ORIGINAL ARTICLE: ANDROLOGY

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® 2016;■:■–■. ©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

Received April 11, 2016; revised and accepted September 9, 2016.
I.A.O. has nothing to disclose. A.-M.A. has nothing to disclose. L.A. has nothing to disclose. N.E.S. has nothing to disclose. E.R.–d.M. has nothing to disclose. N.J. reports a grant from Ferring Pharmaceuticals. A.J. reports grants from Ferring Pharmaceuticals.
Supported by Rigshospitalets research grants (R24-A812 to I.A.O.; 9615.05.8.87 to A.J.; and R42-A1326 to N.J.), ReproUnion (EU InterReg project) and by the Innovation Fund Denmark (Innovations Fonden [Advancing Andrology], grant no. 14-2013-4). The sponsors of the study had no role in study design, data collection, analysis, interpretation, writing the article or in the decision to submit the article for publication.
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Fertility and Sterility® Vol. ■, No. ■, ■ 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.09.015

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, $\geq$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 $\geq$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 $\times$ 22 mm coverslip, placed in the heated stage in the microscope, and examined at $\times$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