intra- and interassay CVs for T were 7.6% and 8.6%, respectively, and for E_2 were 8% and 13%, respectively. Serum inhibin B was measured by a specific two-sided enzyme immunometric assay (Serotec). Intra- and interassay CVs were 15% and 18%, respectively, and LOD was 20 pg/mL.

Free T (cFT) was calculated from T and SHBG concentrations assuming a fixed albumin level of 43 g/L (15). Reference ranges for reproductive hormones with the use of the same assays, including bivariate plots for FSH/inhibin B and LH/T, have previously been published from our laboratory (16, 17).

Genetic Investigations

Karyotype. Lymphocytes were isolated from peripheral blood with the use of routine G-banding and counting of at least ten metaphases, three of which were fully analyzed. All karyotypes were reevaluated for this study by the same clinical geneticist (L.A.). The original metaphase count was unavailable in five cases.

Y-chromosome microdeletions. Deletion mapping of the Y chromosome was carried out according to a method developed in house and carefully validated in a large cohort of infertile patients and normospermic control subjects (18, 19). The test was subsequently adapted to the 2004

TABLE 1

Overview of the characteristics of the study population of infertile men and the subgroups with testicular dysgenesis syndrome (TDS) and idiopathic infertility.

	Infertile						
	All (n = 1,213)		Additional TDS symptoms[a] (n = 147)		Idiopathic (n = 432)		
Variable	n		n		n		*P* value[b]
Age (y)	1,213	33.6 (24.2–49.0)	147	33.0 (24.4–44.5)	432	33.5 (24.2–49.7)	.2[A]
Height (cm)	996	181.8 (166.5–196.3)	119	184.0 (170.0–201.7)	365	180.7 (165.5–195.5)	<.001,*,[A]
Weight (kg)	1,003	84.7 (62.9–122.0)	118	85.4 (66.6–123.4)	370	84.4 (62.5–126.7)	.2[A]
BMI (kg/m^2)	992	25.7 (20.3–36.7)	118	25.1 (20.0–35.0)	364	25.8 (19.6.8–36.6)	.6[A]
Smoker, daily,	1,163	282 (24.2)	141	32 (22.7)	412	100 (24.3)	.7[B]
Smoker, social occasions	1,163	68 (5.8)	141	8 (5.7)	412	26 (6.3)	.8[B]
Alcohol, >21 units per wk	1,105	23 (2.1)	134	2 (1.5)	393	11 (2.8)	.4[C]
Medication within recent 3 mo, unlikely to affect fertility	1,189	188 (15.8)	143	25 (17.5)	422	67 (15.9)	.6[B]
Clinical findings							
Gynaecomastia	1,175	58 (4.9)	143	4 (2.7)	418	18 (4.2)	.7[C]
Varicocele, grade 1	1,192	52 (4.4)	140	2 (1.4)	428	29 (6.7)	.016*,[C]
Testis position (number)	1,167/1,165		135/131		422		
Low, left/right		1,089 (93.3)/1,072 (92.0)		114 (84.4)/104 (79.4)		400 (94.8)	<.001*,[B]
High, left/right		71 (6.1)/86 (7.4)		17 (12.6)/24 (18.3)		22 (5.2)	<.01*,[B]
Inguinal, left/right		7 (0.6)/7 (0.6)		4 (3.0)/3 (2.3)		0	<.001*,[C]
Mean testis size, (mL)	1,155	17.5 (6.0–30.0)	138	15 (5.5–30.0)	417	18.0 (8.0–30.0)	<.001*,[A]
Ultrasonografic finding							
Mean testis size (mL)	1,202	11.3 (3.2–22.0)	143	9.7 (3.1–21.1)	431	11.6 (4.7–22.6)	<.001*,[A]
Microlithiasis	1,201	36 (3.0)	143	11 (7.5)	431	13 (3.0)	.015*,[B]
Intratesticular cyst	1,206	10 (0.8)	144	1 (0.7)	432	3 (0.7)	1.0[C]
Intratesticular Leydig Cell tumor	1,206	2 (0.2)	144	1 (0.7)	432	0	.08[C]
Intratesticular germ cell tumor	1,206	5 (0.4)	144	5 (3.5)	432	0	<.001*,[C]
Hormone parameter							
Total testosterone (nmol/L)	1,212	15.6 (6.7–26.7)	138	14.8 (6.6–25.5)	432	15.9 (8.4–26.6)	.5[D]
Free testosterone (pmol/L)	1,209	315.3 (154.5–520.1)	138	310.7 (141.8–506.7)	429	322.2 (186.0–509.3)	.3[D]
LH (IU/L)	1,212	4.0 (1.4–11.1)	138	4.7 (1.6–13.9)	432	3.8 (1.5–9.8)	<.001*,[D]
SHBG (nmol/L)	1,211	33.0 (12.3–69.7)	138	31.5 (15.5–68.0)	431	34 (12.0–69.2)	.7[D]
Estradiol (pmol/L)	1,210	63.5 (26.3–124.7)	138	65.0 (29.0–129.2)	431	64.0 (27.8–117.6)	.4[D]
Inhibin B (pg/mL)	1,212	138.0 (1.0–370.7)	138	85.0 (1.0–308.6)	432	155.0 (1.0–391.4)	<.001*,[D]
FSH (IU/L)	1,212	5.5 (1.1–28.5)	138	9.1 (2.0–30.9)	432	4.9 (1.3–25.3)	<.001*,[D]
Testosterone/LH ratio	1,212	4.0 (1.0–10.6)	138	3.4 (0.8–8.8)	432	4.3 (1.4–10.2)	<.001*,[D]
Estradiol/testosterone ratio	1,210	4.1 (1.7–11.3)	138	4.3 (2.0–11.6)	431	4.0 (1.8–9.6)	.2[D]
cFT/LH ratio	1,209	80.2 (21.8–220.5)	138	68.0 (16.0–198.1)	429	86.6 (27.7–198.6)	<.001*,[D]
Estradiol/cFT ratio	1,208	0.2 (0.09–0.5)	138	0.2 (0.09–0.5)	429	0.2 (0.09–0.4)	.1[D]
Inhibin B/FSH ratio	1,212	26.0 (0.04–231.2)	138	10.2 (0.03–135.8)	432	34.2 (0.04–233.6)	<.001*,[D]
Semen quality parameter							
Abstinence time of ejaculation (h)	1,176	84.0 (48.0–231.5)	133	90.0 (48.0–223.8)	425	84.0 (48.0–230.1)	.298[D]
Semen volume (mL)	1,184	3.7 (1.0–8.1)	134	3.8 (1.8–8.2)	430	3.8 (1.7–8.1)	.5[D]
Sperm concentration (million/mL)	1,185	4.9 (0.0–86.0)	134	1.8 (0.0–49.8)	432	7.3 (0.0–90.0)	<.001*,[D]
Total sperm count (million)	1,182	17.1 (0.0–282.7)	134	7.2 (0.0–186.8)	432	25.2 (0.0–329.5)	<.001*,[D]

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TABLE 1

Continued.

			Infertile				
	All (n = 1,213)		Additional TDS symptoms[a] (n = 147)		Idiopathic (n = 432)		
Variable	n		n		n		*P* value[b]
Progessively motile (%)	963	38.0 (5.1–73.5)	100	34.3 (10.1–72.5)	372	37.3 (5.2–71.2)	.3[D]
Morphologically normal (%)	961	2.5 (0.0–10.8)	101	2.8 (0.0–10.3)	371	2.5 (0.0–9.7)	.2[D]

Note: Data presented as median (2.5–97.5th percentiles) or n (%). Hormone and semen quality parameters from 8 patients with testicular cancer who received chemo- and/or irradiation therapy have been excluded. BMI = body mass index; cFT = calculated free testosterone; FSH = follicle- stimulating hormone; LH = luteinizing hormone; SHBG = sex hormone-binding globulin.
*$P<.05$, significant; [A]Independent sample T test; [B]Pearson's Chi-square-test; [C]Fisher's exact test; and [D]obtained from regression analysis adjusted for confounders (hormone parameters adjusted for age, BMI, and smoking; and semen quality parameters adjusted for age, BMI, smoking, and ejaculation abstinence time).
[a] Testicular dysgenesis syndrome (TDS) includes patients with poor semen quality and cryptorchidism and/or hypospadias and/or testicular cancer/germ cell neoplasia in situ (GCNIS) testis.
[b] P values resulting from comparing the two subgroups of infertile men with additional TDS or idiopathic infertility.

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guidelines of the European Academy of Andrology (EAA) (20) and expanded to screen for partial AZFc deletions as described previously (21). Since 2001, the laboratory has participated in the external quality control program for the Y-chromosome deletion assay that is run by the European Molecular Genetics Quality Network and EAA. Genomic DNA was extracted from EDTA-treated whole blood samples. The analysis was performed by means of multiplex polymerase chain reaction (PCR)–based amplification of sequence tagged sites or gene-specific sequences spanning the entire azoospermia factor (AZF) region of the Yq arm as well as *SRY* and *ZFY/ZFX* loci on Yp and Yp/Xp, respectively, as previously described (21). The analysis detects deletions in AZFa, AZFb (P5/proximal P1), AZFbc (P5–P4/distal P1), and AZFc (b2/b4) regions, as well as partial deletions in the AZFc region, which are confirmed by quantitative PCR–based analysis of the *DAZ* gene copy number (21).

***CFTR* gene analysis.** Molecular analysis for up to 50 frequent *CFTR* mutations within populations of European origin were performed by means of multiplex allele-specific PCR with the use of a commercially available assay according to the manufacturer's description (CFEu2v1; Elucigene).

Testicular Biopsies

Testicular biopsy was performed as an open surgical biopsy, and in nearly all cases bilateral single biopsies were taken. The tissue samples were fixed overnight at 4°C in Stieve fluid or a similar fixative termed GR-fixative, and subsequently embedded in paraffin. Tissue sections were stained with hematoxylin and eosin or periodic acid–Schiff. In addition, immunohistochemical staining for placental-like alkaline phosphatase, a marker for preinvasive germ cell neoplasia in situ (GCNIS), was performed with the use of a standard immunoperoxidase method. Histologic evaluation of all of the biopsies was performed by two observers as described previously (22).

Statistics

Descriptive statistics for the entire study population and two subgroups of infertile men—idiopathic infertile men and infertile men with additional TDS symptoms—are presented as median and 2.5th–97.5th percentile or number and percentage (Table 1). Between-group differences were calculated for the two subgroups with the use of Pearson chi-square test for categoric variables, and when the assumptions were not met Fisher exact test was used. For the continuous variables, differences between the groups were tested with the use of independent-samples *t* test. Body mass index (BMI) and weight were transformed by natural logarithm to obtain normal distribution. Univariate general linear models were used for all semen quality and hormone parameters. The models were adjusted for abstinence time of ejaculation (piecewise linear functions = linear splines; abstinence time <48 hours, 48–96 hours, or >96 hours), age (continuous), BMI (4 groups: underweight <18.5, normal 18.5–24.9 (reference group), overweight 25–29.9, and obese >30 kg/m^2), and smoking (daily, social occasion, or no).

A *P* value of ≤.05 was considered to be statistically significant. Statistical analyses were performed with the use of the Statistical Package for the Social Science (SPSS) version 20.0.

RESULTS

Descriptions of the study population and baseline characteristics are summarized in Tables 1 and 2. In total, 64.4% of the 1,213 infertile men had one or more possible causes of or contributors to infertility (20% diagnosed during clinical work-up), leaving 35.6% diagnosed as idiopathic infertile (Table 2; Supplemental Fig. 1 [Supplemental Figs. 1–4 and Supplemental Tables 1–3 are available online at www.fertstert.org]). In Table 1 are the characteristics of the subgroups of idiopathic infertile patients (n = 432) and patients with additional TDS symptoms (cryptorchidism, hypospadias, GCNIS/testicular cancer; n = 147) also presented and compared.

Clinical Findings

The most frequent reproductive disorder (other than idiopathic infertility) in the study population was history of cryptorchidism (16.8%; Table 2). Five of these patients were diagnosed for the first time during clinical work-up for

TABLE 2

Reproductive disorders or factors influencing male fertility in the study population (n = 1,213).

Factor	n	%
Testicular cancer, ever diagnosed	26	2.1
Testicular cancer diagnosed during clinical work-up	5	0.4
Germ cell neoplasia in situ (GCNIS) testis diagnosed during clinical work-up	3	0.2
Cryptorchidism, ever diagnosed	204	16.8
Cryptorchidism, diagnosed during clinical work-up	5	0.4
Hypospadias, ever diagnosed	13	1.1
Hypospadias, diagnosed during clinical work-up	7	0.6
Epispadia, diagnosed during clinical work-up	1	0.1
Nongonadal cancer, previously treated	23	1.9
Klinefelter syndrome (47,XXY), diagnosed during clinical work-up	11	0.9
Other chromosomal abnormalities, ever diagnosed[a]	22	1.8
Other chromosomal abnormalities, diagnosed during clinical work-up	19	1.6
Y-chromosome microdeletion, diagnosed during clinical work-up[b]	65	5.4
CFTR mutation, ever diagnosed	13	1.1
CFTR mutation, diagnosed during clinical work-up	8	0.7
Sexually transmitted diseases (STD), previous	161	13.3
Non-STD epididymitis or orchitis, previous	32	2.6
Parotitis including orchitis	4	0.3
Ejaculatory dysfunction, nondiabetic[c]	49	4.0
Diabetes mellitus	18	1.5
Varicocele (2 + 3), ever diagnosed	154	12.7
Varicocele (2 + 3), diagnosed during clinical work-up	132	10.9
Testicular torsion, previous	23	1.9
Orchiectomy, nonmalignant cause[d]	12	1.0
Hypogonadotropic hypogonadism (HH), nonanabolic steroid abuse[e]	6	0.5
Anabolic steroids, ever abuse	24	2.0
Anabolic steroids, current abuse	5	0.4
Medical therapy, current use	305	25.1
Medical therapy likely to affect fertility, current use[f]	117	9.6
Idiopathic infertility[g]	432	35.6

Note: "Ever diagnosed" includes previously diagnosis and factors before clinical work-up and those diagnosed during the clinical work-up. More than one reproductive disorder or factor influencing male fertility can be present in a patient.

[a] Includes autosomal and sex-chromosome anomalies. Details are presented in Supplemental Table 2.

[b] Details are presented in Supplemental Table 3.

[c] Includes obstruction, retrograde ejaculation, and aspermia.

[d] Maldescensus, posttraumatic or misdiagnosed as epididymitis or inguinal hernia.

[e] Includes one patient with Kallmann syndrome, two patients who developed HH after treatment of craniopharyngioma and acromegaly, one patient who had hyperprolactinaemia, and one patient who was previously adrug addict.

[f] Includes, e.g., testosterone, opioids, and cytostatics. Overview of the list of medical therapy is presented in Supplemental Table 1.

[g] Includes medical therapy unlikely to affect fertility, but excludes all other factors in Table 1.

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infertility reasons and another eight patients had persisting cryptorchidism. Hypospadias was ever diagnosed in 13 patients, of whom seven had milder forms that were first diagnosed during clinical work-up. Even more crucial were 26 of the patients ever diagnosed with testicular cancer, including five patients who were diagnosed during the clinical work-up, one of them also having contralateral GCNIS. Additionally, 2 patients were diagnosed with GCNIS.

For the entire study population, the median testis size assessed by means of palpation was within the normal range. The subgroup of infertile men with additional TDS symptoms had smaller testis size with a median at the lower normal limit, also compared with the idiopathic infertile men. Varicocele grade 2 and/or 3 was reported in 154 patients (12.7%) of which a majority were diagnosed during the clinical work-up (10.9%; Table 1). Grade 1 varicocele was not associated with lower semen quality ($P=.9$ [chi-square test]) and therefore not considered as a condition influencing male fertility in this study population. In more than one-third of all men reporting current use of a medical drug, there was some suspicion that it could affect fertility (Supplemental Table 1).

Chromosome and Genetic Aberrations

Chromosome/genetic aberrations were detected in 9.2% of the patients, of which 8.5% were diagnosed during the work-up. Thirty-three patients had chromosomal abnormalities diagnosed during the work-up, including 16 with numeric sex chromosome changes, of which 11 patients had a 47,XXY karyotype. Seventeen patients had autosomal chromosomal abnormalities, including ten with robertsonian or reciprocal translocations, three with inversions, and eight patients with other variants (Supplemental Table 2).

Y-chromosome microdeletions were detected in 65 patients (5.4%); however, in only eight patients (0.7%), complete AZFa, b, or c deletions were identified. The remaining 57 patients had partial AZFc deletions, including 33 with gr/gr deletions, 21 with b2/b3, and three with b1/b3 deletions (Supplemental Table 3). Among other genes associated with male infertility, only *CFTR* was analyzed, and at least one mutation was found in 13 patients.

The distribution of chromosomal and genetic aberrations according to sperm concentrations are shown in Figure 1. All patients with numeric sex chromosomal abnormalities had azoospermia or sperm concentration <1 million/mL, whereas abnormalities in autosomes were seen in a few patients with normal sperm concentration (Fig. 1B).

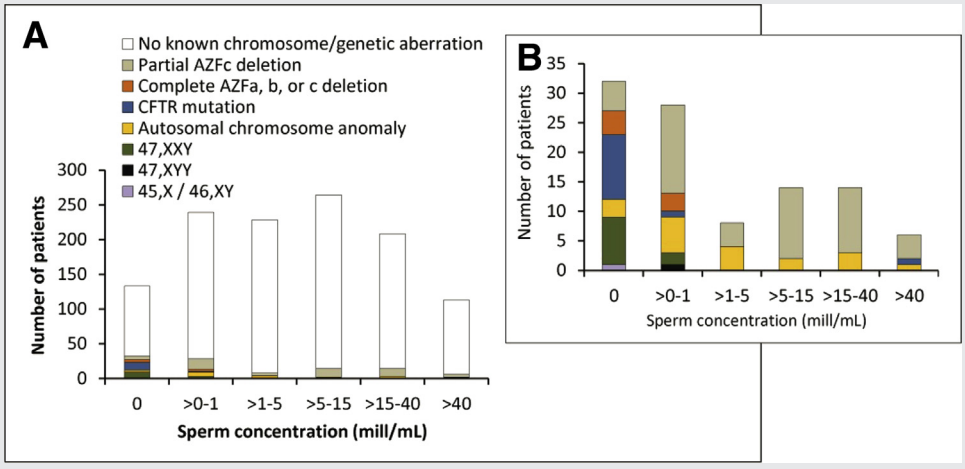
Semen Quality Parameters

Distribution of sperm concentrations among the patients are shown in Figure 1A. Azoospermia was present in 133 patients (10.9% of total group), of which 11 patients had obstruction due to *CFTR* mutation. In the entire group, the median sperm concentration showed oligozoospermic infertility. In patients with idiopathic infertility, median sperm concentration, total sperm count, percentage of progressively motile spermatozoa, and morphologically normal spermatozoa were below the WHO reference ranges. For the infertile men with additional TDS symptoms, the median sperm concentration and total sperm count were even lower than for the idiopathic infertile men, but no differences were found in the morphology or motility (Table 1).

Testicular Biopsy Findings

Fifty-eight men with azoospermia were biopsied and the following histological findings recorded; slightly impaired

FIGURE 1



Distribution of chromosome and genetic aberrations according to sperm concentrations in the entire study population (**A**) and in the subgroup of infertile men with a known chromosome or genetic aberration (**B**).

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spermatogenesis (n = 11), spermatogenic arrest (n = 7), widespread atrophy and impaired spermatogenesis (n = 6), Sertoli cell–only (SCO) syndrome (n = 23), and severely impaired spermatogenesis (n = 11).

Reproductive Hormone Levels

The majority of the idiopathic infertile patients had reproductive hormone levels within the normal reference ranges (Supplemental Fig. 2). However, patients with additional TDS symptoms had higher LH and FSH levels and lower inhibin B levels, inhibin B/FSH ratio, T/LH ratio, and cFT/LH ratio (Table 1), indicating that in this subgroup of infertile men both Leydig cell and Sertoli cell function (and spermatogenesis) are impaired compared with idiopathic infertile men.

The group of patients with sperm concentration <15 million/mL had reduced serum inhibin B levels and/or elevated FSH levels compared with normal reference ranges. It is evident that some of the oligozoospermic and azoospermic men had inhibin B/FSH levels within the normal and even the fertile reference range (Supplemental Fig. 3A). A subgroup of 58 patients with azoospermia had a diagnostic testicular biopsy performed. The majority of these azoospermic men within the normal range of inhibin B/FSH level had either spermatogenic arrest or slightly impaired spermatogenesis, whereas the men diagnosed with widespread atrophy, severely impaired spermatogenesis and SCO syndrome were outside of the normal range and below the detection limit (Supplemental Fig. 3B).

Leydig cell function illustrated by LH-T bivariate plots in relation to normal bivariate ranges are shown stratified by sperm concentration groups in Figure 2 for the 579 infertile patients that were either idiopathic infertile or had additional TDS symptoms. Approximately one-half of these infertile patients represented the group with the poorest sperm concentration of 0–5 million/mL, as did 44% of the idiopathic infertile patients and 66% of those with additional TDS symptoms, respectively. With decreasing sperm concentration, more patients had T and/or LH levels outside the normal ranges, e.g., 20.8% of the idiopathic infertile men and 27.2% of the infertile men with additional TDS symptoms. In the group with the poorest sperm concentration (0–5 million/mL), 32.6% and 34% from these two groups were outside of the normal bivariate plot for LH-T, respectively.

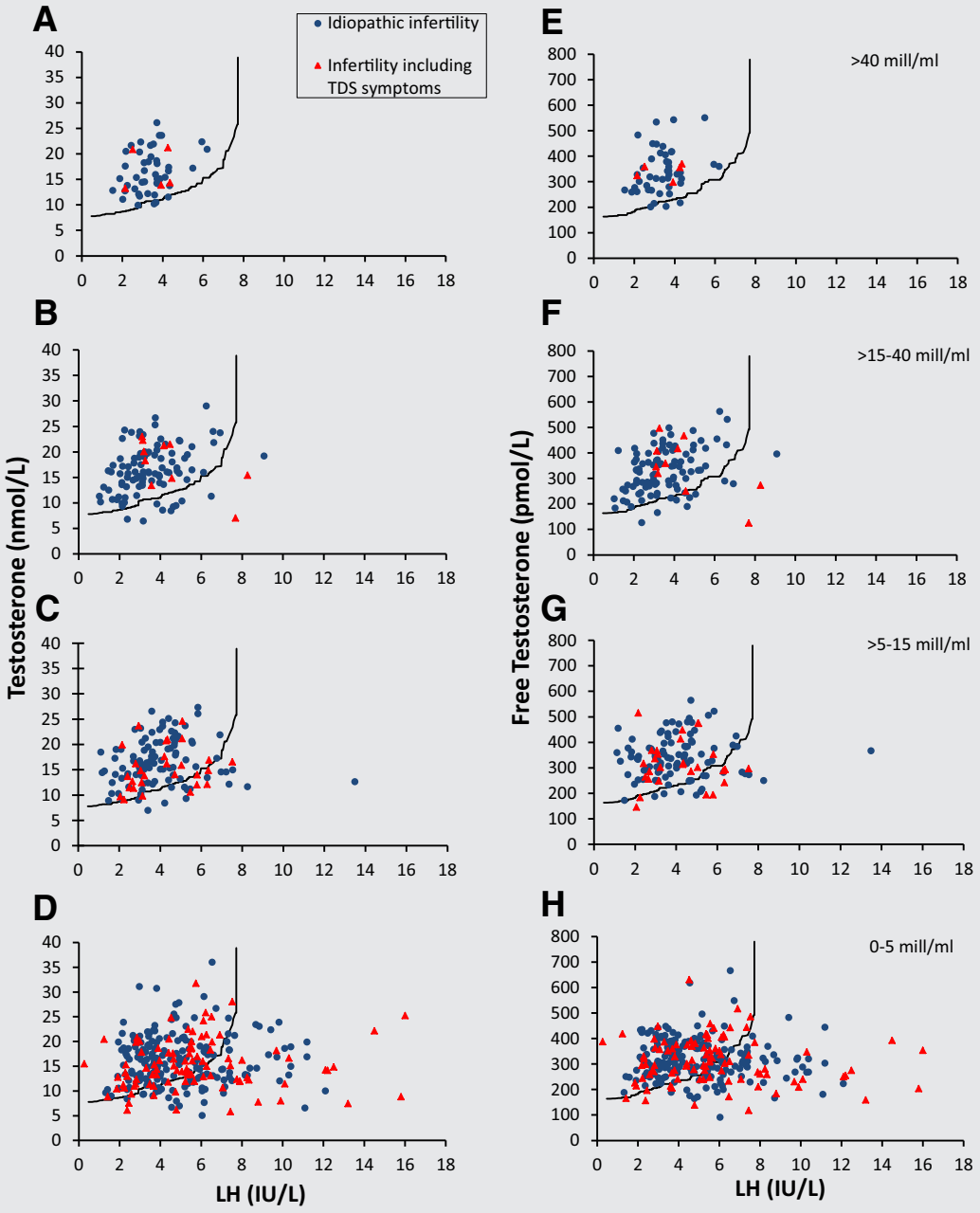
The relative changes in LH, T, cFT, T/LH, and cFT/LH according to sperm concentration (reference group: >40 million/mL) are shown in Supplemental Figure 4.

DISCUSSION

In this cohort of 1,213 infertile men, approximately two-thirds had one or more known underlying causes influencing male fertility, whereas the remaining were considered to have idiopathic infertility. However, even in the latter group subtle signs of Leydig cell dysfunction were apparent. The high frequency and severity of the pathologic findings that we detected during the clinical work-up for male infertility at the center is noteworthy. Notably, we detected testicular cancer and/or GCNIS in seven of the infertile men during their andrologic evaluation. In addition, almost 10% of the men in the entire cohort reported current use of medical drug therapy suspected to affect fertility (23). Some of these men were advised to change the medical therapy when attempting to conceive. If this was not an option, in some cases sperm that were cryopreserved before the medical therapy could be used for assisted reproductive technology (ART).

Cryptorchidism was the most frequent clinical finding known to impair male fertility and was by far the most frequent TDS symptom other than low semen quality. The infertile men with additional TDS symptoms had the poorest testicular function, as evidenced by smaller testis size, lower

FIGURE 2



Serum (**A–D**) testosterone and (**E–H**) calculated free testosterone plotted against LH in two-dimensional charts stratified by sperm concentration in four groups: (**A, E**) >40, (**B, F**) >15–40, (**C, G**) >5–15, and (**D, H**) 0–5 million/mL . Data points represent 432 idiopathic infertile men (*blue circles*), and 147 infertile men with additional testicular dysgenesis syndrome symptoms (*red triangles*). The lines in the graph represent 2.5 percentile for testosterone and cFT and 97.5 percentile for LH.

Olesen. Pathologic findings in infertile men. Fertil Steril 2016.

sperm concentration and inhibin B levels, and higher FSH and LH levels. Testicular cancer or preinvasive-stage GCNIS was found in 2.3% of our infertile population, which is compatible with the fact that male infertility is a high-risk factor for testicular cancer (24). Altogether, these findings support the notion that cryptorchidism, hypospadias, testicular cancer and poor semen quality are associated with each other (25) as part of the TDS hypothesis (2).

The Leydig cell function is reflected by the T-LH ratio; a lower T-LH ratio suggests the need for higher LH drive to attain appropriate T level (26). We found decreasing T/LH in our infertile population with decreasing sperm concentration. Thus, the poorer the semen quality, the poorer the Leydig cell function. Isolated mild LH elevations with normal T levels in the absence of clinical signs or symptoms of T deficiency do not warrant T treatment (27). In fact, T treatment is

contraindicated in men with mild Leydig cell insufficiency when attempting to conceive. However, we believe that a marked LH elevation is a sign of Leydig cell insufficiency, in analogy with marked TSH elevations in mild hypothyroidism with normal T_4. Thus, male infertility may be a marker of present or imminent primary T deficiency (28, 29). The present study can not predict whether or not the men with compensated Leydig cell failure will develop frank T deficiency later in life, but we hypothesize that they are at increased risk. This is in line with other studies (30).

The majority of patients with sperm concentration <15 million/mL had abnormal FSH and inhibin B and fell outside the fertile range in the bivariate FSH–inhibin B plot (56%), although patients with nonobstructive azoospermia due to spermatogenic arrest primarily at spermatocyte level had normal FSH/inhibin B. This confirms our previous reports on the clinical relevance as well as cutoff values of FSH and inhibin B in the evaluation of the infertile man (16, 31, 32).

Despite genetic testing in this study being limited to karyotyping, screening for the Y-chromosome microdeletions, and *CFTR* mutations, in a sizeable proportion of the patients an underlying genetic defect was detected during andrologic work-up of their infertility. The proportion of patients with numeric sex chromosomal abnormalities (including 47,XXY) among infertile men in this series is in accordance with other studies showing that Klinefelter syndrome is the most common genetic finding among patients with azoospermia. Seventeen of our patients had autosomal chromosomal abnormalities. We found that the majority of chromosomal aberrations were detected among patients with azoospermia or severe oligozoospermia. Routine karyotyping of men with sperm concentrations of <10 million/mL has been recommended (33). However, in the present series six patients with sperm concentration >10 million/mL had balanced autosomal anomalies, which supports the recommendation of examining male karyotypes before intracytoplasmic sperm injection regardless of the sperm concentration.

This study confirmed the importance of screening infertile men for Y-chromosome microdeletions. The frequency of complete AZF deletions in this study (0.8%) was lower than the 3.8% in our earlier cohort of 442 Danish patients, but the previous study excluded patients with inflammatory, obstructive, and iatrogenic types of infertility (19). Regarding partial AZFc deletions, these were described in infertile men with sperm concentrations varying from severe oligozoospermia to normal range and in fertile men. Based on numerous association studies, the current consensus is that gr/gr deletions are a genetic risk factor and ought to be screened for in infertile men (34). Our findings in the present study are in general consistent with this consensus, because the patients with gr/gr deletions did indeed have a lower mean sperm concentration than those with b2/b3 deletions. However, we noticed that men with gr/gr deletions had a larger spread of sperm concentrations than the b2/b3-deleted men. Interestingly, all three patients with the rare b1/b3 partial AZFc deletion had severe oligozoospermia, but this observation is uncertain because of the small number of patients.

Numerous lifestyle factors, such as smoking, use of marijuana, alcohol consumption, and BMI, may influence testicular function (35–39). Many of these lifestyle factors are coexisting and may confound possible associations between testicular function and individual risk factors detected in epidemiologic studies. Adult as well as in utero exposure to tobacco seems to be associated with lower sperm concentration and inhibin B levels (35, 36). Conversely, several studies have shown that current smokers have higher T levels than nonsmokers (35). Daily smoking was reported by 24% of our present cohort of infertile men, but adjustment for smoking did not influence any of the reported differences between groups. Interestingly, one-third of our population of infertile men were overweight and 11% obese, so we adjusted all results for BMI in our models. BMI clearly influences total T (40), and an inverse U-shaped association between BMI and sperm concentration has been shown, with normal BMI being associated with the best sperm concentration compared with low or high BMI (39). Likewise, two recent studies report lower sperm concentration among obese subjects (41, 42).

CONCLUSION

We found that two-thirds of the infertile men, whose partners were referred for fertility treatment, presented with known clinical, genetic, and/or biochemical explanatory findings. The high frequency and severity of the pathologic findings that we detected during the clinical work-up for male infertility is noteworthy. Importantly, we detected testicular cancer and/or GCNIS in seven of the infertile men during their fertility evaluation. Karyotyping found previously undiagnosed chromosome aberrations with impact on either the patient himself and/or the fertility treatment with the risk of affecting the fetus. Medical therapy with suspected impact on male fertility may be possible to change or even pause when attempting to conceive, and the patient may be motivated to make some lifestyle changes, which may have a positive effect on fertility. In addition, 20% of the remaining idiopathic infertile men had subnormal Leydig cell function. All of these findings illustrate that male infertility may be a sign of an underlying disease that warrants attention in the clinical practice. In 28% of infertile patients, we did not detect any pathologic findings whatsoever, consistent with the complexity of this condition and calling for more research effort to identify more discrete etiologic factors.

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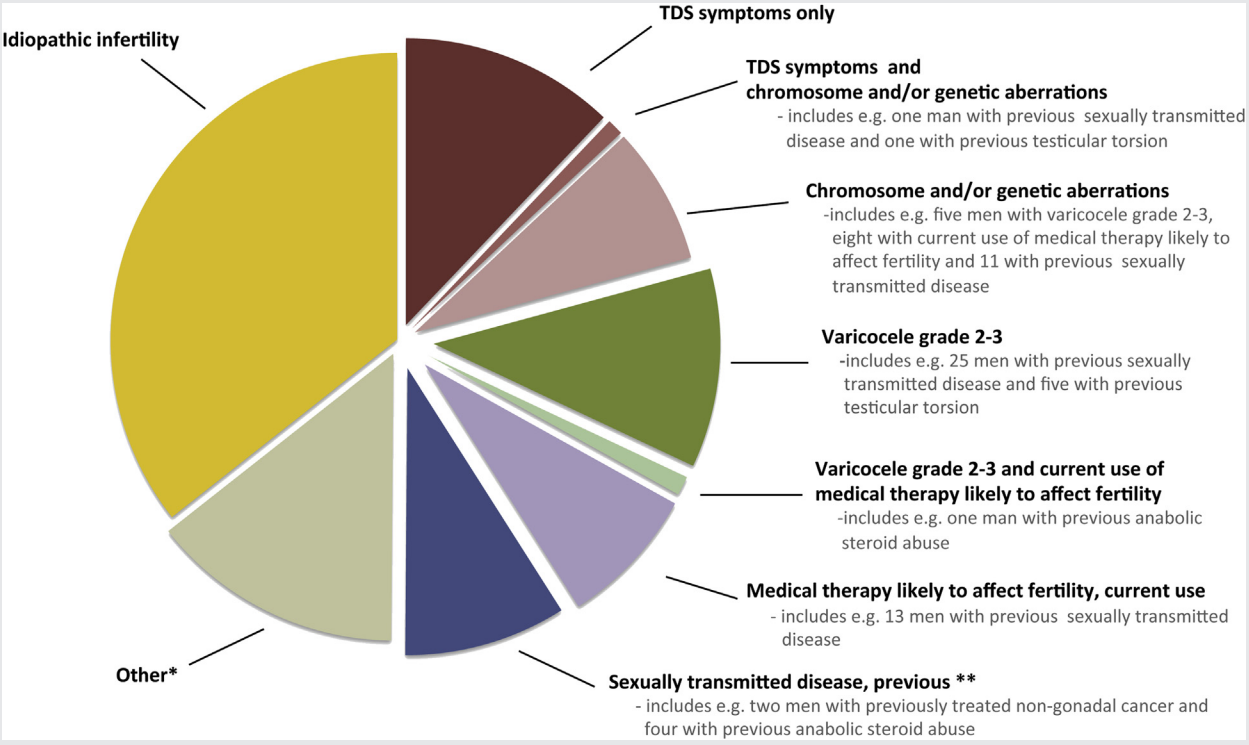
SUPPLEMENTAL FIGURE 1



Reproductive disorders or factors influencing male fertility. In some patients more than one factor or disease occurred, and these conditions may therefore be represented in all categories except for idiopathic infertility and testicular dysgenesis syndrome (TDS) only, as indicated. The most frequent findings are illustrated in the following order: all "idiopathic infertile" men, followed by "TDS symptoms only," "TDS symptoms and chromosome and/or genetic aberrations," "Chromosome and/or genetic aberrations," "varicocele grade 2–3," "varicocele grade 2–3 and current use of medical therapy likely to affect fertility," "current use of medical therapy likely to affect fertility," and "previous sexually transmitted disease." The remaining are categorized as "others" (*nongonadal cancer, previously treated; non-STD epididymitis or orchitis, previous; parotitis including orchitis; ejaculatory dysfunction, nondiabetic; diabetes mellitus; testicular torsion, previous; orchiectomy, nonmalignant cause; hypogonadotrophic hypogonadism, nonanabolic steroid abuse; anabolic steroids abuse). **Chlamydia and/or gonorrhoea. Details and percentages are described in Table 2.

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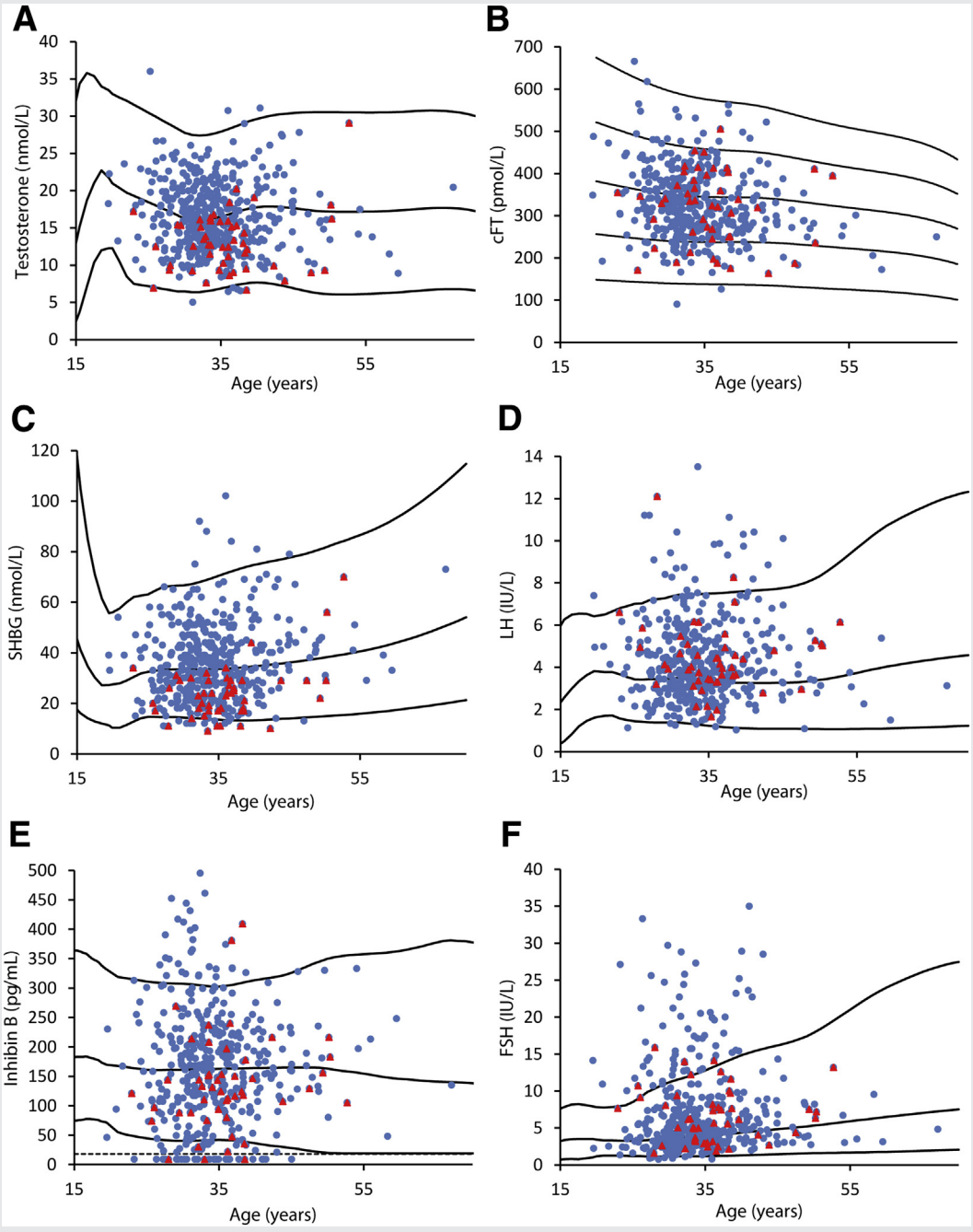
SUPPLEMENTAL FIGURE 2



(**A**) Serum T, (**B**) cFT, (**C**) SHBG, (**D**) LH, (**E**) inhibin B, and (**F**) FSH according to age in 432 idiopathic infertile men. Patients with body mass index >30 kg/m^2 are indicated with *red triangles*. Lines represent mean and 2.5th and 97.5th percentiles.

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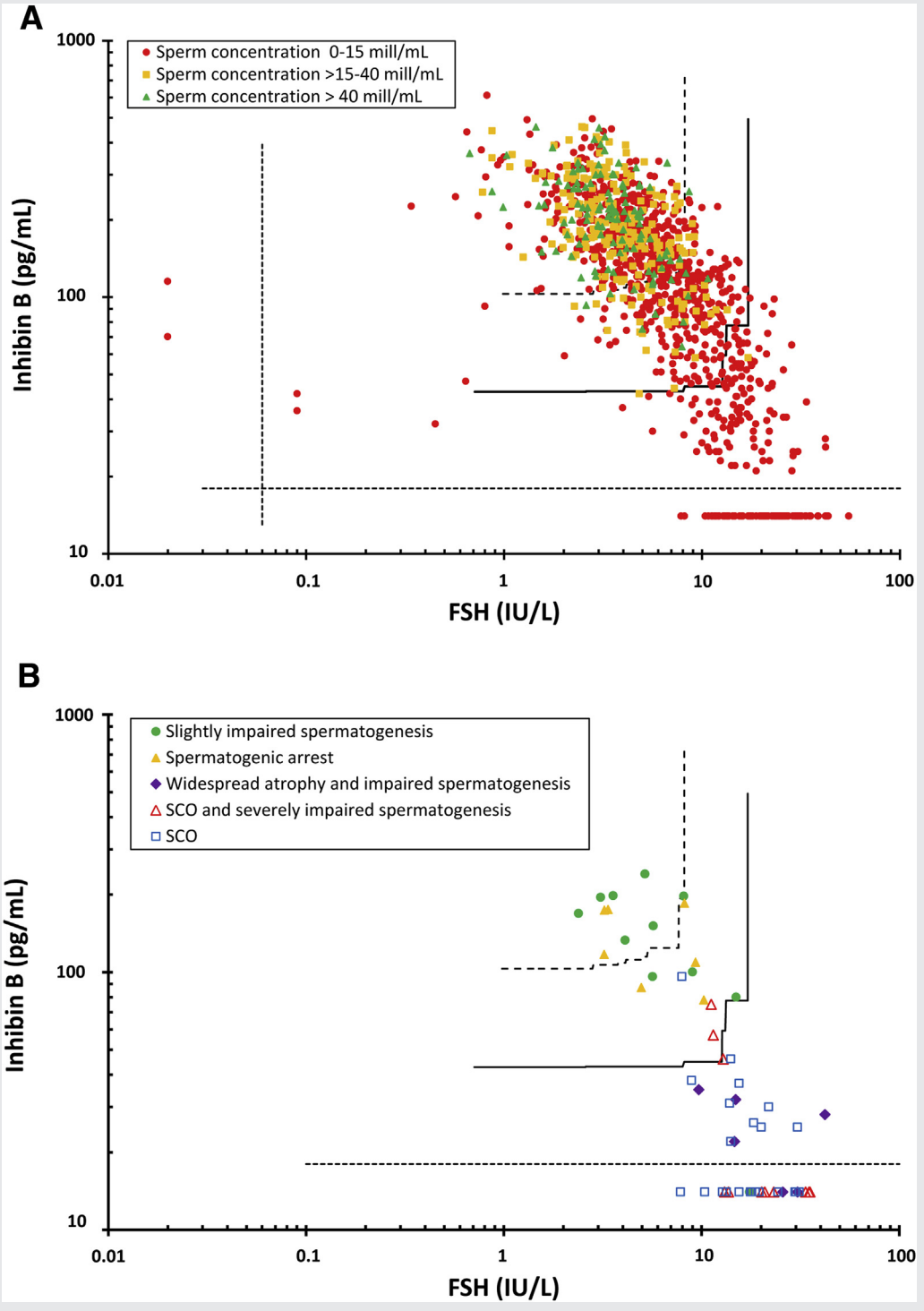
SUPPLEMENTAL FIGURE 3



Serum inhibin B plotted against FSH in a two-dimensional reference chart stratified by sperm concentration (**A**) in the entire study population and (**B**) in a subgroup of the patients with azoospermia stratified by diagnostic testicular biopsy. Lines represent fertile (*dashed*) and normal (*solid*) ranges and limits of detection (*dashed-dotted*) for FSH and inhibin B.

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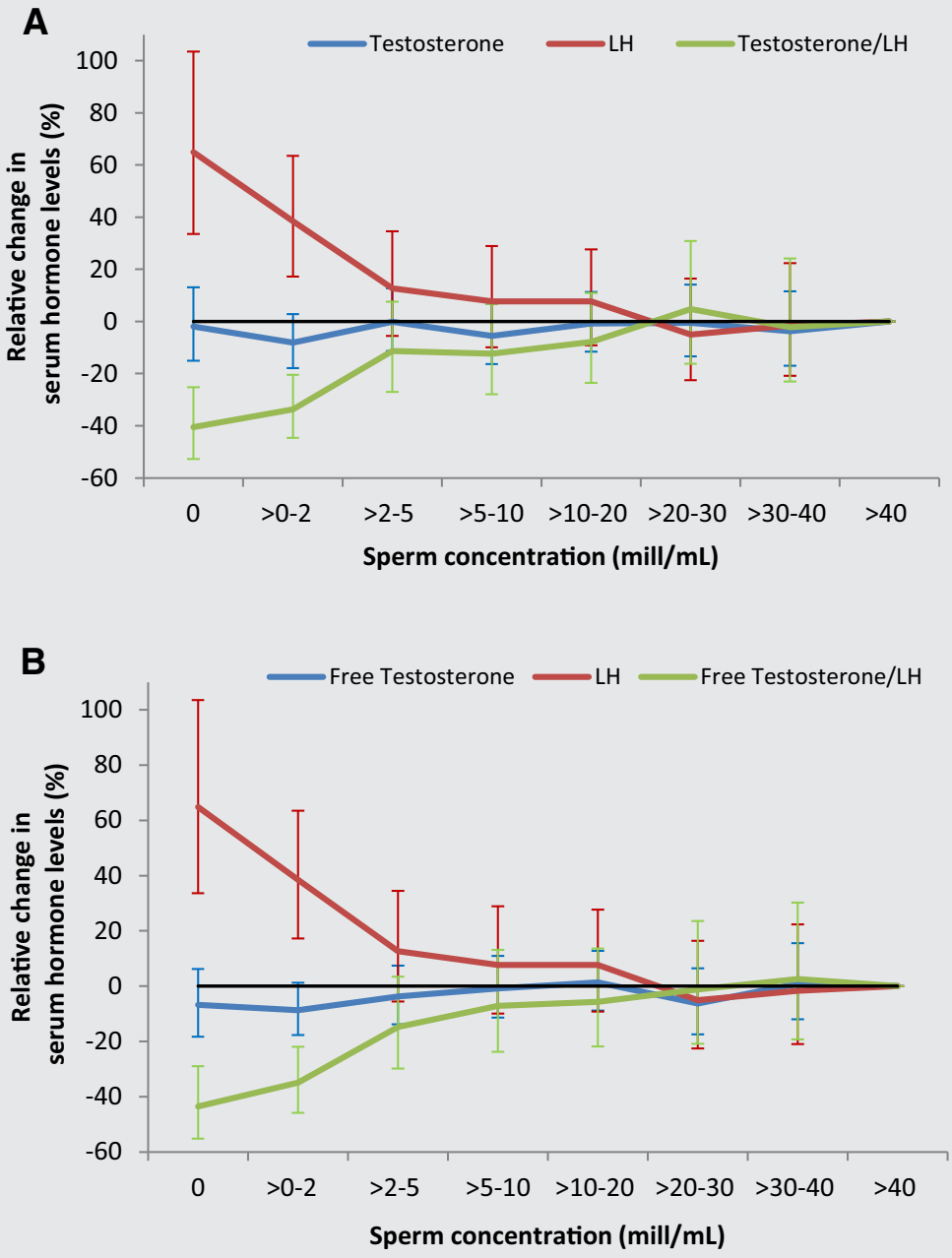
SUPPLEMENTAL FIGURE 4



Relative change in levels of (**A**) serum T, LH, and T/LH and (**B**) calculated free testosterone (cFT), LH, and cFT/LH in various categories of sperm concentration compared with the highest category of >40 million/mL.

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SUPPLEMENTAL TABLE 1

Overview of the current use of medical therapy within recent 3 months in the study population (n = 305).

Medical therapy	n	%
Medical therapy likely to affect fertility		
Finasterid and minoxidil	4	1.3
Antiepileptic drugs	6	2.0
Antiinflammatory drugs	4	1.3
Antiviral drugs	4	1.3
Cytostatis	4	1.3
Glucocorticoids	25	8.2
Immunomodulatory drugs	5	1.6
Immunosuppressive drugs	8	2.6
Opioids	10	3.3
Psychotropic medications	30	9.8
Testosterone	7	2.3
Other	10	3.3
Medical therapy unlikely to affect fertility		
Allergy and asthma medications	93	30.5
Antibiotics	11	3.6
Cialis and Viagra	6	2.0
Insulin	15	4.9
L-Thyroxine	2	0.7
Glycosamine	3	1.0
H2-antagonist	13	4.3
Antihypertensive drugs	6	2.0
Simvastatin	6	2.0
NSAID/paracetamol	33	10.8

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SUPPLEMENTAL TABLE 2

Overview of the chromosomal abnormalities in the study population (n = 33).

Chromosomal abnormality	n
Autosomal chromosomal abnormalities	
Reciprocal translocation (apparently balanced)	
46,XY,t(4;8)(p10;q10)	1
46,XY,t(13;19)(q13;p10)	1
46,XY,t(4;5)(q12;q23)	1
46,XY,t(1;21)(p31;q22)	1
46,XY,t(2;14)(p15;p11)dn	1
Robertsonian translocation (balanced)	
45,XY,der(13;14)(q10;q10)	3
45,XY,der(14;21)(q10;q10)	1
45,XY,der(13;15)(q10;q10)	1
Inversion (apparently balanced)	
46,XY,inv(16)(p11.2q11.2)	1
46,XY,inv(12)(p11p13)mat	1
46,XY,inv(3)(p13p21)	1
Variations	
46,XY,add (13)(p13)	1
47,XY,+mar.ish add(15)(D1521+,WCP15+)	1
47,XY,+mar.ish add(15)(D15Z1+)mat	1
47,XY,+mar.ish add(14)(D14Z1+,WCP14+)mat	1
Sex chromosomal abnormalities	
Numeric abnormalities	
47,XYY	1
47,XXY	11
45,X[12]/46,XY[28]	1
45,x[10]/46,x,idic(y)(q12)[20]	1
Structural abnormalities (apparently balanced)	
46,XY,t(y;5)(q11,2;q31)	1
46,X,t(y;19)(q11.2;q13.3)	1

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SUPPLEMENTAL TABLE 3

Overview of the Y-chromosome microdeletions in the study population (n = 65).

Y-chromosome microdeletion	n (%)	Sperm concentration (million/mL)	Range
AZF a+b+c	1 (1.5)	N.A.[a]	–
AZF a+b+ partial c (gr/gr)	1 (1.5)	0.4	–
AZF b+c	2 (3.1)	0	0–0
AZF b (b2/b3)	1 (1.5)	0	–
AZF c	3 (4.6)	0.008	0–0.1
Partial AZFc deletions			
b1/b3	3 (4.6)	0.015	0–0.4
gr/gr	33 (50.8)	5.9	0–97.3
b2/b3	21 (32.3)	7	0–29.4

[a] No semen sample to analyze.

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