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Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts

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STUDY QUESTION: What are the primary causes of severe male factor infertility?

SUMMARY ANSWER: Although 40% of all patients showed primary causes of infertility, which could be subdivided into three groups based on the severity of their effect, ~75% of oligozoospermia cases remained idiopathic.

WHAT IS KNOWN ALREADY: There are few large-scale epidemiological studies analyzing the causes of male factor infertility.

STUDY DESIGN, SIZE, DURATION: A prospective clinical-epidemiological study was conducted at the Andrology Centre, Tartu University Hospital between 2005 and 2013, recruiting male partners of couples failing to conceive a child for over ≥12 months. Among 8518 patients, 1737 (20.4%) were diagnosed with severe male factor infertility. A reference group of fertile controls was comprised of 325 partners of pregnant women.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The mean age of infertility patients and fertile controls was 33.2 ± 7.3 and 31.7 ± 6.3 years, respectively. All participants were examined using a standardized andrology workup, accompanied by a structured medical interview. Hormonal analysis included serum FSH, LH and testosterone. Semen quality was determined in accordance to the World Health Organization recommendations. Cases with spermatozoa concentrations of ≤5 million/ml were screened for chromosomal aberrations and Y-chromosomal microdeletions.

MAIN RESULTS AND THE ROLE OF CHANCE: The primary cause of infertility was defined for 695 of 1737 patients (~40%). The analyzed causal factors could be divided into absolute (secondary hypogonadism, genetic causes, seminal tract obstruction), severe (oncological diseases, severe sexual dysfunction) and plausible causal factors (congenital anomalies in uro-genital tract, acquired or secondary testicular damage). The latter were also detected for 11 (3.4%) men with proven fertility (diagnoses: unilateral cryptorchidism, testis cancer, orchitis, mumps orchitis). The causal factors behind the most severe forms of impaired spermatogenesis were relatively well understood; causes were assigned: for aspermia in 46/46 cases (100%), for azoospermia in 321/388 cases (82.7%), and for cryptozoospermia in 54/130 cases (41.5%). In contrast, 75% of oligozoospermia cases remained unexplained. The main cause of aspermia was severe sexual dysfunction (71.7% of aspermia patients). Azoospermia patients accounted for 86.4% of all cases diagnosed with secondary hypogonadism and 97.1% of patients with seminal tract obstruction. Of patients with a known genetic factor, 87.4% had extreme infertility (azoo-, crypto- or aspermia). The prevalence of congenital anomalies in the uro-genital tract was not clearly correlated with the severity of impaired sperm production. Previously defined 'potential contributing factors' varicocele and leukocytospermia were excluded as the primary causes of male infertility. However, their incidence was >2-fold higher (31.0 vs 13.5% and 16.1 vs 7.4%; $P < 0.001$) in the idiopathic infertility group compared to controls. In addition, the proportions of overweight (or obese) patients and patients suffering from a chronic disease were significantly increased in almost all of the patient subgroups.

LIMITATIONS REASONS FOR CAUTION: The study included only subjects with reduced total spermatozoa counts. Thus, these findings cannot be automatically applied to all male factor infertility cases.

WIDER IMPLICATIONS OF THE FINDINGS: The novel insights and improved clarity achieved in the comprehensive analysis regarding the absolute, causative and plausible factors behind male infertility, as well as the 'potential contributing factors', will be valuable tools in updating the current clinical guidelines. The study highlights knowledge gaps and reiterates an urgent need to uncover the causes and mechanisms behind, and potential treatments of, oligozoospermic cases, representing the majority of idiopathic infertility patients (86.3%).

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Introduction

Approximately 15% of couples of reproductive age fail to achieve a wanted pregnancy within a 12-month period, despite regular unprotected sexual intercourse. In 50% of involuntarily childless couples, abnormal semen parameters point to a male-infertility-associated factor (Jungwirth *et al.*, 2012). There is an apparent rising need to dissect the heterogeneous causes behind male infertility and to provide more personalized treatment of the condition. Currently, there are only a few large-scale epidemiological studies addressing this topic (WHO, 1997; Pierik *et al.*, 2000; Tüttelmann *et al.*, 2011). The published studies suffer from significant methodological shortcomings, such as insufficient efforts towards standardization of clinical and laboratory examinations, different referral levels of participating centers or missing relevant control groups. In addition, the rapid spread of invasive management solutions based on assisted reproductive technology approaches has reduced the interest among clinicians to properly investigate, diagnose and treat the causes of male infertility. There is an increasing tendency to neglect state-of-art high-quality and potentially informative andrological analysis of male partners of infertile couples before opting for ART. As a consequence, men with medical pathologies as the primary cause of their suboptimal semen quality are not properly diagnosed and treated. Inadequate assessment of the causes of male infertility leads to a common situation where the female partner is subjected to invasive, stressful and expensive ART procedures without a consideration of alternative solutions to manage the couple's infertility.

Estonia, a country of 1.3 million inhabitants, has a unique structure of andrological service in Europe. The Andrology Centre at Tartu University Hospital (AC-TUH) initiated its service in 2005 and has acted as one of the European Academy of Andrology (EAA) accredited centres since 2006. The AC-TUH serves as the country's non-referral university andrology clinic and performs a standardized clinical assessment of the majority of male counterparts of infertile couples in Estonia (800–1100 new patients per year). According to our best estimate, over 90% of Estonian infertile men diagnosed with severe male factor infertility have been assessed in the AC-TUH. The uniform quality of clinical examination is guaranteed by a special 4-year residential training program in andrology–urology provided since 1992 by the University of Tartu together with its hospital.

The current clinical-epidemiological analyses included 1737 patients with severe male factor infertility (sperm count <39 million per ejaculate; WHO, 2010) analyzed at the AC-TUH across a 9-year period. As a reference group of fertile controls, 325 partners of pregnant women were examined using an andrology workup identical to that of the infertility patients. The study aimed at (i) comparing the prevalence of generally accepted causal and potential contributing factors between the cases with severe male factor infertility and fertile controls and (ii) profiling the distribution of suspected primary causes of severe male factor infertility for patient subgroups based on semen analysis (aspermia, azoospermia, cryptozoospermia, severe and moderate oligozoospermia). To our knowledge, this is the first such large-scale, country-wide, prospective, monocentre study.

Materials and Methods

Ethics statement

The study was approved by the Ethics Review Committee on Human Research of the University of Tartu, Estonia. The study was conducted according to the Declaration of Helsinki principles. Written informed consent for evaluation and use of their clinical data for scientific purposes was obtained from each patient prior to recruitment.

The male factor infertility study group

The AC-TUH with its two branch offices (Tartu, Tallinn) served at the time of study group formation as both a primary centre (for self-referred patients) and referral centre for disorders of male reproductive health with the service area covering the whole Estonia. The recruitment phase of the current prospective study lasted 9 years (January 2005 till December 2013) and included male partners of couples failing to conceive a child over a period of ≥12 months. The total number of infertile men investigated between 2005 and 2013 was 8518. During this period, the AC-TUH performed 74.8% of semen analyses in the country (Supplementary Table SI). The study group of severe male factor infertility was formed based on reduced spermatozoa count (<39 million per ejaculate) in at least in two consecutive semen analyses. In case of substantial fluctuations

in the spermatozoa counts in alternative analysis, the inclusion/exclusion decision was based on the best semen analysis with optimal abstinence time (3–4 days or closest to this window). In total, 1737 (20.4%) men aged 33.2 ± 7.3 years fulfilled the inclusion criteria (Table I). All study participants were of white European ancestry with an exception of a one man (African).

In addition to the semen analysis, the study subjects went through a structured medical interview (100% of the study group) and were subjected to physical examinations (99.2%; refused or missing data: $n = 14$) and blood tests for hormonal analysis (98.4%; refused: $n = 27$). Of the patients with spermatozoa concentrations ≤5 million/ml ($n = 1216$), the majority ($n = 1074$) underwent karyotype analysis to identify large chromosomal abnormalities and screening for the Y-chromosomal microdeletions. For 86 patients (7.1%), genetic tests were not ordered as the definite cause of the infertility condition had already been diagnosed. There were 56 patients (4.6%) who refused genetic tests. In 2005–2013, the AC-TUH ordered 94.5% of Y-chromosomal microdeletion analyses in Estonia, indicating the concentration of the most severe cases of male infertility (Supplementary Table SI). Genetic analysis for the CTFR (Cystic fibrosis transmembrane conductance regulator) gene mutations was performed only for the cases of azoospermia accompanied with low semen volume and/or maldevelopment of seminal ducts and/or seminal vesicles.

The control group 'Partners of pregnant women'

The study group 'Partners of pregnant women' represented a reference sample for fertile control men, recruited in Estonia ($n = 325$; 31.7 ± 6.3 years; Table I). The group's composition is detailed in a recent publication (Ehala-Aleksejev and Punab, 2015). Briefly, in 2010–2014, male partners of informed pregnant women ($n = 3800$) at the Women's Clinics of TUH and West-Tallinn Central Hospital were invited to participate in the study and ~30% of eligible men agreed. The final recruitment and clinical assessment was conducted at both branches of the AC-TUH (Tartu and Tallinn). The participants had a choice to only complete a structured medical questionnaire or to additionally pass a standard andrological physical examination along with blood hormone and/or semen analysis. The full dataset including semen and blood samples, completed questionnaire and physical examination (identical to that of the infertility patients) was collected for 364 men (30% of participants recruited by AC-TUH). Among these, three men were excluded from the current study as the pregnancy of the couple had been achieved by IVF. Additional 10 men were excluded due to borderline oligozoospermia and 26 men due to extended (>12 months) time taken to achieve pregnancy. The final group analyzed as a reference sample for fertile control men was comprised of 325 men.

Semen analysis and applied nomenclature to define semen quality

Semen samples were obtained by patient masturbation and all semen values were determined in accordance to the World Health Organization (WHO) recommendations at the time of recruitment (WHO, 1999; WHO, 2010). In brief, after ejaculation, the semen was incubated at 37°C for 30–40 min for liquefaction. Semen volume was estimated by weighing the collection tube with the semen sample and subsequently subtracting the predetermined weight of the empty tube and assuming 1 g = 1 ml. For assessment of the spermatozoa concentration, the samples were diluted in a solution of 0.6 mol/l NaHCO3 and 0.4% (v/v) formaldehyde in distilled water. The spermatozoa concentration was assessed using the improved Neubauer haemocytometers. Motility was assessed in order to report the number of progressively motile spermatozoa (WHO motility classes A + B). Smears for morphology assessment were made. Following fixation and Papanicoulaou staining morphology was assessed according to strict criteria (Menkveld *et al.*, 1990). Semen smears were made for detecting white blood cells (WBC). The smears were air-dried, Bryan-Leishman stained, and examined with the use of oil immersion microscopy (magnification: ×1000) by an experienced microscopist. Polymorphonuclear leukocytes were differentiated from spermatids by the presence of segmented nuclei, bridges between lobes of nucleus, and specific granulation of the cytoplasm. The WBC concentration in semen was calculated by using the known spermatozoa concentration. In cases of low semen volume (<1.5 ml) and in clinical cases experiencing orgasm with missing antergade ejaculation, the retrograde ejaculation was confirmed by examining a sample of post-ejaculatory urine for the presence of spermatozoa.

The study group with severe male factor infertility ($n = 1737$) was subdivided based on the severity of semen impairment: (i) Aspermia was defined for cases not able to deliver semen; (ii) Azoospermia refers to spermatozoa missing in the ejaculate; (iii) Cryptozoospermia was defined in the current study as a spermatozoa count of <1 million/ejaculate; (This definition was applied as it enables definition of a clear-cut study group, although it differs from WHO (2010) criteria referring to a few sperm in the ejaculate identifiable only after concentration.) (iv) Severe oligozoospermia refers to a spermatozoa count of 1–10 million/ejaculate; (v) Moderate oligozoospermia refers to a spermatozoa count of 10–38 million/ejaculate.

Clinical examination

Patients were examined by six specialist clinicians, who had received the respective training in clinical assessment and standardized andrological workup, locally and in collaboration with the other EAA accredited centers (Carlsen *et al.*, 2000). Overall 59.9% and 19.8% of all subjects were investigated by two senior clinicians, MP and OP, respectively. The remaining patients (20.3%) were examined by four clinicians. The subject's height and weight for all patients were recorded by an assisting nurse.

Physical examination for the assessment of genital pathology and testicular size was performed with the man in standing position. If necessary, pathologies were clarified further with the men in supine position. The orchidometer (made of birch wood, Pharmacia & Upjohn, Denmark) was used for the assessment of testicular size. The total testes volume was the sum of right and left testicles. The position of the testicles in the scrotum, pathologies of the genital ducts (epididymis and ductus deference) and the penis, presence and grade of varicocele were registered for each study participant. Varicocele was graded according to a traditional system (Dubin and Amelar, 1970) as follows: Grade 1—palpated only on the Valsalva maneuver; Grade 2—venous distension easily palpable but not visible; Grade 3—venous plexus bulges through the scrotal skin, visible and palpable. Varicoceles were classified according to the highest assigned grade, independent from the affected side. Objective physical examination and interviewing on the patient's medical history was applied to diagnose existing and retrospective cases of cryptorchidism and document past operations due to inguinal hernia.

Hormone assays

Venous blood of the patient was drawn from the cubital vein in the morning from 08.00 to 10.30 and serum was separated immediately. FSH, LH and total testosterone levels of blood serum were measured using the Immulite automated chemiluminescence immunoassay analyser (Immulite; Diagnostic Products Corp., Los Angeles, CA, USA) according to manufacturer's instructions, at the United Laboratories, Tartu University Hospital. For IMMULITE 1000 system analytical sensitivity was 0.1 IU/L for both LH and FSH. The intra- and inter-assay CVs were 4.2 and 8% for FSH, 4.0 and 7.1% for LH, 6.3 and 9.4% for testosterone and 7.5 and 13% for estradiol.

Table 1 Characteristics of patient group with severe male factor infertility compared to the group of men with proven fertility.

	Severe male factor infertility (n = 1737)		Partners of pregnant women (n = 325)		Among groups
	Mean (SD)	Median (5–95)	Mean (SD)	Median (5-95)	*P*-value*
General parameters					
Age (years)	33.2 (7.3)	32.3 (23.2–46.6)	31.7(6.3)	31 (22.9–44)	0.001
Height (cm)	181.4 (7.4)	182 (170–193)	180.8 (6.4)	181 (171–192)	0.132
Weight (kg)	87.9 (7.49)	85.5 (64–118)	83.4 (12.94)	82 (64–107)	<0.001
BMI	26.7 (4.6)	26 (20.2–35.1)	25.5 (3.66)	24.8 (20.3–32.2)	<0.001
Duration of infertility (yrs)	3.1 (3.1)	2 (1–10)	NA	NA	
Testicular and seminal parameters					
Total testis volume (ml)	36.1 (12.8)	37 (9–51)	47.1 (10.0)	47 (34–62)	<0.001
Abstinence time (days)	3.8 (2.2)	3 (2–7)	4.33 (4.6)	3 (2–8)	0.738
Ejaculate volume (ml)	3.56 (1.89)	3.3 (0.7–7)	4.15 (1.78)	3.79 (1.8–7.9)	<0.001
Sperm concentration (million/ml)	4.1 (5.1)	3 (0–13)	100.5 (79.57)	80 (19–247)	<0.001
Total sperm count (million)	12.2 (12.5)	8 (0–36)	394.4 (328.8)	303 (67–985)	<0.001
Progressive motility (%)	22.3 (17.2)	20 (0–54)	50.3 (12.2)	51 (30–70)	<0.001
Normal morphology (%)	2.2 (3.2)	1 (0–8)	10.1 (5.5)	10 (2–20)	<0.001
Neutrophil count (million/ml)	0.62 (2.7)	0.1 (0–2.4)	0.34 (1.6)	0.1 (0–1.4)	0.388
Hormonal parameters					
FSH (IU/L)	10.7 (11.7)	6.8 (1.7–35.1)	4.0 (2.2)	3.5 (1.4–7.6)	<0.001
LH (IU/L)	5.5 (4.4)	4.4 (1.5–13.9)	3.8 (1.8)	3.7 (1.5–6.8)	<0.001
FSH/LH ratio	1.97 (1.3)	1.63 (0.55–4.36)	1.2 (0.6)	1.0 (0.4–2.4)	<0.001
Testosterone (nmol/l)	17.2 (7.3)	16.1 (7.5–30.4)	17 (5.9)	16.4 (8.7–27.4)	0.794
Prevalence of known causal factors for male inferility (n, %)					
Cryptorchidism	165	9.5%	6	1.8%	<0.001
Testis cancer	27	1.6%	1[b]	0.3%	0.075
Orchitis/epididymitis	66	3.8%	2	0.6%	0.003
Mumps orchitis	18	1.0%	2	0.6%	0.477
Prevalence of potential contributing factors to infertility (n, %)					
Varicocele (total)	615	35.4%	79	24.3%	<0.001
grade3	81	4.7%	5	1.5%	<0.001
grade2	370	21.3%	39	12.0%	<0.001
grade1	148	8.5%	33	10.2%	0.340
operated	16	0.9%	2	0.6%	0.587
Leukocytospermia	233	13.4%	24	7.4%	<0.001
Testis trauma	116	6.7%	27	8.3%	0.289
Hernia inguinalis operation	86	4.3%	16	4.9%	0.626
Chronic diseases	424	24.4%	32	9.8%	<0.001
Overweight[a]	893/1479	60.4%	158	48.6%	<0.001
Obesity[a]	325/1479	22.0%	44	13.5%	0.001

Severe male factor infertility is defined based on reduced (<39 million) total spermatozoa count. The group 'Partners of pregnant women' represent controls with proved fertility.
* Statistical significance between the two groups was assessed using Mann–Whitney *U*-test for quantitaitve parameters (A-C) and Pearson's Chi-Square test for categoric parameters (D-E).
[a]Data missing for 258 infertility patients.
[b]Teratoma with no chemo/radiation therapy.
BMI, body mass index; NA, not applicable.

Genetic analyses

Tests for the known genetic causes of male infertility were performed at the United Laboratories of Tartu University Hospital. Karyotype analysis to identify large chromosomal abnormalities and screening for the Y-chromosomal microdeletions were performed.

Karyotyping in the cytogenetic analysis was carried out on the metaphase chromosome spreads derived from cultured peripheral blood

lymphocytes. Y-chromosome microdeletions (AZFa, AZFb, AZFc) were analyzed according to the EAA guidelines, eligible at the time of the analysis (Simoni et al., 2004).

Genetic analysis for the CTFR included three most prevalent causative CTFR mutations in Estonia (p.F508del, 394delTT, IVS8 5T/7T/9T) (Teder et al., 2000). From the year 2008 onward, all patients subjected to CTFR analysis have been screened in parallel with an APEX (Arrayed Primer EXtension) technology using a targeted microarray designed for the CTFR mutations (Schrijver et al., 2005). The obtained genotypes of the three common variants were 100% concordant between the traditional single mutation analysis and the APEX approach. All detected rare variants exhibited heterozygote status and were of unknown clinical significance. No patients were detected as homozygous carriers of a rare mutation, as a compound heterozygote for a common (p.F508del, 394delTT, IVS8 5T/7T/9T) and a rare mutation, or as a compound heterozygote of rare variants.

Four dedicated study nurses (two in each center) entered the collected epidemiological, laboratory and clinical examination data into two separate, but identically structured, databases. Prior to statistical analysis, the two databases were merged and duplicate entries were eliminated. The entered laboratory data were counter-controlled from primary sources (lab databases) and, if needed, edited by a specially trained researcher (PP). Clinical data relevant to define the cause of male infertility were controlled retrospectively for all study subjects from their medical case histories (2005–2008 in paper format, from 2009 onward in electronic format) one-by-one by the corresponding author of the study (MP).

Definition of causal factors for severe male factor infertility

Causal factors were defined as known clinical and genetic factors with unequivocal or major negative effect on male reproductive function. Hierarchical ordering of causal factors was applied in order to define the specific primary cause of severe male factor infertility for each patient. The following order of considered causal factors from the strongest towards the least influential effects was applied (hierarchy defined by MP): (i) genetic causes, (ii) secondary hypogonadism, (iii) congenital anomalies: systemic and/or in uro-genital tract, (iv) oncological diseases, (v) serious sexual dysfunctions, (vi) seminal tract obstruction, (vii) other testicular factors (details in Table II). The patient was diagnosed with idiopathic infertility when no generally accepted known causal factor for male infertility could be identified.

Every clinical case was diagnosed with only one primary cause. In patients that had been assigned two known causal factors, the primary cause was defined according the factor which had a hierarchically higher classification position in the current study. An example of the hierarchical classification is a patient with Klinefelter syndrome and cryptorchidism, where the primary causal factor is the genetic cause. Also subjects with CTFR gene mutations causative for seminal tract obstruction were listed in the genetic origin group as the primary cause. All subjects included in this category were either homozygous for common mutations (p.F508del, 394delTT, IVS8 5T/7T/9T) or compound heterozygous for two common mutations. For a patient with cryptorchidism and a history of epididymitis or treatment for testis cancer in his later life, the defined primary causal factor is congenital anomalies in uro-genital tract. Men with suboptimal sperm counts, but normal testicular biopsy or TESE results (sufficient number of spermatozoa in one TESE specimen) were diagnosed with seminal tract obstruction. The only exception in hierarchical order is in the group of severe sexual dysfunction, where two subjects had also cryptorchidism, but according to a consensus clinical decision, the primary cause of current infertility problem was still sexual dysfunction. Diagnosis of sexual dysfunction excluded the cases with a lack of sexual interest and erectile dysfunctions as treatable conditions not affecting semen quality.

Among the group with the diagnosis of infertility due to other testicular factors, were included testis traumas and genital tract operations only if the testicular damage was followed by an immediate substantial post-event decrease in testicular volume. Oncological cases were included only if they received systematic chemotherapy, radiotherapy, operative treatment affecting anatomical integrity of the genital tract or combinations of these treatment modalities. Azoospermia cases with normal or borderline testis volume, FSH and FSH/LH ratio, but unavailable testis biopsy or TESA results ($n = 6$), were included into idiopathic infertility group.

Patients diagnosed with more than one causal factor

In total 28 (1.6%) of the analyzed severe infertility cases were diagnosed with more than one causal factor. Among the 135 patients with an identified major genetic cause, there were concomitant cases of cryptorchidism (seven patients), anejaculation (1), orchitis (2) and anabolic steroid abuse (2). Among 22 men diagnosed with secondary hypogonadism, one patient had an accompanying cryptorchidism and another anorgasmia. The large group of congenital anomalies in uro-genital tract ($n = 186$) included patients with concomitant anamnesis testis cancer (one case), obstruction (1), orchitis (5), mumps orhitis (1) and a long-lasting salasopyrin usage (1). Among the patients with the anamnesis of oncological diseases there were two cases of post-treatment severe sexual dysfunctions, and one case of abused anabolic steroids. Two men with the severe sexual dysfunction had an unrelated medical history of cryptorchidism.

Definition of additional potential contributing factors to male infertility

We also analyzed the prevalence of additional clinical factors considered as potential contributors to male infertility. Although the harmful effects of these risk factors on male reproductive potential are acknowledged, these do not often cause infertility. Thus, in this report we use the term 'potential contributing factors'. The analyzed clinical conditions in relation to male infertility included varicoceles (WHO, 1997; WHO, 1992), leukocytospermia (Jungwirth et al., 2012), testicular traumas (Lin et al., 1998), hernia operations (Gulino et al., 2012), chronic diseases (Baker, 1998; De Sanctis et al., 2013) and overweight/obesity (Ehala-Aleksejev and Punab, 2015). Testicular trauma was defined as traumatic event causing testicular swelling or scrotal skin bruising. Leukocytospermia was defined according to WHO, 2010 definition for the neutrophil count >1 million/ml. Chronic diseases were defined based on the retrospective clinical anamnesis of the patient as a previously diagnosed and treated non-genital disease with a duration of at least 3 months. Most common chronic disorders included cardiovascular diseases, various endocrinopathies, asthma, neurological disorders and depression, renal, gastrointestinal and joint diseases. 'Overweight' was defined if the BMI was >25 and 'obesity' was defined if the BMI >30. The definition of contributing factors for each clinical case was supervised and corroborated by MP.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 22.0 for Windows.

Results

Clinical profile of patients with severe male factor infertility compared to partners of pregnant women: the generally accepted causal factors are not explicit

Among the 8518 male partners of infertile couples examined at the AC-TUH in 2005–2013, 20.4% represented patients with severe male

Table II Distribution of known causal factors for severe male factor inferility in the full study group and in the subgroups of the patients with reduced semen quality.

Causal factors for severe male infertility	Full group *n* = 1737		Aspermia *n* = 46		Azoospermia *n* = 388		Cryptozoospermia[a] *n* = 130		Severe oligozoosp.[b] *n* = 360		Moderate Oligozoosp.[c] *n* = 813	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
1. Genetic causes	135	7.8	4	8.7	100	25.8	14	10.8	9	2.5	8	1.0
1.1. Autosomal aberrations	13	0.7			2	0.5	2	1.5	6	1.7	3	0.4
Inversions	3	0.2			1	0.3			1	0.3	1	0.1
Marker chromosome	4	0.2					1	0.8	2	0.6	1	0.1
Translocations	6	0.3			1	0.3	1	0.8	3	0.8	1	0.1
1.2. CTFR mutations	11	0.6			10	2.6	1	0.8				
Obstruction	10	0.6			10	2.6						
Subobstruction	1	0.1					1	0.8				
1.3. Sex chromosome abnormalities	71	4.1	4	8.7	63	16.2			2	0.6	2	0.2
46,XX male	1	0.1	1	2.2								
47,XXY	60	3.5	3	6.5	57	14.7						
Mosaicism 47,XXY/46,XY	5	0.3			4	1.0					1	0.1
47,XYY	4	0.2			1	0.3			2	0.6	1	0.1
Mosaicism 47,XYY/46,XY	1	0.1			1	0.3						
1.4. Y chromosome microdeletions	40	2.3			25	6.4	11	8.5	1	0.3	3	0.4
AZFb and *AZFc* deletion	6	0.3			6	1.5						
AZFb deletion only	2	0.1			2	0.5						
AZFc deletion only	32	1.8			17	4.4	11	8.5	1	0.3	3	0.4
2. Secondary hypogonadism	22	1.3	2	4.3	19	4.9	1	0.8				
2.1. Hypothalamic	14	0.8	1	2.2	13	3.4						
Kallmann syndrome	3	0.2			3	0.8						
Isolated hypogonadotropic hypogonadism	6	0.3	1	2.2	5	1.3						
Secondary GnRH deficiency	5	0.3			5	1.3						
2.2. Hypopituitarism	7	0.4	1	2.2	5	1.3	1	0.8				
Craniopharyngioma operation	1	0.1			1	0.3						
Prolactinoma operation	3	0.2	1	2.2	2	0.5						
Traumatic	1	0.1			1	0.3						
Unknown	2	0.1			1	0.3	1	0.8				
2.3. Other	1	0.1			1	0.3						
Congenital adrenal hyperplasia	1	0.1			1	0.3						

Continued

Table II *Continued*

Causal factors for severe male infertility	Full group *n* = 1737		Aspermia *n* = 46		Azoospermia *n* = 388		Cryptozoospermia[a] *n* = 130		Severe oligozoosp.[b] *n* = 360		Moderate Oligozoosp.[c] *n* = 813	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
3. Congenital anomalies in uro-genital tract	186	10.7	3	6.5	55	14.2	17	13.1	37	10.3	74	9.1
3.1. Systemic	17	1.0	1	2.2	7	1.8	5	3.8	1	0.3	3	0.4
Unilateral renal & seminal vesicles agenesis	2	0.1			1	0.3			1	0.3		
Unilateral renal & seminal vesicles Agenesis+ cryptorchidism	1	0.1			1	0.3						
Spina bifida aperta + microanomalies	4	0.2					3	2.3			1	0.1
Bladder exstrophy	1	0.1	1	2.2								
Renal hypoplasia	2	0.1					1	0.8			1	0.1
Renal hypoplasia + bilateral cryptorchidism	1	0.1			1	0.3						
Severe hypospadias + bilateral cryptorchidism	1	0.1			1	0.3						
Anorectal malformations	1	0.1					1	0.8				
CHARGE syndrome	1	0.1									1	0.1
Fabry disease	1	0.1			1	0.3						
Klippel-Trenaunay-Weber Syndrome	1	0.1			1	0.3						
Multiple microanomalies, unspecified	1	0.1			1	0.3						
3.2. Testicular	169	9.7	2	4.3	48	12.4	12	9.2	36	10.0	71	8.7
3.2.1. Cryptorchidism	143	8.2	1	2.2	43	11.1	9	6.9	34	9.4	56	6.9
Bilateral	43	2.5	1	2.2	21	5.4	1	0.8	11	3.1	9	1.1
Unilateral	69	4.0			8	2.1	6	4.6	18	5.0	37	4.6
Unilateral + contralateral agenesis	2	0.1			2	0.5						
Unilateral untreated	26	1.5			9	2.3	3	2.3	4	1.1	10	1.2
Bilateral untreated	3	0.2			2	0.5			1	0.3		
3.2.2. Congenital anorchia	7	0.4	1	2.2	3	0.8			1	0.3	2	0.2
Bilateral	2	0.1	1	2.2	1	0.3						
Unilateral	5	0.3			2	0.5			1	0.3	2	0.2
3.2.3. Triorchidism	1	0.1									1	0.1
3.2.4. Unilateral developmental disorder of testis	18	1.0			2	0.5	3	2.3	1	0.3	12	1.5
4. Oncological diseases	59	3.4	3	6.5	31	8	3	2.3	9	2.5	13	1.6
4.1. Before operation and/or gonadotoxic treatment	6	0.3			2	0.5	1	0.8	1	0.3	2	0.1
Testis cancer, unilateral	5	0.3			2	0.5	1	0.8	1	0.3	1	0.1
Prostate carcinoma	1	0.1									1	0.1

4.2. After operation and/or gonadotoxic treatment	53	3.1	3	6.5	29	7.5	2	1.5	8	2.2	11	1.5
Testis cancer, unilateral	19	1.1	2	4.3	6	1.5	1	0.8	5	1.4	5	0.6
Testis cancer, bilateral	1	0.1			1	0.3						
Hematologic cancer with seconadary testis cancer	1	0.1			1	0.3						
Hematological cancers	22	1.3			17	4.4	1	0.8	2	0.6	2	0.2
Bone cancer	3	0.2			3	0.8						
Thyroid cancer	3	0.2							1	0.3	2	0.2
Carcinoma recti	1	0.1									1	0.1
Carcinoma colon	1	0.1									1	0.1
Laryngeal carcinoma	1	0.1			1	0.3						
Brain carcinoma	1	0.1	1	2.2								
5. Severe sexual dysfunction	76	4.4	33	71.7	3	0.8	6	4.6	11	3.1	23	2.8
5.1. Anorgamia (in case of vaginal sex)	8	0.5	4	8.7					1	0.3	3	0.4
Spinal trauma	3	0.2	3	6.5								
Diabetes mellitus	1	0.1	1	2.2								
Masturbation successful, idiopathic	3	0.2									3	0.4
Masturbation successful, spinal trauma	1	0.1							1	0.3		
5.2. Anejaculation	20	1.2	20	43.5								
Spinal trauma	13	0.7	13	28.3								
Diabetes mellitus	2	0.1	2	4.3								
Sclerosis multiplex	1	0.1	1	2.2								
Epilepsy	1	0.1	1	2.2								
Idiopathic	3	0.2	3	6.5								
5.3. Retrograde ejaculation (total)	9	0.5	9	19.6								
Diabetes mellitus	6	0.3	6	13								
Post TURP	1	0.1	1	2.2								
Idiopathic	2	0.1	2	4.3								
5.4. Retrograde ejaculation (partial)	39	2.2			3	0.8	6	4.6	10	2.8	20	2.5
Diabetes mellitus	5	0.3			1	0.3			1	0.3	3	0.4
Spinal trauma	4	0.2					1	0.8	1	0.3	2	0.2
Hypospadia operations	1	0.1			1	0.3						
Post TURP	1	0.1									1	0.1
Serious pelvic trauma	1	0.1									1	0.1

Continued

Table II *Continued*

Causal factors for severe male infertility	Full group *n* = 1737		Aspermia *n* = 46		Azoospermia *n* = 388		Cryptozoospermia[a] *n* = 130		Severe oligozoosp.[b] *n* = 360		Moderate Oligozoosp.[c] *n* = 813	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Idiopathic	27	1.6			1	0.3	5	3.8	8	2.2	13	1.6
6. Seminal tract obstruction	103	5.9			100	25.8	2	1.5			1	0.1
Epididymal (defined per exclusionem)	99	5.7			97	25	2	1.5				
Prostate	1	0.1			1	0.3						
Pelvic trauma	1	0.1			1	0.3						
Vasectomy	1	0.1			1	0.3						
Status post vaso-epididymostomy	1	0.1									1	0.1
7. Other testicular factors	114	6.6	1	2.2	13	3.4	11	8.5	28	7.8	61	7.5
7.1. Acquired testicular damage (TD)	86	5.0	1	2.2	7	1.8	6	1.7	22	6.1	50	6.2
Exposure to high dose radiation in Chernobyl[d]	1	0.1									1	0.1
Testis trauma with volume change	11	0.6	1	2.2	1	0.3	1	0.8	4	1.1	4	0.5
Mumps orchitis	17	1.0			5	1.3			4	1.1	8	1.0
Orchitis, epididymitis	34	2.0			1	0.3	2	1.5	10	2.8	21	2.6
Testicular torsion	6	0.3					2	1.5			4	0.5
Hernia operation with ipsilateral TD	8	0.5							3	0.8	5	0.6
Epididymal cyst operation with ipsilateral TD	5	0.3							1	0.3	4	0.5
Hydrocele operation with ipsilateral TD	3	0.2									3	0.4
Other testis operation with ipsilateral TD	1	0.1					1	0.8				
7.2. Secondary testicular damage	28	1.6			6	1.5	5	3.8	6	1.7	11	1.4
Anabolic steroids	20	1.2			6	1.5	4	3.1	4	1.1	6	0.7
Medication – salasopyrin, trexan	6	0.3					1	0.8			5	0.6
Status diagnosed post kidney transplantation	2	0.1							2	0.6		

[a]Cryptozoospermia refers in this study to spermatozoa count <1 million/ejaculate.
[b]Severe oligozoospermia refers to spermatozoa count 1–10 million/ejaculate.
[c]Moderate oligozoospermia refers to spermatozoa count 10–38 million/ejaculate.
[d]Estonian residents, who had participated in the crisis management on-site of the Chernobyl disaster immediately after the 1986 nuclear plant accident in the Ukraine (former Ukraine SSR belonging to the USSR).
CAVD, Congenital absence of the vas deferens; CTFR, Cystic fibrosis transmembrane conductance regulator gene; TURP, Transurethral resection of the prostate.

factor infertility (n = 1737; <39 million spermatozoa/ejaculate; aged 33.2 ± 7.3 years) (Table I). The status of infertility had lasted 3.1 ± 3.1 years, and it was primary or secondary in 83.2% and 16.8% of cases, respectively. Compared to the partners of pregnant women representing the referral group for fertile men (n = 325, 31.7 ± 6.3 years), a higher proportion of infertile patients were overweight (60.4 vs 48.6%; $P < 0.01$) or obese (22.0 vs 13.5%; $P < 0.001$). As expected, infertile men differed significantly from the controls in seminal and testicular parameters, and in the increased FSH and LH levels ($P < 0.001$). The patient and control groups did not differ in abstinence time, semen neutrophil count and testosterone measurements.

Notably, among the partners of pregnant women there were 11 cases with four generally accepted known causal factors for male infertility: clinical history of cryptorchidism (six cases; all unilateral), testis cancer (one case), orchitis/epididymitis (2) and mumps orchitis (2) (Table I). Both cases of mumps orchitis had a substantially reduced size of the affected testis, but a high volume of the contralateral testis (7 + 40 ml, 8 + 25 ml, respectively). In total, these four diagnoses were assigned to 3.4% of fertile controls compared to 15.9% among patients. In addition, there was no statistical difference between the groups for the prevalence of testes cancer and mumps orchitis. We conclude that although on most occasions these four diagnoses represent causal factors for severe male factor infertility, the risk is not absolute, especially when only one testis is affected.

Among the 'potential contributing factors', infertility patients exhibited a 2-fold increased prevalence of varicocele (Grade 2 and 3: 26.0 vs 13.5% in controls), leukocytospermia (13.4 vs 7.4%) and a medical history of chronic diseases (24.4 vs 9.8%; all comparisons, $P < 0.001$). Grade 1 and operated varicocele, history of testes trauma or hernia inguinalis operation without substantial testicular damage were not identified as risk factors for infertility.

The primary causes of severe male infertility differ among the clinical subgroups of patients

For 695 of 1737 infertility patients (40%), the currently applied analyses were able to define the primary cause of infertility, whereas 1042 (60%) remained idiopathic (Tables II–III). The prevalence of known causal factors for severe male factor infertility showed a clear gradient from more extreme towards less severe cases of impaired sperm parameters. In the aspermia subgroup, the primary causal factor for the condition was identified for all 46 patients (100%; Table III). The clinical diagnosis of the causative factor was assigned for 82.7% of azoospermia (n = 321/388), 41.5% of cryptozoospermia (n = 54/130), 26.1% of severe (n = 94/360) and 22.1% of moderate (n = 180/813) oligozoospermia groups (Table III). Among the oligozoospermia patients, ~75% were diagnosed as idiopathic.

There were substantial differences in the distribution of the major causes of infertility among the clinical subgroups (Table II). In the aspermia group (n = 46), the main causative factor was severe sexual dysfunction (71.7% of cases; n = 33). Secondary hypogonadism (n = 19/22; 86.4% of the diagnosis group) and seminal tract obstruction (n = 100/103; 97.1% of the diagnosis group) were identified

Table III Proportion of causal factor and idiopathic infertility among patients grouped based on the severity of semen impairment.

Subgroups based on diagnosis	All patients		Aspermia		Azoospermia		Cryptozoospermia[a]		Severe oligozoosp.[b]		Moderate Oligozoosp.[c]	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Patients with causal factor for infertility	695	40	46	100	321	82.7	54	41.5	94	26.1	180	22.1
Idiopathic infertility	**1042**	**60**	**0**	**0**	**67**	**17.3**	**76**	**58.5**	**266**	**73.9**	**633**	**77.9**

[a]Cryptozoospermia refers in this study to spermatozoa count <1 million/ejaculate.
[b]Severe oligozoospermia refers to spermatozoa count 1–10 million/ejaculate.
[c]Moderate oligozoospermia refers to spermatozoa count 10–38 million/ejaculate.

almost exclusively among azoospermia patients. Overall, seminal tract obstruction and gross genetic aberrations explained more than half of the azoospermia cases (each diagnosis 25.8%; $n = 100/388$). Known genetic factors caused extreme infertility (azoo-, crypto- or aspermia) in 87.4% of cases of genetic disease ($n = 118/135$). There were five subjects with 47,XYY syndrome (including one mosaic case), which is seldom counted as a causal factor for male infertility (Kim *et al.*, 2013). The prevalence of congenital anomalies in the uro-genital tract was not clearly correlated with the severity of impaired sperm production. More than one causal factor was assigned to 28 (1.6%) patients (see Methods section).

Role of 'potential contributing factors' in severe male factor infertility

The proportion of patients with chronic disease, overweight and obesity was increased in both the causal factor and idiopathic infertility groups ($P < 0.001$) compared to fertile men (Table IV-A). In contrast, Grade 2–3 varicocele (31.0 vs 13.5% in controls) and leukocytospermia (16.1 vs 7.4%) exhibited a >2-fold higher prevalence only among idiopathic infertility patients ($P < 0.001$), whereas the causal factor group did not differ from the controls.

Next, we analyzed the prevalence of the 'potential contributing factors' among the causal factor diagnosis subgroups (Table IV-B). Significantly increased prevalences of leukocytospermia and varicocele were detected among the patient group with the diagnosis of 'seminal tract obstruction' (18.4 vs 4.5–9.1% for other diagnoses) and 'other testicular disorders' (23.7 vs 13.6–19.7% for other diagnoses), respectively. This observation may possibly reflect the specific causative chain of these clinical conditions. Diagnosis of chronic diseases was ~2-fold elevated compared to controls only in cases of infertility caused by 'other testicular factors' (26.3%), 'congenital anomalies of uro-genital tract' (24.6%) and 'genetic causes' (23.0%) (all compared to 9.8% in controls; $P < 0.001$). The highest proportion of obese patients (BMI > 30) was observed in the subgroup of 'secondary hypogonadism' (35.0 vs 13.5% in controls; $P < 0.05$).

Varicocele and leukocytospermia appeared to minimally affect aspermia and azoospermia, which are mostly caused by explicit causal factors (Tables II; IV-C). The diagnosis of varicocele was assigned to almost every third man with cryptozoospermia (27.7%), severe (32.2%) and moderate oligozoospermia (28.5%) (all compared to 13.5% in controls; $P < 0.001$). The prevalence of leukocytospermia was elevated only among the oligozoospermia patients (14.7–15.5% vs 7.4% in controls; $P < 0.01$).

Table IV Distribution of additional 'potential contributing factors' to male infertility.

		Varicocele, Grade 2–3		Leukocytospermia		Chronic disease		Overweight[a]		Obesity[a]	
	n	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
A. All study subjects ($n = 1737$)											
Male partners of pregnant women	325	44	13.5	24	7.4	32	9.8	158	48.6	44	13.5
Infertility, causal factor identified	695	128	18.4	65	9.3	141	20.3***	364	63.9***	128	22.5***
Infertility, idiopathic cases	1042	323	31.0***#	168	16.1***#	283	27.2***#	529	58.2**#	197	21.7**
B. Infertile patients subgrouped based on identified known causal factor ($n = 695$)											
1. Genetic causes	135	24	17.8	9	6.7	31	23.0***	64	58.7	28	25.7*
2. Secondary hypogonadism	22	4	18.2	1	4.5	3	13.6	13	65.0	7	35*
3. Congenital anomalies in uro-genital tract	186	32	17.1	17	9.1	46	24.6***	99	60.4*	33	20.1
4. Oncological diseases	59	8	13.6	6	8.5	8	13.6	41	71.9*	10	17.5
5. Serious sexual dysfunction	76	15	19.7	5	6.6	8	10.5	32	69.6*	10	21.7
6. Seminal tract obstruction	103	18	17.5	19	18.4**	15	14.6	53	65.4*	14	17.3
7. Other testicular factors	114	27	23.7*	9	7.9	30	26.3***	62	66.6**	26	28.0**
Kruskall–Wallis test for overall distribution, *P*-value			$P < 0.001$		$P < 0.001$		$P = 0.002$		NS		NS
C. All patients according to the category of semen quality ($n = 1737$)											
1. Aspermia	46	4	8.7	N/A	N/A	4	8.7	19	63.3	7	33.3
2. Azoospermia	388	63	16.2	43	11.1	76	19.6***	205	63.3***	76	23.5**
3. Cryptozoospermia	130	36	27.7***	53	8.5	93	30.7***	77	72***	28	26,2**
4. Severe oligozoospermia	360	116	32.2***	53	14.7**	93	25.8***	189	61.6**	64	20.8*
5. Moderate oligozoospermia	813	232	28.5***	126	15.5***	211	26***	403	56.7*	150	21.1**
Kruskall–Wallis test for the overall distribution, *P*-value			$P < 0.001$		$P < 0.001$		$P = 0.004$		$P = 0.022$		NS

[a]Overweight is defined as BMI >24.9; Obesity is defined as BMI >29.9 ; BMI values are missing for 258 infertility patients.
***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ compared to fertile controls; Pearson's Chi-square test.
#$P \leq 0.003$ compared to causal factor infertility; Pearson's Chi-square test; NS, $P > 0.2$; N/A, not applicable.

Table V Summary of generally accepted causal factors and additional potential contributing factors on severe male factor infertility ordered according to the severity of the effect on sperm parameters and fertility potential.

Classification	Subclass	Factor[a]	Fertile men (%)	Infertile men (%)	Effect among infertility patients
Causal factors	Absolute	Secondary hypogonadism	0	1.3	100% aspermia, azoospermia or cryptozoospermia
		Seminal tract obstruction	0	5.9	99% aspermia, azoospermia or cryptozoospermia
		Known genetic causes[b]	0	7.8	87.4% aspermia, azoospermia or cryptozoospermia
	Severe	Oncological diseases	0.3	1.6	62.7% aspermia, azoospermia or cryptozoospermia 37.3% oligozoospermia
		Severe sexual dysfunction	0	4.4	55.3% aspermia, azoospermia or cryptozoospermia 44.7% oligozoospermia
	Plausible	Congenital anomalies in uro-genital tract	1.8	10.7	40.3% aspermia, azoospermia or cryptozoospermia 59.7% oligozoospermia
		Acquired or secondary testicular damage	1.2	6.6	21.9% aspermia, azoospermia or cryptozoospermia 78.1% oligozoospermia
Potential contributing factors	Testicular health	Varicocele, Grade 2-3	12.0	26	Increased prevalence in idiopathic infertility and 'Other testicular factors' groups. Increased prevalence among all patients with detectable ($n > 0$) sperm counts, i.e. except for aspermia and azoospermia.
		Leukocytospermia	7.4	13.5	Increased prevalence in idiopathic infertility and 'Seminal tract obstruction' groups. Increased prevalence only in oligozoospermia cases.
	General health	Chronic disease	9.8	24.4	Increased prevalence in idiopathic infertility, 'Genetic causes', 'Congential anomalies in uro-genital tract' and 'Other testicular factors' groups. Contribution to aspermia excluded.
		Overweight (Obesity)	48.6 (13.5)	60.4 (22.0)	Increased prevalence in idiopathic infertily and in the majority of the causal factor subgroups.

[a]Included clinical diagnoses are detailed in Table II.
[b]Autosomal and sex chromosomal abnormalities, Y-chromosomal micordeletions, CTFR gene mutations.

Discussion

We report the largest long-term, prospective monocenter study for the causes of male factor infertility. Across a 9-year study period, one in five investigated men (1737/ 8518) was diagnosed with reduced sperm counts. A specific value of our study arises from an unbiased recruitment and well-standardized analysis of the study subjects; the AC-TUH represents the non-referral andrology clinic investigating >90% of all cases of severe male infertility in Estonia. Thus, there was no pre-selection of patients either by specialist doctors or GPs. A unique aspect of our study was the analysis of the aspermia group, for which there is relatively limited previous knowledge in the epidemiology of male infertility (Mehta and Sigman, 2015). An additional strength in our analysis was the inclusion of a reference group of men with proven fertility ($n = 325$), who had passed an identical andrological examination to that of the infertility patients.

Firstly, the study showed that the current well-established guidelines and routine work-up in the andrology clinic are able to assign the primary cause of infertility for only 40% of patients (Tables II–III). In the oligozoospermia group, three in four cases remained idiopathic. This data highlights an obvious gap in our current understanding of the causes, biological mechanisms and pathways behind impaired spermatogenesis and male reproductive physiology.

Additionally, our study brought novel insights into the clinical consequences arising from known causes behind male infertility. The exhaustive investigation of each involved subject suggests that the causal factors for infertility could be further divided based on the severity of their effect as absolute, severe and plausible causal factors (Table V). Whereas absolute and severe causes lead to mostly extremely impaired sperm production and infertility, plausible factors are more prevalent among the oligozoospermia cases and are also occasionally detected among fertile men. From a clinical perspective, these diagnoses exhibit a potential (but also pose a challenge) to develop appropriate personalized interventions to improve spermatogenic efficiency.

Finally, the data of the current study also enabled the preclusion of a number of previously assigned causative factors for male infertility. These 'potential contributing factors' (Grade 2–3 varicocele; leukocytospermia; chronic disease; overweight/obesity) were also prevalent among the men with proven fertility (Tables IV–V). Their role in enhancing the imbalance of reproductive physiology towards reduced sperm counts is most probably dependent on the overall health and functional capacity of the testis. However, as all these risk factors (although not causative) were significantly enriched in idiopathic infertility patients and especially in the oligozoospermia group, they are not to be ignored in the clinical practice. Varicocele represents the most

frequent potentially treatable genital disease in infertile men and high prevalence of this condition among idiopathic cases supports clinical decision-making towards appropriate management strategies. In the literature, varicocele is one of the most controversial factors contributing to male subfertility. Consistent with our data (13.5% in controls; 31% in idiopathic infertility), the prevalence of varicocele in general population has been estimated 15–20% compared to 30–40% among men attending infertility clinics (Jarow, 2001). The current results are in good accordance with our earlier analysis showing that only Grade 2–3 varicocele (but not Grade 1) cause deterioration of testis function (Punab, 2007).

The study strengthened the previous knowledge (Ehala-Aleksejev and Punab, 2015; Tarín et al., 2015) that male infertility is accompanied by increased prevalence of chronic diseases and overweight/obesity compared to fertile men (Table III). Still, no conclusions can be drawn about whether these are true 'potential contributing factors' or a consequence of impaired physiology and health. As it has been suggested that severe male infertility is interrelated not only with various general health problems, but also with reduced longevity (Jensen *et al.*, 2009; Eisenberg *et al.*, 2014), large-scale multicenter studies are urgently needed to move forward with this important knowledge gap on the role and optimal management of chronic disease and increased BMI among infertile men.

There are a limited number of previous exhaustive analyses on the causes of male infertility which could be used as a comparative context for our data. An exception to this is the azoospermia subgroup, which has been analyzed in detail for the infertile men attending the Centre of Reproductive Medicine and Andrology, University Clinics of Münster (Germany) (Tüttelmann *et al.*, 2011). The overall distribution of the diagnosed primary causes of azoospermia among the Estonian and German patients overlapped. One difference was the proportion of diagnosed obstructive azoospermia cases in Münster (11%) compared to Estonia (26%). The majority of our azoospermic cases had epididymal obstruction, which has most probably been caused by sexually transmitted diseases (STD) in the past. A high prevalence of STD among our patients can be explained by the 'STD epidemic' in Estonia during the post-communist transition period from 1990 to 1995 (Põder and Bingham, 1999), which coincides with the age group of the majority of our study subjects. Another major difference was a lower prevalence of Y-chromosomal microdeletions in Münster compared to Estonian patients. The most probable reason for this discrepancy is that this retrospective analysis had included patients, who had attended the University Clinics of Münster before *AFZa-c* analyses were introduced into routine andrological workup. The detected prevalence of Y-chromosomal microdeletions in Estonian patients (6.4% and 8.9% in total and non-obstructive azoospermia groups, respectively; 8.5% in cryptozoospermia) is consistent with the reported 5–10% prevalence among azoospermia cases in other populations (Krausz *et al.*, 2015).

Overall, the typical design of the limited number of such studies in the field is that of a retrospective analyses of patients, who have been clinically phenotyped and assigned a diagnosis >10–20 years ago, when the infertile men workup did not include several current diagnostic tests (e.g. genetic tests). In addition, the distribution and prevalence of diagnosis among the attending patients has also been dependent on the centre's clinical level (level of referral) and its finer specialization. Thus, the definition of causal factors varies between the published studies.

The earliest large study on the causes of male infertility was carried out in 1982–1985 encompassing 33 centers worldwide and 7273 male partners of infertile couples (WHO, 1997). The most frequent diagnostic categories were seminal abnormalities of unknown cause (i.e. idiopathic infertility; >45%), varicocele (22.6%), accessory gland infection (12.4%), immunological infertility (5.4%), congenital abnormalities such as cryptorchidism (3.0%), systemic causes (2.6%) and sexual dysfunctions (2.3%). In 2000, a monocenter study was published by a tertiary referral andrology center in Rotterdam ($n = 1549$) (Pierik *et al.*, 2000). It also reported seminal abnormalities of unknown cause as the most frequent diagnosis (idiopathic infertility; >40%), followed by varicocele (14.2%), immunological infertility (11%), accessory gland infection (5.3%), cryptorchidism and other congenital abnormalities of the male reproductive system (9.0%) and sexual dysfunction (4.6%). However, as genetic factors had not been analyzed, oncological causes and sexual dysfunctions were not specified, the study missed important data on these patients. Inclusion of the control group of men with proven fertility in the current study allowed a clear-cut exclusion of several previously suggested factors as primary causes of severe male infertility, such as varicocele, inguinal hernia operations, accessory gland infection/leukocytospermia and possibly also immunologic factors.

Conclusions and perspectives

Our 9-year prospective, monocenter study for the causes of male infertility has revealed large knowledge gaps in this important clinical field. For 60% of the patients, the primary causal factor could not be assigned. Whereas the causal factors behind the most severe forms of male infertility (aspermia, azoospermia, cryptozoospermia) are quite well understood and diagnosed in the clinical practice, there is an urgent need for multilayered synergetic clinical and basic research (e.g. (epi) genetics/genomics) to uncover the causes and mechanisms behind oligozoospermia, representing the majority (86.3%) of idiopathic infertility cases. Studies uncovering novel mechanisms and biological pathways may also provide innovative solutions for male infertility treatment. Although these studies are challenged by the heterogeneity of the disorder, successful outcomes may lead to rewarding solutions in the improvement of the clinical management of the condition.

The currently applied guideline for male infertility, developed by European Association of Urology (Jungwirth *et al.*, 2012), refers in its epidemiology section to the analysis of andrology patients at the Centre of Reproductive Medicine and Andrology, University Clinics of Münster. However, the referred chapter 'Classification of andrological disorders' and dataset in the major andrology textbook from year 2010 (Tüttelmann and Nieschlag, 2010) also reports patients who had attended the clinic for other reasons besides male infertility, such as hypogonadism and sperm cryopreservation requests due to malignant disease. Due to a missing appropriate control group representing fertile men, some of the factors such as varicocele are misleadingly classified as 'Infertility of known (possible) cause'. Novel insights and improved clarity achieved in the current comprehensive analysis regarding the absolute, causative and plausible factors behind male infertility, as well as the suggested 'potential contributing factors' is expected to serve in updating the current clinical guidelines.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

Study concept and design: M.P. Acquisition of data: M.P., O.P., P.P., V.V., K.P., R.L., P.K. Statistical analysis: M.P. Interpretation and synthesis of data: M.P., M.L. Drafting the manuscript: M.P. Supervision and critical revision of the manuscript for important intellectual content: M. L. Agreement with the manuscript's results and conclusions: O.P., P. P., V.V., K.P., R.L., P.K. All authors have read, and confirm that they meet, the authorship criteria.

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Conflict of interest

None declared.

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