ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

ORIGINAL ARTICLE

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Keywords:
hypogonadism, infertility, metabolic syndrome, semen analysis

Received: 5-May-2015
Revised: 24-Apr-2016
Accepted: 10-May-2016

doi: 10.1111/andr.12232

Metabolic syndrome in white European men presenting for primary couple's infertility: investigation of the clinical and reproductive burden

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SUMMARY

Despite complex interactions between obesity, dyslipidemia, hyperinsulinaemia, and the reproductive axis, the impact of metabolic syndrome on human male reproductive function has not been analysed comprehensively. Complete demographic, clinical, and laboratory data from 1337 consecutive primary infertile men were analysed. Health-significant comorbidities were scored with the Charlson Comorbidity Index (categorised 0 vs. 1 vs. 2 or higher). NCEP-ATPIII criteria were used to define metabolic syndrome. Semen analysis values were assessed based on the 2010 World Health Organisation (WHO) reference criteria. Descriptive statistics and logistic regression models tested the association between semen parameters and clinical characteristics and metabolic syndrome. Metabolic syndrome was found in 128 (9.6%) of 1337 men. Patients with metabolic syndrome were older ($p < 0.001$) and had a greater Charlson Comorbidity Index of 1 or higher (chi-square: 15.6; $p < 0.001$) compared with those without metabolic syndrome. Metabolic syndrome patients had lower levels of total testosterone ($p < 0.001$), sex hormone-binding globulin ($p = 0.004$), inhibin B ($p = 0.03$), and anti-Müllerian hormone ($p = 0.009$), and they were hypogonadal at a higher rate (chi-square: 32.0; $p < 0.001$) than patients without metabolic syndrome. Conversely, the two groups did not differ significantly in further hormonal levels, semen parameters, and rate of either obstructive or non-obstructive azoospermia. At multivariate logistic regression analysis, testicular volume (OR: 0.90; $p = 0.002$) achieved independent predictor status for WHO pathological semen concentration; conversely, age, Charlson Comorbidity Index scores, metabolic syndrome, and inhibin B values did not. No parameters predicted normal sperm morphology and total progressive motility. Metabolic syndrome accounts for roughly 9% of men presenting for primary couple's infertility. Although metabolic syndrome patients have a lower general male health status, semen analysis values seem independent of the presence of metabolic syndrome.

INTRODUCTION

In addition to the well-known association between male infertility and a higher risk of developing testicular germ cell tumours, colorectal cancer, melanoma, and prostate cancer (Jacobsen *et al.*, 2000; Walsh *et al.*, 2010), it has become clear that infertile men share a significantly lower health status (Salonia *et al.*, 2009). More specifically, a deranged metabolism was shown to be actively involved in affecting male reproductive function (Michalakis *et al.*, 2013). In this context, substantial evidence indicates that complex interactions underlie the pathologic relationship among obesity, metabolic syndrome (MetS), and the reproductive axis (Michalakis *et al.*, 2013). Obesity is known to affect fertility in women and is likely involved in men (Winters & Walsh, 2014). An excess of adipose tissue is responsible for hormonal imbalance, especially when considering the hypothalamic–pituitary–gonadal axis (Donato *et al.*, 2011). Nevertheless, evidence linking obesity with impaired semen parameters is still conflicting and far from conclusive (Fejes *et al.*, 2005; Kasturi *et al.*, 2008; Hammiche *et al.*, 2012; Eisenberg *et al.*, 2014). Belloc *et al.* (2014) described in a large cohort study including 10,665 men how increased BMI was associated with decreased semen volume, concentration, and motility. Likewise, Hammiche *et al.* (2012) reported how overweight and obese men have a significantly lower ejaculate volume and sperm

count. Similar findings were described by Sermondade *et al.* (2013). Obesity, along with environmental toxicants, cryptorchidism, and varicocoele is an inducer of oxidative stress-derived testicular damage, which leads to an increase in germ cell apoptosis and therefore hypospermatogenesis. Such stresses can cause changes in the dynamics of testicular microvascular blood flow, endocrine signalling, and germ cell apoptosis (Turner & Lysiak, 2008). Similarly, diabetes mellitus (DM) also perturbs both sexual and reproductive hormonal homeostasis and is reported to affect spermatogenesis at various levels (Bhattacharya *et al.*, 2014).

MetS represents a clinical entity of several cardiovascular and metabolic alterations (i.e. high blood pressure, obesity, faulty glucose metabolism, hypertriglyceridaemia, and low levels of high-density lipoprotein-cholesterol [HDL-C]) whose common ground is believed to lie in insulin resistance, irrespective of the MetS definition. Although single components related to MetS were shown to have a detrimental effect on male reproductive health, the impact of MetS on male reproductive function has never been analysed comprehensively in white Europeans. Likewise, the lack of previous clinical evidence and the increasing prevalence of MetS (Scuteri *et al.*, 2014) with its potential impact on the hormonal milieu and overall health status prompted us to investigate the eventual role of MetS in male infertility. Therefore, we assessed the prevalence of MetS, the correlations of MetS with clinical characteristics, and its impact on semen and hormonal parameters in a cohort of white European men presenting for primary couple's infertility.

MATERIALS AND METHODS

Patients

From September 2005 to April 2013, 1337 consecutive white European men affected by primary couple's infertility (non-interracial infertile couples only) were enrolled in this cross-sectional study. Patients were enrolled if they were between 18 and 60 years of age and had either male factor infertility (MFI) or mixed factor infertility (MxFI). MFI was defined after a comprehensive diagnostic evaluation of all the female partners. To this aim, a comprehensive gynaecological work up was requested for all female partners belonging to our infertile couples. As per protocol of the IVF center of our academic hospital, history records of possible infertility factors (e.g. endometriosis, PCOS, etc.) and ovarian reserve were assessed for every woman. In this context, as for WHO definition, whenever those aspects were suggestive for a female factor, the couple was depicted as affected by a mixed infertility factor. According to the World Health Organisation (WHO) criteria, infertility is defined as not conceiving a pregnancy after at least 12 months of unprotected intercourse regardless of whether or not a pregnancy ultimately occurred (WHO web chapter on couple's infertility, 2014). Primary infertility is defined when a couple has never been able to conceive (WHO web chapter on couple's infertility, 2014).

Patients were assessed with a thorough medical history including age and comorbidities. Comorbidities were scored with the Charlson Comorbidity Index (CCI) (Charlson *et al.*, 1987), including myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, peptic ulcer disease, liver disease, diabetes, hemiplegia, moderate or severe renal disease, tumour without metastasis, leukaemia (either acute or chronic), lymphoma, metastatic solid tumours and AIDS. We used the International Classification of Diseases, 9th revision. For the specific purpose of the analysis, CCI was categorized as 0, 1, or 2 or higher.

Weight and height were measured for each participant, and body mass index (BMI), defined as weight in kilograms by height in square meters, was calculated. Waist circumference was measured for every patient (Han *et al.*, 1995). Testes volume was assessed through a Prader orchidometer, calculating the mean value between the two sides. Only patients without genetic alterations (karyotype abnormalities, Y chromosome microdeletions, CFTR gene mutations) were included in the study.

Patients underwent at least two consecutive semen analyses, both showing below standard values for normal semen parameters according to the WHO criteria (WHO. Laboratory Manual for the Examination and Processing of Human Semen, 2010). For the specific purpose of this study, the worst of the two semen analyses was used for the statistical evaluation.

Venous blood samples were drawn from each patient between 7 AM and 11 AM after an overnight fast. In all cases, fasting glucose levels were measured via a glucose oxidase method (Aeroset Abbott, Rome, Italy). Total cholesterol, HDL-C, and triglyceride levels were measured with the automated enzymatic colorimetric method (Aeroset Abbott). Follicle-stimulating hormone (FSH); luteinizing hormone (LH), prolactin (PRL), thyroid-stimulating hormone (TSH), and 17-β-estradiol (E_2) were measured using a heterogeneous competitive magnetic separation assay (Bayer Immuno 1 System; Bayer Corp., Tarrytown, NY, USA). Inhibin B (InhB) and anti-Müllerian hormone (AMH) were measured by an enzyme-linked immunosorbent assay (Beckman Coulter AMH Gen II ELISA, High Wycombe, UK). Total testosterone (tT) levels were measured via a direct chemiluminescence immunoassay (ADVIA Centaur; Siemens Medical Solutions Diagnostics, Deerfield, IL, USA), and sex hormone-binding globulin (SHBG) levels were measured via a solid-phase chemiluminescent immunometric assay on Immulite 2000 (Medical Systems SpA, Genoa, Italy). Calculated free testosterone (cfT) was derived from the Vermeulen formula. Hypogonadism was defined as tT less than 3 ng/mL (Bhasin *et al.*, 2010). The same laboratory was used for all patients.

MetS was defined according to the 2004 updated National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (ATP III) criteria (at least three of the following criteria: waist circumference greater than 102 cm; triglycerides equal to or greater than 150 mg/dL (1.7 mmol/L); HDL less than 40 mg/dL (1.03 mmol/L); blood pressure equal to or greater than 130/85 mm Hg or use of medication for hypertension; fasting glucose equal to or greater than 100 mg/dL (5.6 mmol/L) or use of medication for hyperglycaemia) (Grundy *et al.*, 2004).

Data collection was done following the principles outlined in the Declaration of Helsinki; after local Ethic Committee approval, all patients signed an informed consent agreeing to deliver their own anonymous information for future studies.

Statistical analyses

Data are presented as means (medians; IQ ranges). The statistical significance of differences in medians and proportions was

tested with the Mann-Whitney U test and Pearson chi-square test, respectively. Exploratory analyses were initially applied to all variables; variables were then kept where appropriate as clinically significant to the results. Analysis of covariance (ANCOVA) was applied for the multivariate assessment of continuous sperm parameters differences between MetS+ and Mets− subgroups; similarly, this difference was evaluated for single MetS components.

Logistic regression models tested the association between clinical predictors (age; MetS; categorized CCI; FSH; InhB; mean testis estimated volume; varicocoele) and pathologic semen parameters as the WHO 2010 criteria (WHO. Laboratory Manual for the Examination and Processing of Human Semen, 2010). Statistical tests were performed using SPSS v.19 (IBM Corp., Armonk, NY, USA). All tests were two sided, with a significance level set at 0.05.

RESULTS

Table 1 lists the characteristics and the descriptive statistics of the entire cohort of patients. MFI and MxFI were found in 1091 patients (81.6%) and 246 patients (18.4%), respectively. As a whole, MetS was found in 128 (9.6%); MetS was less common in men with MFI (95 [8.7%]) as compared with those with MxFI (33 [13.4%]) (chi-square: 5.1; $p = 0.02$).

Table 2 depicts the characteristics and the descriptive statistics according to a segregation of the presence of MetS vs. the absence of MetS. Patients with MetS were significantly older, had a higher BMI, and were found to share a heavier burden of comorbidities (all $p < 0.001$). Conversely, no differences were observed in testicular volume between the two groups. Regarding hormonal milieu, patients with MetS showed lower InhB, AMH, tT, and SHBG circulating levels compared with those without MetS (all $p < 0.05$). Hypogonadism was more common in patients with MetS as compared with their non-MetS counterpart ($p < 0.001$); moreover, whose two groups did not differ in terms of FSH, LH, cfT, E2, tT:E2 ratio, TSH, and PRL values. Similarly, no significant differences were observed in terms of semen parameters (Table 2).

Table 3 details the results of the ANCOVA multivariate analysis testing the adjusted differences in terms of sperm parameters in the entire cohort of patients after grouping into MetS+ and MetS− subgroups; moreover, this analysis was repeated for each single MetS component. At all times ANCOVA was adjusted for patient age, CCI, mean testis volume, FSH, and inhibin B. No differences were observed in sperm parameters between all the analysed conditions, except for reduced PT motility in patients with waist circumference >102 cm (Table 3).

Table 4 details logistic regressions models testing the associations between clinical predictors and pathologic sperm parameters. At logistic UVA, higher FSH but lower InhB levels and lower right testis volumes were associated with pathologic sperm concentrations (all $p \leq 0.02$). Conversely, age, CCI, and positive MetS were not. Similarly, at logistic UVA, CCI of 2 or higher and higher FSH levels were significantly associated with pathologic progressive motility (all $p \leq 0.02$). At logistic MVA, only higher FSH and lower mean testis volume reached independent predictor status for pathologic sperm concentration (all $p \leq 0.05$). Testicular volume emerged as independent predictor of pathological

Table 1 Characteristics and descriptive statistics of patients (No = 1337)

No. of patients	1337
Age (years)	
Mean (median)	36.5 (36)
IQ Range	33–40
BMI (kg/m^2)	
Mean (median)	25.77 (25.31)
IQ Range	23.45–27.68
CCI [No. (%)]	
CCI 0	1242 (92.9)
CCI 1	48 (3.6)
CCI ≥2	47 (3.5)
Mean testis volume (Prader estimation)	
Mean (median)	17.7 (17.5)
IQ Range	13.5–22.5
Varicocele [No. (%)]	461 (34.4)
MetS components [No. (%)]	
Elevated BP (BP ≥130/85 mmHg or therapy)	476 (35.6)
Central obesity (waist circumference >102 cm)	345 (25.8)
Reduced HDL cholesterol (<40 mg/dL) or therapy	366 (27.4)
Elevated triglycerides (≥150 mg/dL) or therapy	197 (14.7)
Elevated fasting glucose (≥100 mg/dL)	292 (21.8)
FSH (mUI/mL)	
Mean (median)	10.0 (5.6)
IQ Range	3.1–12.7
LH (mUI/mL)	
Mean (median)	5.2 (4.1)
IQ Range	2.7–6.2
InhB (pg/mL)	
Mean (median)	94.3 (84.2)
IQ range	24.6–141.1
AMH (ng/mL)	
Mean (median)	7.3 (5.6)
IQ Range	3.6–8.9
tT (ng/mL)	
Mean (median)	5.0 (4.7)
IQ Range	3.6–5.9
cfT (ng/mL)	
Mean (median)	0.21 (0.10)
IQ Range	0.07–0.12
tT <3 ng/mL [No. (%)]	187 (14.0)
cfT <0.06 ng/mL [No. (%)]	164 (12.3)
E_2 (pg/mL)	
Mean (median)	34.0 (32.0)
IQ Range	24.9–42.0
tT–E_2 ratio	
Mean (median)	0.17 (0.15)
IQ Range	0.11–0.20
SHBG (nmol/L)	
Mean (median)	30.5 (28.0)
IQ Range	21.4–37.0
PRL (ng/mL)	
Mean (median)	14.4 (8.2)
IQ Range	3.2–18.4
TSH (μUI/mL)	
Mean (median)	1.87 (1.62)
IQ Range	1.14–2.25
Semen volume (mL)	
Mean (median)	2.2 (2.0)
IQ Range	0.1–3.5
Semen volume <1.5 mL [No. (%)]	185 (13.8)
Sperm concentration	
Mean (median)	30.8 (14.0)
IQ Range	3.7–42.4
Sperm concentration ≤15 × 10^6/mL [No. (%)]	681 (50.9)
Progressive motility	
Mean (median)	26.2 (25.0)
IQ Range	10.0–40.0
Progressive motility ≤32% [No. (%)]	945 (65.6)
Normal morphology	
Mean (median)	10.5 (4.0)
IQ Range	0.0–12.0
Normal morphology ≤4% [No. (%)]	693 (51.8)

Table 1 (Continued)

Total sperm count	
Mean (median)	52.2 (28.5)
IQ Range	6.3–74.2
Non-obstructive azoospermia [No. (%)]	143 (10.7)
Obstructive azoospermia [No. (%)]	48 (1.5)

Data are BMI, body mass index; CCI, Charlson Comorbidity Index; FSH, follicle stimulating hormone; LH, luteinizing hormone; InhB, inhibin B; AMH, antimüllerian hormone; tT, total testosterone; cfT, calculate free testosterone; E_2, 17β estradiol; tT–E_2 ratio, total testosterone/17β estradiol ratio; SHBG, sex hormone binding globulin; PRL, prolactin; TSH, thyroid-stimulating hormone.
Non-obstructive azoospermia and obstructive azoospermia were defined according to European Association of Urology 2015 guidelines (http://uroweb.org/wp-content/uploads/EAU-Guidelines-Male-Infertility-20151.pdf.

sperm morphology ($p = 0.02$), whereas no variable reached statistical significance for pathologic progressive motility (Table 4).

DISCUSSION

We tested the rate of MetS cross-sectionally in a large cohort of white European men seeking a first medical referral for primary couple's infertility. We also investigated the impact of MetS in terms of clinical and semen characteristics in the same cohort. Our interest was fuelled, on the one hand, by previous data showing the increasing prevalence of MetS among European men (Scuteri *et al.*, 2014), its potential impact on the hormonal milieu (Corona *et al.*, 2011; Michalakis *et al.*, 2013) and overall health status (Alberti *et al.*, 2009), and, on the other, by the lack of exhaustive published observations on the correlation between MetS and male infertility.

Our findings showed for the first time that almost 1 of 10 men presenting for primary couple's infertility meets NCEP-ATP III criteria for MetS. This rate seems concordant with the findings previously reported in an unselected male sample (Lotti *et al.*, 2014); conversely, this prevalence appears higher than that observed in the general population of the same age range (Miccoli *et al.*, 2005). We also observed that MetS is responsible for a lower general male health status, as depicted by a higher CCI, which may be considered a reliable proxy of general health status regardless of the aetiology of infertility (Salonia *et al.*, 2009). Conversely, semen parameters were not affected by the positivity for MetS.

We chose NCEP-ATP III criteria to define MetS because they are the most widely used and readily available to physicians, thus facilitating their clinical and epidemiological use. Moreover, this definition does not harbour any preconceived notion of the underlying cause of MetS, whether it is insulin resistance or obesity. To the best of our current knowledge, these data report findings of the largest cohort of primary infertile men ever studied in this context. Currently adopted stringent enrolment criteria allowed us to select a consistently homogeneous white European male population, thus minimizing the impact of potential unpredictable genetic biases.

Men with pure MFI showed a lower incidence of MetS compared with those in couples with MxFI. In this context, whether MetS in an infertile man may contribute to exacerbating a female partner's predisposition to infertility, or it plays a standalone role as an MFI determinant, or eventually it is irrelevant in terms of reproductive outcomes are questions we cannot answer with our available data. In this context, our data account for a slightly low MxFI prevalence in our cohort as compared with published data. In our country, a consistent share of men belonging to couple with a mixed factor is mostly and directly intercepted by IVF clinics and therefore they eventually miss a chance to be comprehensively evaluated in a uro-andrological setting, therefore decreasing the final prevalence of MxFI in our cohort.

Current findings highlighted that patients with MetS were older and had a higher rate of comorbidities and of biochemical hypogonadism compared with their non-MetS counterpart. Of clinical importance, we used CCI (Charlson *et al.*, 1987) to assess patients' comorbidities, considered the most valid and reliable hospital-based comorbidity index used by health researchers to assess the impact of comorbid disease status in health care databases. In this context, CCI was originally designed to assess comorbidities typically associated with 1-year mortality; therefore for its specific purpose, CCI includes medical conditions that are more frequently found in an older or even elderly population and usually not in the younger population (i.e. 31–36 years of age) that characterizes our cohort of individuals. Thus, by definition and by its inherent limits, CCI completely excludes any item related to blood hypertension or sexually transmitted diseases, which, in contrast, may be relevant medical conditions in young infertile men in the real-life setting. Furthermore, because DM is the only component shared by both CCI and NCEP-ATP III criteria for MetS, CCI scores allowed us to consider the impact of comorbidities not attributed to MetS per se in our predictive model.

Salonia *et al.* (2009) previously demonstrated that infertile men have a lower general health status compared with fertile controls. Eisenberg *et al.* (2013) reported an increased risk of cancer among azoospermic men compared with non-azoospermic infertile patients. Walsh *et al.* (2010) previously observed a higher risk of both testicular germ cell cancer and high-grade prostate cancer among infertile men. Similarly, current findings would suggest that patients with MetS appeared to belong to an even less healthy subgroup of individuals in the general primary infertile population.

The second major aspect of these findings is related to patient age. To the best of our knowledge, this analysis was the first to document a significantly higher age in primary infertile men with MetS compared with those without MetS. Similar results have been previously reported in an unselected male sample by Lotti and colleagues (Lotti *et al.*, 2013). Salonia *et al.* (2012) highlighted a worrisome trend towards delayed fatherhood in white European men. In this context, it was previously shown that semen volume, sperm motility, and sperm morphology decrease with age (Sartorius & Nieschlag, 2010). In contrast, the relationship between increasing age and sperm concentration remains unclear (Sartorius & Nieschlag, 2010). A significant role for paternal age has been postulated for a number of genetic factors, thus including numerous and severe age-dependent structural chromosomal aberrations, with several X-linked recessive and autosomal dominant disorders that have been already clearly confirmed (Sartorius & Nieschlag, 2010; Salonia *et al.*, 2012).

Advancing male age has been also associated with a potential alteration of human sperm apoptosis outcome (Sartorius & Nieschlag, 2010), thus possibly having a negative impact on naturally occurring control mechanisms that serve to select healthy sperm (Sartorius & Nieschlag, 2010). Similarly, delayed fatherhood has been linked with a more significant proportion of sperm carrying an abnormal rate of DNA fragmentations (Hammiche *et al.*,

Table 2 Characteristics and descriptive statistics of patients according to positivity NCEP/ATPIII criteria for MetS

	+MetS	−MetS	*p* value*
No. of patients	128	1209	
Age (years)			
Median (IQR)	38 (34–42)	36 (33–39)	<0.001
BMI (kg/m^2)			
Median (IQR)	27.6 (25.9–30.1)	25.0 (23.3–27.3)	<0.001
CCI [No. (%)]			
CCI 0	109 (85.2)	1133 (93.7)	<0.001 (χ^2, 15.6)
CCI 1	12 (9.4)	36 (3.0)	
CCI ≥2	7 (5.5)	40 (3.3)	
Mean testis volume (Prader estimation)			
Median (IQR)	20 (13.5–25)	17.5 (13.5–22.5)	0.08
Varicocele [No. (%)]	40 (31.3)	421 (34.8)	0.42 (χ^2, 0.7)
MetS components [No. (%)]			
Elevated BP (BP ≥130/85 mmHg or therapy)	94 (73.4)	382 (31.6)	<0.001 (χ^2, 88.4)
Central obesity (waist circumference > 102 cm)	102 (79.7)	243 (20.1)	<0.001 (χ^2, 214.6)
Reduced HDL cholesterol (<40 mg/dL) or therapy	85 (66.4)	281 (23.2)	<0.001 (χ^2, 108.5)
Elevated triglycerides (≥150 mg/dL) or therapy	78 (60.9)	119 (9.8)	<0.001 (χ^2, 240.5)
Elevated fasting glucose (≥100 mg/dL)	65 (50.7)	227 (18.7)	<0.001 (χ^2, 69.5)
FSH (mUI/mL)			
Median (IQR)	5.2 (3.3–17.0)	5.7 (3.1–12.7)	0.50
LH (mUI/mL)			
Median (IQR)	4.0 (2.8–6.6)	4.1 (2.7–6.1)	0.66
InhB (pg/mL)			
Median (IQR)	40.0 (27.3–114.7)	85.8 (24.3–142.9)	0.03
AMH (ng/mL)			
Median (IQR)	4.1 (1.6–5.4)	4.7 (2.4–9.6)	0.009
tT (ng/mL)			
Median (IQR)	3.8 (2.7–5.3)	4.7 (3.6–6.0)	<0.001
cfT (ng/mL)			
Median (IQR)	0.03 (0.02–0.05)	0.05 (0.02–0.08)	0.28
tT <3 ng/mL [No. (%)]	39 (30.4)	148 (12.2)	<0.001 (χ^2, 32.0)
cfT < 0.06 ng/mL	33 (25.7)	131 (10.8)	<0.001 (χ^2, 24.0)
E_2 (pg/mL)			
Median (IQR)	32 (24–41)	32 (25–42)	0.71
tT–E_2 ratio			
Median (IQR)	0.13 (0.09–0.18)	0.15 (0.11–0.21)	0.06
SHBG (nmol/L)			
Median (IQR)	23.4 (18.3–33.8)	29.0 (22.0–37.5)	0.004
PRL (ng/mL)			
Median (IQR)	8.5 (3.4–18.2)	8.0 (3.0–18.0)	0.79
TSH (μUI/mL)			
Median (IQR)	1.7 (1.2–2.6)	1.6 (1.1–2.2)	0.30
Semen volume (mL)			
Median (IQR)	2.0 (0.1–3.0)	2.0 (0.1–2.5)	0.09
Semen volume <1.5 mL [No. (%)]	18 (14.1)	167 (13.8)	0.94 (χ^2, 0.1)
Sperm concentration			
Median (IQR)	13.8 (2.2–40.8)	14.2 (3.8–44.1)	0.50
Sperm concentration <15 × 10^6/mL [No. (%)]	66 (51.5)	615 (50.9)	0.88 (χ^2, 0.02)
Progressive motility			
Median (IQR)	25 (11–44)	25 (10–40)	0.81
Progressive motility <32% [No. (%)]	79 (61.7)	798 (66.0)	0.33 (χ^2, 0.9)
Normal morphology			
Median (IQR)	5 (0–16)	4 (0–12)	0.96
Normal morphology <4% [No. (%)]	69 (53.9)	624 (51.6)	0.62 (χ^2, 0.2)
Total sperm count			
Median (IQR)	25.3 (5.7–72.8)	28.7 (6.3–75.4)	0.19
Non-obstructive azoospermia [No. (%)]	12 (9.3)	131 (10.8)	0.61 (χ^2, 0.3)
Obstructive azoospermia [No. (%)]	1 (0.8)	19 (1.6)	0.48 (χ^2, 0.5)

Data are +MetS, positive criteria for metabolic syndrome; −MeTs, negative criteria for metabolic syndrome; BMI, body mass index; CCI, Charlson Comorbidity Index; FSH, follicle stimulating hormone; LH, luteinizing hormone; InhB, inhibin B; AMH, antimüllerian hormone; tT, total testosterone; cfT, calculate free testosterone; E_2, 17β estradiol; tT–E_2 ratio, total testosterone/17β estradiol ratio; SHBG, sex hormone binding globulin; PRL, prolactin; TSH, thyroid-stimulating hormone. **p* value according to Mann–Whitney *U*-test or χ^2 test, as indicated.

2011). As a consequence, advanced paternal age may be associated with higher frequencies of aneuploidies, point mutations, and breaks in sperm DNA that, in turn, correlate with fertilization, impaired pre-implantation development, impaired late post-implantation development, and poor pregnancy outcomes, regardless of whether the insemination is natural or artificial (Sartorius & Nieschlag, 2010). Overall, infertile patients are delaying fatherhood (Salonia *et al.*, 2012), with all the possible

Table 3 *p* value for adjusted differences in continuous seminal parameters in the whole cohort (n = 1337) at analysis of covariance (ANCOVA)

	Sperm concentration	Progressive motility	Normal morphology	Semen volume	Total sperm count
MetS (yes vs. no)	0.67	0.58	0.58	0.31	0.89
BP ≥130/85 mmHg or therapy (yes vs. no)	0.53	0.15	0.31	0.35	0.46
Waist circumference >102 cm (yes vs. no)	0.75	0.005	0.12	0.41	0.35
HDL cholesterol <40 mg/dL or therapy (yes vs. no)	0.53	0.21	0.80	0.19	0.32
Triglycerides ≥150 mg/dL or therapy (yes vs. no)	0.71	0.24	0.81	0.81	0.99
Fasting glucose ≥100 mg/dL (yes vs. no)	0.31	0.55	0.26	0.36	0.94

Data are BP, blood pressure; HDL, high density lipoprotein. ANCOVA is adjusted for age, CCI, FSH, InhB, mean testis volume.

Table 4 Logistic regression models predicting pathologic sperm parameters according to WHO 2010 criteria (OR; *p* value [95% CI]) in the whole cohort (n = 1337)

	Sperm concentration <15 × 10^6/mL		Progressive motility <32%		Normal morphology <4%	
	UVA model	MVA model	UVA model	MVA model	UVA model	MVA model
Age	0.99; 0.42 [0.97–1.01]	0.98; 0.54 [0.92–1.05]	1.01; 0.42 [0.98–1.04]	0.97; 0.40 [0.90–1.04]	0.97; 0.02 [0.94–0.99]	0.97; 0.35 [0.90–1.04]
CCI 0	–; 0.46	–; 0.66	–; 0.53	–; 0.98	–; 0.11	–; 0.73
CCI 1	0.72; 0.35 [0.36–1.44]	0.83; 1.23 [0.20–7.59]	0.75; 0.48 [0.33–1.67]	0.83; 0.84 [0.13–5.34]	0.73; 0.42 [0.34–1.58]	2.24; 0.49 [0.23–21.56]
CCI ≥2	1.33; 0.43 [0.65–2.73]	0.32; 0.37 [0.03–3.81]	5.58; 0.02 [1.29–24.12]	–	2.73; 0.54 [0.98–7.59]	1.64; 0.69 [0.14–19.45]
+MetS	0.97; 0.89 [0.65–1.46]	1.08; 0.89 [0.40–2.92]	0.89; 0.65 [0.55–1.46]	1.32; 0.61 [0.46–3.84]	0.73; 0.92 [0.58–1.47]	1.51; 0.46 [0.51–4.48]
Right testis volume	0.87; 0.02 [0.84–0.90]	0.90; 0.002 [0.85–0.96]	0.98; 0.13 [0.95–1.01]	1.01; 0.75 [0.94–1.09]	0.97; 0.07 [0.94–1.00]	0.92; 0.02 [0.86–0.98]
FSH	1.13; <0.001 [1.09–1.16]	1.11; 0.005 [1.03–1.19]	1.06; 0.01 [1.01–1.10]	1.01; 0.73 [0.95–1.08]	0.99; 0.92 [0.97–1.03]	1.01; 0.79 [0.95–1.08]
InhB	0.99; <0.001 [0.98–0.99]	1.00; 0.96 [0.99–1.01]	1.01; 0.55 [0.99–1.10]	1.01; 0.56 [1.00–1.02]	1.00; 0.05 [1.00–1.01]	1.01; 0.05 [1.00–1.02]

Data are UVA, univariable analysis; MVA, multivariable analysis; CCI, Charlson Comorbidity Index; +MetS, positive criteria for metabolic syndrome; FSH, follicle stimulating hormone; InhB, inhibin B.

detrimental consequences, but infertile patients with MetS are even at higher risk. Current findings showed that they are older than infertile patients not sharing criteria for MetS.

Our analyses confirmed the association between MetS and male hypogonadism in the general population (Muller *et al.*, 2005). Similar findings were also reported by studies specifically considering infertile patients (Lotti *et al.*, 2013; Leisegang *et al.*, 2014). Current analyses showed that tT was reduced in MetS patients compared with those without MetS. In contrast, cfT did not seem to be affected by this condition. In contrast, Lotti *et al.* (2013) reported decreased values of both tT and free testosterone (fT), and Leisegang *et al.* (2014) only reported a fT reduction in this specific setting. Along with tT, SHBG was also found to be reduced in our subset of patients with MetS. Although obesity and MetS are known to lower SHBG levels (Svartberg *et al.*, 2004), the actual impact on fT is still under debate. For instance, the Massachusetts Male Aging Study showed no difference in terms of fT in overweight men (Mohr *et al.*, 2006). MacDonald *et al.* (2010) reported the results of a meta-analysis showing a negative relationship for tT, SHBG, and fT with increased BMI. Contextual decreases in both SHBG and tT may partially account for unmodified cfT levels in our patients; this appears important because we have emphasized that their age was considerably younger than that reported in the studies just cited, thus potentially disguising an age-related effect on T levels (Camacho *et al.*, 2013).

Obesity-related and MetS-related hypogonadism is known to be accompanied by a plethora of factors simultaneously acting centrally and peripherally (Michalakis *et al.*, 2013). However, the impact of MetS on endocrine testicular function does not appear to be restricted to T homeostasis only. We observed that InhB and AMH levels are both reduced in patients with MetS according to previous findings (Robeva *et al.*, 2012).

Our findings did not show a potential role of MetS in affecting semen parameters. In contrast, Lotti *et al.* (2013) showed an association between MetS poor sperm morphology and several other factors in men broadly presenting for couple infertility; however, Lotti *et al.* (2013) used the International Diabetes Federation & American Heart Association/National Heart, Lung, and Blood Institute Classification, which was previously criticized for its emphasis on obesity rather than insulin resistance in pathophysiologic terms. Preclinical models of infertility showed that a high-fat diet inducing obesity may result in reduced sperm motility and a decreased percentage of spermatozoa with normal morphology (Palmer *et al.*, 2012). Therefore, focusing mainly on an obesity-based definition of MetS might somehow disguise results in terms of sperm abnormalities, losing sight of the correct MetS-induced alterations. These observations were partially contradicted by more recent analyses which have been obtained using a different animal model. Indeed, Marchiani *et al.* (2015) used a well-established high-fat diet rabbit model resembling human MetS, including development of hypogonadism; in this context, the authors demonstrated that high-fat diet decreased sperm motility, morphology and acrosome reaction in response to progesterone and increased sperm cholesterol content. All the above parameters showed an univariable

association with most MetS features, its severity and plasma T levels. Overall these findings indicated that the development of MetS produced a number of detrimental effects on sperm quality and functionality by inducing metabolic disorders leading to alterations in testis and epididymis (Marchiani *et al.*, 2015).

As a whole, MetS emerged as a powerful modifier of the endocrine milieu in the current cohort of patients. Conversely, MetS per se did not seem to have a negative impact on semen parameters. Whether this may be because of the fact that current findings are an expression of a cross-sectional study in which we have considered patients whose testicular function is already impaired beyond the eventual influence of MetS or we are simply not focusing on the proper proxy of testis exocrine function is a question we cannot answer. Molecular alterations in spermatogenesis, assessed for instance through DNA sperm fragmentation analysis, will perhaps provide more detailed information.

Our study is not devoid of limitations. This was a hospital-based study, raising the possibility of selection bias. The cohort was recruited from a single academic outpatient clinic, and despite the fact that this particular research probably had the largest consistently homogeneous same-race cohort of primary infertile men (restricted to non-interracial infertile couples), several larger studies across different centres and populations will be needed to substantiate our findings. Second, current analyses were implemented in a cross-sectional setting that lacked a comparison vs. a same-race, age-matched cohort of fertile individuals. Third, the analyses lacked data regarding potential molecular alterations in spermatogenesis, which might be of importance in investigating the eventual impact of MetS on semen health.

CONCLUSIONS

Our analyses showed novel evidence that almost 1 of 10 white European men presenting for primary couple's infertility meets NCEP-ATP III criteria for MetS. In the same cohort of individuals, MetS is responsible for a lower general male health status, as depicted by a higher CCI, along with a higher rate of hypogonadism. Conversely, semen parameters were not affected by the positivity for MetS.

ACKNOWLEDGEMENTS

The authors thank Dragonfly Editorial for reviewing the language in this manuscript.

AUTHORS' CONTRIBUTIONS

Study Concept and Design: Andrea Salonia, Eugenio Ventimiglia, Michele Colicchia; Data collection: Eugenio Ventimiglia, Paolo Capogrosso, Michele Colicchia, Luca Boeri, Alessandro Serino, Giulia Castagna, Maria Chiara Clementi, Giovanni La Croce, Cesare Regina, Andrea Salonia; Statistical analyses and data interpretation: Eugenio Ventimiglia, Marco Bianchi, Andrea Salonia; Drafting of the manuscript: Eugenio Ventimiglia, Andrea Salonia; Critical revision of the manuscript: Rocco Damiano, Francesco Montorsi; Responsibility; Guarantor's name: Andrea Salonia.

CONFLICT OF INTEREST

None.

FINANCIAL SUPPORT

None.

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