ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

ORIGINAL ARTICLE

Correspondence:
Daniele Gianfrilli, Department of Experimental Medicine, Sapienza University of Rome, Viale del Policlinico 155, Rome, Italy.
E-mail: daniele.gianfrilli@uniroma1.it

'Amico-Andrologo' Study Group details are given in Acknowledgements section.

Keywords:
adolescence, alcohol, andrological health, health risk behaviours, prevention, testicular development

Received: 2-Apr-2019
Revised: 6-May-2019
Accepted: 6-May-2019

doi: 10.1111/andr.12659

Risk behaviours and alcohol in adolescence are negatively associated with testicular volume: results from the Amico-Andrologo survey

[1]D. Gianfrilli, [2]A. Ferlin, [1]A. M. Isidori, [3]A. Garolla, [4] M. Maggi, [5]R. Pivonello, [6]D. Santi, [1]A. Sansone, [7]G. Balercia, [6]A. R. M. Granata, [8]A. Sinisi, [9]F. Lanfranco, [10]P. Pasqualetti, [3] C. Foresta, [1]A. Lenzi and 'Amico-Andrologo' Study Group

[1]Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza University of Rome, Rome, Italy, [2]Department of Clinical and Experimental Sciences, Unit of Endocrinology and Metabolism, University of Brescia, Brescia, Italy, [3]Unit of Andrology and Reproductive Medicine, Department of Medicine, University of Padova, Padova, Italy, [4]Sexual Medicine and Andrology, Dipartimento Scienze Biomediche Sperimentali e Cliniche 'Mario Serio', University of Florence, Firenze, Italy, [5]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II di Napoli, Naples, Italy, [6]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy, [7]Endocrinology, Department of Clinical and Molecular Sciences, Polytechnic University of Marche, Ancona, Italy, [8]Andrology Unit, Department of Medical, Surgical, Neurological, Metabolic and Aging Sciences, University Hospital,L. Vanvitelli University of Campania, Naples, Italy, [9]Andrology Unit, Division of Endocrinology, Diabetology and Metabolism, Department of Medical Sciences, University of Turin, Torino, Italy, and [10]Service of Medical Statistics and Information Technology, Fatebenefratelli Foundation for Health Research and Education, Rome, Italy

ABSTRACT

Background: Risk factors established during adolescence affect health outcomes in adulthood, although little is known about how adolescent health risk behaviours (HRBs) affect testicular development and reproductive health.

Objectives: To assess prevalence of HRBs among last year high school students; to describe the most prevalent andrological disorders in this cohort; to explore HRBs associated with andrological disorders and investigate factors possibly associated with impaired testicular development in puberty.

Materials and methods: The Amico-Andrologo Survey is a permanent nationwide surveillance programme conducted by the Italian Society of Andrology and Sexual Medicine and supported by the Ministry of Health. A nationally representative survey of final-year male high school students was conducted using a validated structured interview (n = 10124) and medical examination (n = 3816).

Results: Smoking (32.6%), drinking (80.6%) and use of illegal drugs (46.5%) are common in adolescence. 16.6% of subjects were overweight, 3.1% were underweight and 2.3% were obese. Among sexually active students (60.3%), unprotected sex was very common (48.3%). Only 11.6% had been treated for andrological disorders, despite an abnormal clinical examination in 34.6%. Bilateral testicular hypotrophy (14.0%), varicocoele (27.1%) and phimosis (7.1%) were the most prevalent disorders; 5.1% complained of premature ejaculation and 4.7% had an STI. Underweight and heavy alcohol or drug use were associated with testicular hypotrophy. HRBs emerged as significant predictors of testicular hypotrophy, explaining up to 9.6% of its variance. Limitations include risk of selection bias for voluntary physical examination and recall bias for the self-compiled questionnaire.

Discussion: There is an emerging global adverse trend of HRBs in male high school students. A significant proportion of adolescent males with unsuspected andrological disorders engage in behaviours that could impair testicular development.

Conclusion: Greater attention to the prevention of andrological health in adolescence is needed.

INTRODUCTION

Sexual and reproductive health has recently emerged as an important healthcare need that involves a variety of clinical and public health issues, including sexually transmitted infections (STIs), declining fertility and rising rates of testicular cancer (Khabbaz *et al.*, 2014; Slater & Robinson, 2014; Nigam *et al.*, 2015; Stephen *et al.*, 2016).

Most studies investigating overlapping risk factors such as smoking, alcohol consumption and physical activity in relation to male sexual and reproductive health have been performed in middle-aged men, while those in adolescents are small and inconsistent. Adolescence is a key time for prevention of health risk behaviours (HRBs) such as alcohol and tobacco use, drug abuse and unprotected sex (Sawyer *et al.*, 2012). Male adolescents are a group of particular concern, as they have higher levels of unmet healthcare needs (Mulye *et al.*, 2009) and less interest in prevention. Testicular examination is rarely performed by primary care providers (Bell *et al.*, 2013), and self-examination is rarely suggested (Thornton, 2016); however, incidence of testicular cancer is at its peak in the third decade of life, just after the end of puberty. While more complex imaging techniques, such as testicular contrast-enhanced magnetic resonance (Manganaro *et al.*, 2015) or elastography (Pozza *et al.*, 2016), might help diagnose more suspicious lesions, self-examination of the testis should be recommended for early prevention (Aberger *et al.*, 2014). The US Centers for Disease Control and Prevention (CDC) Healthy 2020 Objectives identified several targets relevant to adolescents, focusing on weight, substance use and abuse, smoking and sexual and reproductive health (U.S. Department of Health and Human Services, 2013). Alcohol use was estimated as the seventh leading risk factor for disability-adjusted life-years in 2016, accounting for 6.8% (5.8–8.0) of age-standardized male deaths (GBD 2016 Alcohol Collaborators, 2018).

Adolescence is considered a vulnerable time for the development and maturation of the genitourinary tract (Abreu & Kaiser, 2016), but there is a clear gender gap in research and prevention programmes for reproductive health and sexuality (Bell *et al.*, 2013). We carried out a nationwide andrological health surveillance programme using an adaptation of the Youth Risk Behaviours Surveillance (YRBS) validated questionnaire, hence enabling a comparison with US-CDC data.

This observational, cross-sectional study (i) provides estimates of the prevalence of health risk behaviours in young male adolescents in the last year of high school; (ii) describes the most frequent andrological disorders found in this large population; (iii) explores HRBs associated with andrological disorders; and (iv) investigates possible factors associated with impaired testicular growth during puberty.

SUBJECTS AND METHODS

The Amico-Andrologo Survey (AA Survey) is a permanent national project conducted by the Italian Society of Andrology and Sexual Medicine (SIAMS) since 2010 in young men in their final year of high school. All subjects are invited to complete an anonymous, self-administered written questionnaire and, if willing, to undergo a voluntary onsite andrological examination performed by trained clinicians. The study is approved by the local Institutional Board Review, Regional School Authorities and the Ministry of Health (protocol 19251/P - 'Prevenzione in Andrologia' signed 28 April 2008).

Sample demographics and data collection

The AA Survey achieved a 4.6% sampling of the entire male final-year school population. The student participation rate was > 99.5%. A post-stratification adjustment was performed using demographic distributions from the most recent data obtained from the Italian Census, the standard for measuring demographic and other trends in Italy (see Appendix S1). All subjects completed the questionnaire before undergoing the voluntary onsite blinded andrological examination. No formal sample size calculation was performed in advance and the number of students was set on the basis of feasibility criteria. However, a sample size of about 10 thousand units (questionnaire) allows to reach a precision for prevalence estimates of 0.005 in case of largest variability ($p = 0.5$), resulting in a 95% confidence interval of 0.49–0.51. In addition, a sample size of about 3816 units (medical examination) allows to perform reliable multivariable analysis. More in detail, in our model with 14 independent variables (as for linear regression model with global testicular volume as dependent variable), the Case-per-Variable (CPV) was 3816/14 = 272, largely higher than the conventional suggested threshold (CpV = 10). Similarly, in our model with 12 independent variables (as for logistic regression with bilateral testicular hypotrophy as binary dependent variable), the Event-per-Variable was 534/12 = 45, again largely higher than the conventional suggested threshold (EpV = 10).

Main outcome measures

The AA Survey questionnaire is based on the validated CDC YRBS questionnaire (Centers for Disease Control and Prevention (CDC), 2018) (addressing age/family composition, anthropometrics, physical activity, smoking/alcohol/drug use, sexual activity, use of prescribed medications) implemented with items addressing past and present andrological and psychosexual complaints. During the clinical examinations, the parameters registered were weight, height, waist circumference, testicular position and volume (by Prader orchidometer) (Chipkevitch *et al.*, 1996), presence and grading of varicocoele (Isidori & Lenzi, 2017) and presence of penile disorders (curvature, phimosis, warts, hypospadias).

Statistical analysis

Detailed statistical methods are reported in the Appendix S1. Briefly, normality was assessed by the Shapiro–Wilk test ($p > 0.05$); transformation was used when appropriate. The association between bitesticular volume (BTV) BMI and HRBs (smoking, alcohol, drugs and physical activity) was assessed by a fractional polynomial method. Logistic regression models were used when using hypotrophy as a dependent variable.

The proportion of variance explained by each variable (HRBs, BMI, etc.) was assessed as R^2. The contribution of each factor above other covariates was determined through assessment of mean squared errors for full and reduced models. Regression analyses were repeated after excluding subjects with previous or current andrological conditions (Schafer, 1997). All analyses were performed using STATA software (version 11.0; Stata Corporation, College Station, TX, USA) and SPSS 17.0 (Chicago, IL, USA). All comparisons were performed using 2-tailed significance tests, with $p \leq 0.05$ considered statistically significant.

Role of the funding source

The Italian Ministry of Health was not directly involved in any phase of this study, from data collection to manuscript preparation.

RESULTS

A total of 10124 self-completed questionnaires were collected; 185 (1.8%) were excluded due to inconsistent completion. A total of 3816 subjects volunteered to undergo a medical interview and examination (Table 1). Both the questionnaire and the medical examination populations were representative of the population of young men in the last year of high school. No statistical difference was found between self-reported or clinician-measured anthropometric measurements. Overall, 4.9% (CI 3.9–6.1) of all examined subjects had a waist circumference above 102 cm. Comparison with the US-YRBS matched cohort (Table S1) revealed that, for a similar height, US subjects weighed more, with a mean BMI score 2.1 (CI 1.8–2.3) points higher.

Health risk behaviours

Just over half (51%) of subjects reported having tried *smoking* (average number of smoked cigarettes: 40–50 per week, 6.5/day). Occasional *alcohol consumption* was extremely high (80%), while heavy drinking was reported by 30%. Half had tried an *illegal drug*, with marijuana/hashish being the most common (45–50%), followed by alkyl nitrites (12–17%) and cocaine (8–10%); 29% had taken an illegal drug in the month before the survey. Comparison between the questionnaires and MD interviews revealed a similar prevalence for most items (Table 1). Subjects undergoing medical examination reported slightly increased consumptions of alcohol, recreational drugs and current smoking. The similarities between Italian and US data on HRBs (Tables S1 and S2) revealed a widespread trend, with only marginal differences (ecstasy 5% in Italy vs. 10% in United States; methamphetamines 3.5% in Italy vs. 5.5% in United States). However, use of injected drugs was 2–3 times lower in Italy than in the United States (Table S2).

Overall, the prevalence of sexual activity was 60.3% among the subjects answering the questionnaires, and 59.7% for subjects undergoing medical examination. Precocious sexual activity (before the age of 13) and multiple partners (>4) were more frequent in the United States than Italy (6.4 vs. 3.3 and 22.7 vs. 12.9, $p < 0.01$). In contrast, unprotected sex was more common in Italy, with 47% to 65% of self-reporting subjects and 41% of examined subjects reporting having had unprotected sex (Table 1). Reported age at first sexual intercourse was 16.12 and 16.58 years, respectively, in questionnaires and visits.

Andrological disorders on clinical examination

Testicular examination data were available for 3816 subjects (Table 2). Left testicular volume was on average smaller than the right. BTV fell below the target for adults in 22.8% of subjects

Table 1 Anthropometric data and health risk behaviours on self-reported questionnaires and MD-recorded clinical examinations. Categorical data are presented as percentage (99% CI); continuous variables are presented as mean (99% CI)

	Self-reported AAS questionnaires (n = 9939)	MD clinical examination record (n = 3816)
Age		
18 years old (%)	90%	89%
19 years old (%)	9%	10%
>20 years old (%)	<1%	<1%
Weight (kg)	72.75 (72.49–73.02)	72.8 (72.44–73.15)
Height (cm)	178.51 (178.35–178.67)	178.42 (178.21–178.63)
BMI	22.8 (22.73–22.88)	22.74 (22.83–22.93)
Underweight (BMI < 18.5)	3.07 (2.68–3.53)	3.4 (2.76–4.3)
Normal weight (BMI 18.5–25)	78 (76.96–79)	78.4 (76.64–80.1)
Overweight (BMI 25–30)	16.62 (15.73–17.56)	15.6 (14.13–17.18)
Obese (BMI > 30)	2.31 (1.96–2.70)	2.5 (1.95–3.29)
Waist circumference (cm)	–	82.9 (82.6–83.36)
Waist > 102 cm (%)	–	4.9 (3.9–6.13)
Tobacco use		
Ever tried cigarette smoking (%)	51.31 (50.14–52.48)	55.3 (53.0–57.5)
Smoked at least one cigarette every day for last 30 days (current smoker) (%)	32.6 (31.52–33.7)	38.33 (36.51–40.19)
Smoked a whole cigarette for the first time before age 13 years (all) (%)	23.21 (21.74–24.76)	10.5 (8.8–12.6)
Smokes more than 10 cigarettes per day (among current smokers) (%)	30.83 (28.97–32.75)	13.94 (12.46–15.56)
Alcohol use		
Had at least one drink of alcohol on at least 1 day (in the 30 days before the survey, current drinking) (%)	80.58 (79.61–81.51)	80.1 (78.5–81.53)
Had five or more drinks of alcohol in a weekend or twelve drinks or more in a month (in the 30 days before the survey) (%)	28.46 (27.3–29.65)	29.9 (27.9–32.06)
Mean number of drinks per weekend	2.32 (2.24–2.40)	2.67 (2.56–2.78)
Drug use		
Ever tried any illegal drug (%)	46.53 (45.23–47.84)	50.3 (48.05–52.57)
Anabolic steroid use		
Ever used anabolic agents (%)	1.8 (1.2–2.4)	1.1 (0.3–1.9)
Sexual activity		
Ever had sexual intercourse (%)	60.33 (59.17–61.47)	59.7 (57.83–61.54)
Had sexual intercourse for the first time before age 13 years (%)	3.33 (2.76–4.01)	0.31 (0.12–0.93)
Had sexual intercourse with four or more persons (in the last year) (%)	12.95 (11.8–14.2)	9.8 (8.02–11.91)
Age at first sexual intercourse (years)	16.11 (16.07–16.16)	16.58 (16.36–16.8)
Ever had unprotected sexual intercourse (%)	48.29 (46.96–49.63)	50.1 (47.6–52.58)

(Table 2). Up to 14% had both testes below the target volume for young adults. Left varicocoele occurred in 27% of subjects (50% grade I, 30% grade II, 20% grade III). Higher grade left varicocoele might have been a causative factor for ipsilateral hypotrophy; however, right varicocoele was much less frequent than right hypotrophy, suggesting either a contralateral influence of left disease or other contributing factors (see next section). Hypotrophy was defined as testicular volume < 14 mL.

Figure 1 describes the distribution of BTV in relation to BMI in all patients. The prevalence of testicular hypotrophy was higher in underweight than in normal-weight subjects (21.7% vs. 27.1%, $p = 0.037$).

Phimosis was also a frequent finding (4.8%), while treated and untreated morphological abnormalities in the external urethral meatus were found in a small (1.4%) but relevant number of subjects. The most prevalent complaints were premature ejaculation (6.1% of interviewed and 6.6% of clinically examined subjects), erectile dysfunction (2.0% and 1.6%, respectively) and delayed ejaculation (1.46% and 2.26%, respectively).

Contributors to andrological health

BTV was considered the best single indicator of andrological health in the study population (Figures 1–4). Alcohol was the factor with the greatest negative effect, with a greater than 4- or 5-mL reduction in BTV, respectively, observed in moderate (mean of 2 alcohol units per day during weekends) or heavy (mean of ≥ 3 alcohol units per day during weekends) vs. occasional drinkers (mean of < 2 alcohol units per day during weekends) (Table 3, Fig. 2). Smoking was associated with a marginally higher BTV (1.5 ± 0.4 mL, Table 3); however, the mean testicular volume in heavy smokers was lower than in moderate smokers (-2.1 ± 0.5 mL) (Fig. 3). Heavy drug use was associated with a mean 1.8 ± 0.4 mL reduction in BTV (Fig. 3).

Grades 2 and 3 left varicocoele were associated with significantly lower BTV (Fig. 4). There was a difference of -5.0 ± 0.5 and -5.9 ± 0.6 between the BTV of subjects with grade 3 left varicocoele and those with no or with grade 1 varicocoele. The adjunct of an HRB such as alcohol further lowered the BTV, confirming that these were independent but concurrent factors affecting testicular development (Fig. 4). Multivariable analysis showed no significant varicocoele–HRB interaction.

Regression models are reported in Tables 3 and 4 for all subjects and for the subset without past or present andrological disorders ($n = 2235$). Figure 5 shows the variance explained by each factor or group of factors. Overall, the full-adjusted model accounted for 14.5% of inter-individual variation in BTV. Lifestyle factors accounted for 9.6% of the variability over and above

Table 2 Andrological disorders found on clinical examination. Categorical data are presented as percentage (99% CI); continuous variables are presented as mean (99% CI)

	n = 3816
Testicular disorders	
Left testicular volume (mL)	16.16 (16.04–16.28)
Right testicular volume (mL)	16.94 (16.82–17.07)
Global testicular hypotrophy: right + left testicular volumes < 28 mL (%)	22.81 (21.08–24.64)
Bilateral testicular hypotrophy (both < 14 mL) (%)	14.01 (12.60–15.53)
Left varicocoele (total)	27.11 (25.27–29.03)
– I grade	– 50.47 (44.90–56.03)
– II grade	– 30.25 (25.37–35.61)
– III grade	– 19.28 (15.25–24.07)
Right varicocoele (total)	3.62 (2.91–4.50)
– I grade	– 84.42 (71.11–92.26)
– II grade	– 12.99 (6.01–25.84)
– III grade	– 2.6 (0.51–12.21)
Penile disorders	
Phimosis	7.11 (6.10–8.27) [133/1882]
Penile infections and cutaneous lesions	4.73 (3.88–5.84)
Abnormal urethral external meatus (hypospadias, epispadias)	0.76 (0.47–1.12)

Figure 1 Distribution of global testicular volume against BMI plotted on a Log_{10} scale. Bitesticular volume is the sum of right and left testicular volume. The dotted lines represent the lower limit of normal global testicular volume (28 mL).

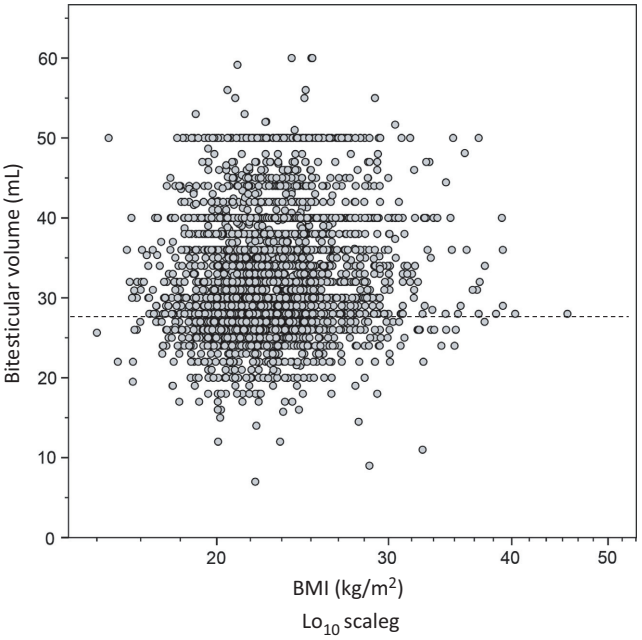
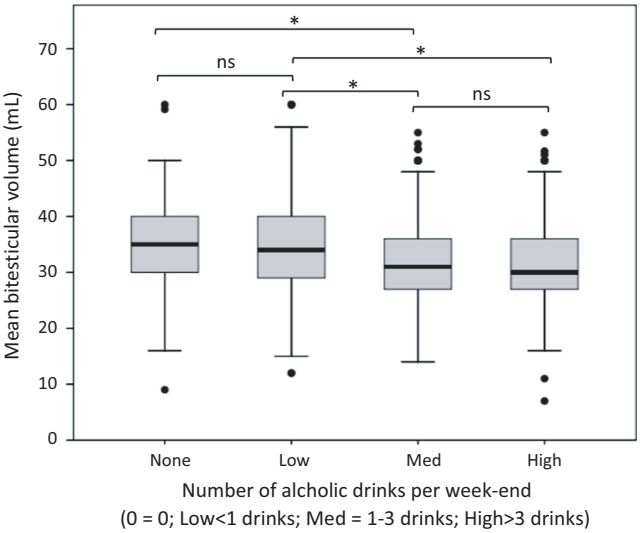


Figure 2 Box-plot of mean bitesticular volume against the level of weekend alcohol consumption. ns = non-significant difference, *$p < 0.01$.



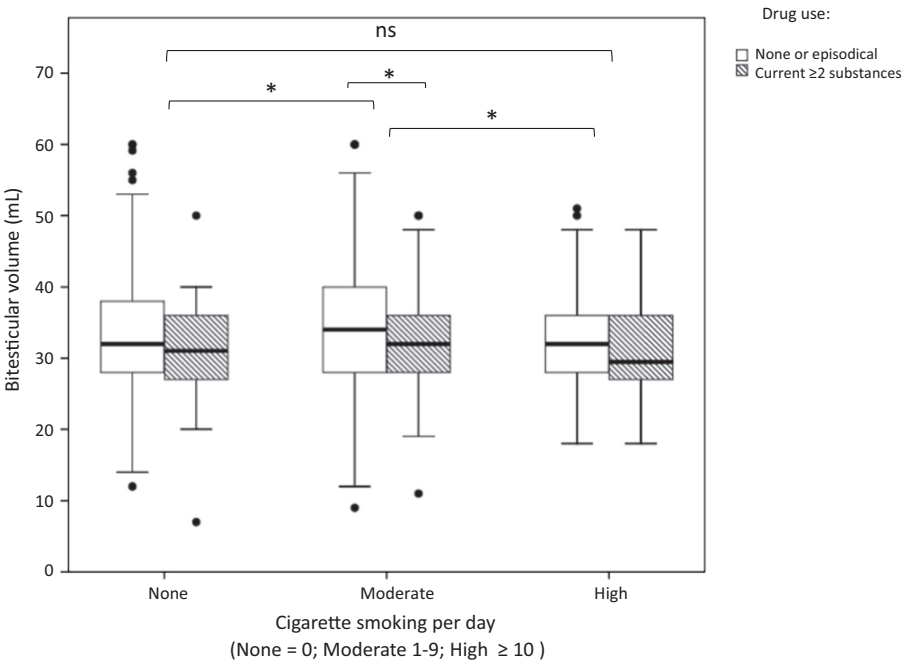


Figure 3 Box-plot of mean bitesticular volume against the level of cigarette smoking and concomitant use of illicit substances. ns = non-significant, *$p < 0.01$.

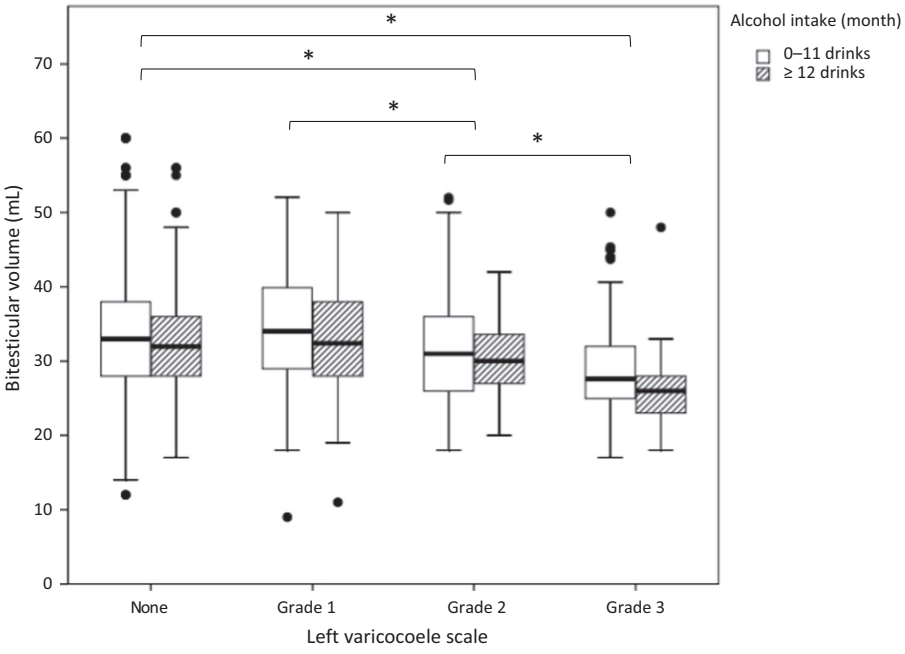


Figure 4 Box-plot of mean bitesticular volume showing the combined effect of the severity of varicocoele and drinking per month. *$p < 0.01$.

other significant contributors such as age, BMI and varicocoele; heavy alcohol use accounted for 2.9% of variability over that explained by other contributors. Individual factors such as BMI, age at puberty and past medical disorders were all significant contributors (Table 3). Logistic regression analysis was used to predict global testicular hypotrophy (Table 4), confirming that the most important categorical contributors to testicular hypotrophy were the severity of left varicocoele followed by heavy alcohol intake and drug use, but not smoking.

DISCUSSION

According to the WHO's 2004 Global Burden of Disease study, the main risk factors for incident disability-adjusted life year (DALYs) in 10–24-year-olds are alcohol (7% of DALYs), unprotected sex (4%), lack of contraception (2%) and illicit drug use (2%) (Gore *et al.*, 2011). Risk factors and lifestyles adopted in adolescence may negatively affect adult health as well as that of future generations, through epigenetics (Jenkins *et al.*, 2016). Prevention is the most efficacious way of improving sexual and reproductive health, as further confirmed by the high prevalence of undiagnosed (and hence untreated) andrological disorders.

A specific 'window of vulnerability' of male genitalia in relation to pubertal exposure has been hypothesized (Richiardi *et al.*, 2007). Ours is the first study addressing the association between drug/alcohol abuse and testicular volume (Nieschlag *et al.*, 2010; Foresta *et al.*, 2013). We found slightly higher mean

Table 3 Association of global testicular volume (GTV) and covariates included in the analysis. Linear regression coefficients (β, unstandardized), standard error (SE), and *p*-value

Variables	Overall (n = 3816) r^2 = 14.51%[a]			Excluding subjects with past or present andrological disorders (n = 2235) r^2 = 13.93%[b]		
	β	SE(β)	*p*-value	β	SE(β)	*p*-value
(Constant)	33.384	2.025	0.000	32.811	2.660	0.000
Alcohol (moderate vs. no or mild)	−4.164	0.427	0.000	−4.093	0.538	0.000
Alcohol (heavy vs. no or mild)	−5.500	0.460	0.000	−5.742	0.570	0.000
Smoking (none/moderate/heavy)	1.500	0.372	0.000	1.667	0.482	0.001
Drug use (heavy)	−3.451	0.389	0.000	−3.767	0.512	0.000
Age at puberty (years)	−0.374	0.115	0.001	−0.413	0.146	0.005
BMI (kg/m^2)	0.281	0.059	0.000	0.349	0.083	0.000
Sexual activity	1.062	0.362	0.003	0.982	0.468	0.036
Current andrological disorders (other than varicocoele)	−2.955	0.501	0.000	–	–	–
Left varicocoele (mild/moderate)	2.075	0.543	0.000	–	–	–
Left varicocoele (severe)	−2.048	0.972	0.035	–	–	–
Right varicocoele (mild/moderate)	1.089	0.432	0.012	–	–	–
Right varicocoele (severe)	0.787	1.417	0.579	–	–	–
Cryptorchidism	−2.191	0.953	0.016			

[a]Adj r^2 without lifestyle factors: 4.85% (excluding alcohol, smoking and sexual activity).
[b]Adj r^2 without lifestyle factors: 3.03% (excluding alcohol, smoking and sexual activity).

Table 4 Prediction of bilateral testicular hypotrophy by covariates included in the analysis. Logistic regression analysis with categorical covariates (β, unstandardized), standard error (SE) and *p*-value

Variables	Overall (n = 3816)			
	B	SE(β)	*p*-value	CI
Constant				
Alcohol (occasional)	1.688	0.179	0.000	1.371–2.078
Alcohol (heavy)	1.450	0.179	0.003	1.138–1.847
BMI (kg/m^2)	0.940	0.013	0.000	0.914–0.967
Left varicocoele (mild/moderate)	0.563	0.071	0.000	0.439–0.720
Left varicocoele (severe)	2.100	0.378	0.000	1.475–2.989
Right varicocoele (mild/moderate)	1.172	0.118	0.114	0.963–1.428
Right varicocoele (severe)	0.832	0.245	0.532	0.467–1.481
Smoking (none/moderate/heavy)	0.724	0.067	0.000	0.604–0.867
Drug use (light)	1.343	0.155	0.011	1.071–1.684
Drug use (heavy)	1.442	0.266	0.047	1.005–2.069
Current diseases	2.145	0.246	0.000	1.713–2.686
Past medical history	1.246	0.153	0.075	0.978–1.586

testicular volumes than reported in the largest Italian survey, whereas the prevalence of undescended testis was consistent (Foresta *et al.*, 2013). Left varicocoele was more prevalent than right varicocoele, with Dubin III grade recorded in 19% of subjects, and only a quarter of these had been previously diagnosed and/or treated (Table S3). Several studies have reported ipsilateral hypotrophy in adolescent varicocoele. However, we found a significant proportion of subjects (14%) with both testicles below 14 mL. The present survey suggests an additive effect of HRBs and varicocoele toward reduced testicular volume.

Contrasting evidence has emerged concerning smoking and male fertility (Curtis *et al.*, 1997; Henriksen *et al.*, 2004; Sansone *et al.*, 2018a). The EMAS (Wu *et al.*, 2008) found higher total testosterone in ageing men than in non-smokers, primarily due to an increase in SHBG with a compensatory rise in LH and no effect on free testosterone. Smoking impairs sexual function (Biebel *et al.*, 2016). Maternal use of nicotine patches and/or smoking during pregnancy have been associated with bilateral cryptorchidism and reduced spermatozoa quality in the male offspring (Main *et al.*, 2010). A significant association between marijuana use and non-seminoma testicular germ cell tumours was reported in users who started before the age of 18 years (OR, 2.8; 95% CI, 1.6–5.1), suggesting vulnerability to testicular carcinogenesis during puberty (Richiardi *et al.*, 2007; Daling *et al.*, 2009).

The questionnaire revealed a substantial number of precocious and/or heavy cigarette smokers. The percentage reporting ever having tried a cigarette was similar to that reported in the United States, although the number of heavy smokers was much higher in Italy. Smoking had no adverse effect on BTV, but heavy smoking was an independent negative predictor of testicular hypotrophy. The use of stimulating illicit drugs was also similar to that reported in the United States, with up to 9% having tried cocaine. The trend for marijuana use (reported by up to 50% of respondents) also seems to be global.

Another concern for gonadal health is steroid abuse (Sansone *et al.*, 2018b), as recently stated by the Endocrine Society (Pope *et al.*, 2014b). About 2% of American high school students reported having used anabolic agents abuse in the previous 12 months (Pope *et al.*, 2014b), compared to less than 1 to 2% of Italian students. Use of such agents in fact rarely begins at school age (Pope *et al.*, 2014a), but mostly between 22 and 24 years (Pope *et al.*, 2014b).

The WHO estimates that adolescent alcohol abuse is increasingly widespread (Gore *et al.*, 2011). Binge drinking is common in this age group (Townshend & Duka, 2005) and is associated with reduced testosterone levels (Frias *et al.*, 2000). *In vivo* and *in vitro* studies have demonstrated that excessive alcohol intake suppresses the hypothalamic–pituitary–gonadal axis (La Vignera *et al.*, 2013; Rachdaoui & Sarkar, 2013). In adolescents, even moderate alcohol consumption impairs testicular endocrine function far beyond the period of consumption (Diamond *et al.*, 1986). In our study, around 30% of our subjects reported weekend alcohol consumption. We found a strong association between alcohol abuse and reduced BTV or testicular hypotrophy, possibly suggesting testicular function impairment; however, whether these findings correlate with worse spermatogenesis or endocrine function has yet to be confirmed.

Alcohol can suppress hypothalamic secretion of LHRH (Emanuele *et al.*, 2002) and GHRH (Dees *et al.*, 2000) and kisspeptin responsiveness to IGF-1 (Hiney *et al.*, 2010), thereby delaying puberty. Our data seemingly support alcohol-induced disturbances in HPG axis activity during puberty, possibly slowing gonadal maturation. The data are consistent with a recent report correlating drinking intensity and smoking during adolescence with subsequent (2 to 5 years later) arterial changes relevant to atherosclerosis progression, with independent and additive effects on vascular damage (Charakida *et al.*, 2018).

In adults, the effects of light or moderate alcohol consumption are controversial (Sansone *et al.*, 2018a), while there is a

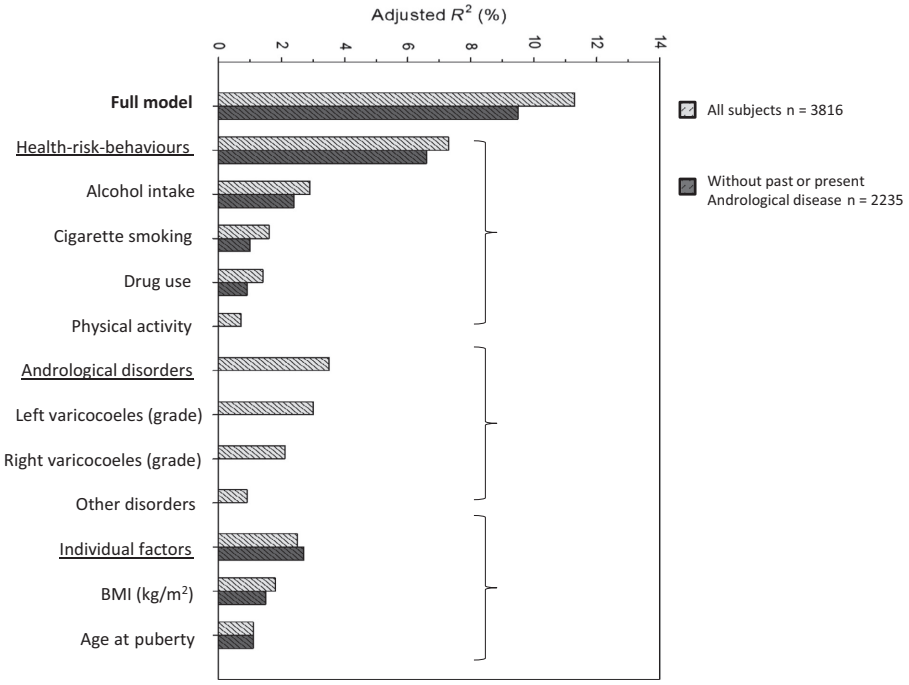


Figure 5 Modelling of contribution of youth risk behaviours, age at puberty and BMI to testicular volume in the entire cohort and in those without andrological disorders.

consensus on the detrimental effect of heavy drinking on fertility (Curtis *et al.*, 1997; Henriksen *et al.*, 2004). However, the responsiveness of the HPG axis during puberty is rather different (Isidori *et al.*, 2008; Giannetta *et al.*, 2012). In our multivariable analysis, high alcohol intake was additive to the coexistence of disorders such as varicocoele and, independently of other risk factors or the age of pubertal onset, explained up to 8% of variance in testicular volume.

Limitations

Our survey has advantages, but also limitations. The AA Survey was based on the YRBS (Centers for Disease Control and Prevention (CDC), 2018), an excellent example of a validated, reliable school survey. The questionnaires were randomly sampled to be representative of a national cohort of young men attending the last year of high school; however, the clinical examinations were performed on a voluntary basis, possibly biasing the prevalence of pathological findings. Approximately one-third of the interviewed subjects underwent an andrological examination: the demographics, anthropometrics and risk behaviours of the interviewed subjects were nearly identical, suggesting minimal bias. The validity of our findings was further confirmed by repeating the statistical modelling in subjects without a history of andrological disorders or treatments (Table 3). Finally, multivariable analysis reduced other confounding factors.

As with most questionnaires, we had to rely on self-reported data, although anonymization of the questionnaire should have enabled the true figures to be collected. Over-reporting is unlikely, because adolescents do not generally perceive their reproductive function as vulnerable. Overall, the two samples (interviews and examinations) were large and the response rate and degree of data completion were satisfactory. Even with its limitations, the AA Survey cohort is the largest data collection available in Italy since mandatory military screening was abolished, and one of the largest in Europe.

Additionally, as this was an observational study, we could not establish undisputable aetiological connections between HRBs and testicular volume. The effects of HRBs, such as alcohol, on testicular health and function have been proven by an ever-increasing number of papers; however, we cannot rule out that both HRBs and testicular impairment could be the result of a third, external factor, such as environmental, social or behavioural factors.

CONCLUSIONS

We provide the first evidence that substance abuse, particularly alcohol, during adolescence is associated with impaired testicular volumetric development. The additive association of HRBs and andrological disorders in the transition from adolescence to adulthood suggests that some pubertal disorders may be linked to lifestyle changes in recent decades (Buck Louis *et al.*, 2008). Preventive interventions could improve the chance of healthy development (Catalano *et al.*, 2012). Greater attention to the importance of andrological health in adolescence is needed; strategies that place the adolescent years at centre stage could offer valuable opportunities to improve fertility later in life. Longitudinal observation is needed to establish whether the 10% reduction in testicular volume linked to HRBs during adolescence that we observed affects the fatherhood of this cohort of subjects.

ACKNOWLEDGEMENTS

The Amico-Andrologo Study Group: Antonio Anastasia, Nicola Cacco, Melissa Cutini, Carla Di Dato, Vilma Duretto, Dario Esposito, Francesco Garifalos, Antonio RM Granata, Davide Menafra, Marianna Minnetti, Giovanna Motta, Carlotta Pozza, Riccardo Pofi, Giulia Rastrelli, Jiska Ristori, Gianmaria Salvio, Andrea Sansone, Daniele Santi, Umberto Valente.

Language revision: Marie-Hélène Hayles.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the local Institutional Review Board, the regional school authorities and the Ministry of Health (protocol 19251/P/2008).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

The datasets analysed during the current study are not publicly available, but are available from the corresponding author on reasonable request subject to authorization by the Italian Ministry of Health.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests in relation to this work.

AUTHORS' CONTRIBUTIONS

Daniele Gianfrilli, Alberto Ferlin, Andrea M. Isidori, Carlo Foresta and Andrea Lenzi contributed to the study design, data acquisition and interpretation, and drafting of the report; Andrea Garolla, Mario Maggi, Rosario Pivonello, Daniele Santi, Andrea Sansone, Antonio RM Granata, Giancarlo Balercia, Antonio Agostino Sinisi and Fabio Lanfranco contributed to the data acquisition and review of the report, and Patrizio Pasqualetti contributed to statistical analysis and review of the report.

FUNDING

This research was funded by the National Centre for Disease Prevention and Control of the Italian Ministry of Health as part of the 'Prevention in Andrology' project (protocol 19251/P – 'Prevenzione in Andrologia' signed April 28, 2008, PI Prof. Andrea Lenzi, Sapienza – University of Rome). None of the authors received any forms of compensation by pharmaceutical companies or agencies for their work in this project. The authors had full access to all data in the study and share full responsibility for the decision to submit for publication.

REFERENCES

Aberger M, Wilson B, Holzbeierlein JM, Griebling TL & Nangia AK. (2014) Testicular self-examination and testicular cancer: a cost-utility analysis. *Cancer Med* 3, 1629–1634.

Abreu AP & Kaiser UB. (2016) Pubertal development and regulation. *Lancet Diabetes Endocrinol* 4, 254–264.

Bell DL, Breland DJ & Ott MA. (2013) Adolescent and young adult male health: a review. *Pediatrics* 132, 535–546.

Biebel MG, Burnett AL & Sadeghi-Nejad H. (2016) Male Sexual Function and Smoking. *Sex Med Rev* 4, 366–375.

Buck Louis GM, Gray LE Jr, Marcus M, Ojeda SR, Pescovitz OH, Witchel SF, Sippell W, Abbott DH, Soto A, Tyl RW, Bourguignon JP, Skakkebaek NE, Swan SH, Golub MS, Wabitsch M, Toppari J & Euling SY. (2008) Environmental factors and puberty timing: expert panel research needs. *Pediatrics* 121(Suppl 3), S192–S207.

Catalano RF, Fagan AA, Gavin LE, Greenberg MT, Irwin CE Jr, Ross DA & Shek DT. (2012) Worldwide application of prevention science in adolescent health. *Lancet* 379, 1653–1664.

Centers for Disease Control and Prevention (CDC). (2018) Youth Risk Behavior Surveillance System (YRBSS) questionnaires. Available at: https://www.cdc.gov/healthyyouth/data/yrbs/questionnaires.htm.

Charakida M, Georgiopoulos G, Dangardt F, Chiesa ST, Hughes AD, Rapala A, Davey Smith G, Lawlor D, Finer N & Deanfield JE. (2018) Early vascular damage from smoking and alcohol in teenage years: the ALSPAC study. *Eur Heart J* 21, 345–353.

Chipkevitch E, Nishimura RT, Tu DG & Galea-Rojas M. (1996) Clinical measurement of testicular volume in adolescents: comparison of the reliability of 5 methods. *J Urol* 156, 2050–2053.

Curtis KM, Savitz DA & Arbuckle TE. (1997) Effects of cigarette smoking, caffeine consumption, and alcohol intake on fecundability. *Am J Epidemiol* 146, 32–41.

Daling JR, Doody DR, Sun X, Trabert BL, Weiss NS, Chen C, Biggs ML, Starr JR, Dey SK & Schwartz SM. (2009) Association of marijuana use and the incidence of testicular germ cell tumors. *Cancer* 115, 1215–1223.

Dees WL, Dissen GA, Hiney JK, Lara F & Ojeda SR. (2000) Alcohol ingestion inhibits the increased secretion of puberty-related hormones in the developing female rhesus monkey. *Endocrinology* 141, 1325–1331.

Diamond F, Ringenberg L, Macdonald D, Barnes J, Hu CS, Duckett G, Sweetland M & Root A. (1986) Effects of drug and alcohol-abuse upon pituitary testicular function in adolescent males. *J Adolesc Health* 7, 28–33.

Emanuele N, Ren J, LaPaglia N, Steiner J & Emanuele MA. (2002) EtOH disrupts female mammalian puberty: age and opiate dependence. *Endocrine* 18, 247–254.

Foresta C, Garolla A, Frigo AC, Carraro U, Isidori AM, Lenzi A & Ferlin A. (2013) Anthropometric, penile and testis measures in post-pubertal Italian males. *J Endocrinol Invest* 36, 287–292.

Frias J, Torres JM, Rodriguez R, Ruiz E & Ortega E. (2000) Effects of acute alcohol intoxication on growth axis in human adolescents of both sexes. *Life Sci* 67, 2691–2697.

GBD 2016 Alcohol Collaborators. (2018) Alcohol use and burden for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 392, 1015–1035.

Giannetta E, Gianfrilli D, Barbagallo F, Isidori AM & Lenzi A. (2012) Subclinical male hypogonadism. *Best Pract Res Clin Endocrinol Metab* 26, 539–550.

Gore FM, Bloem PJ, Patton GC, Ferguson J, Joseph V, Coffey C, Sawyer SM & Mathers CD. (2011) Global burden of disease in young people aged 10-24 years: a systematic analysis. *Lancet* 377, 2093–2102.

Henriksen TB, Hjollund NH, Jensen TK, Bonde JP, Andersson AM, Kolstad H, Ernst E, Giwercman A, Skakkebaek NE & Olsen J. (2004) Alcohol consumption at the time of conception and spontaneous abortion. *Am J Epidemiol* 160, 661–667.

Hiney JK, Srivastava VK & Les Dees W. (2010) Insulin-like growth factor-1 stimulation of hypothalamic KiSS-1 gene expression is mediated by Akt: effect of alcohol. *Neuroscience* 166, 625–632.

Isidori AM & Lenzi A. (2017) *Ultrasound of the Testis for the Andrologist - Morphological and Functional Atlas*. Springer International Publishing, Cham, Switzerland.

Isidori AM, Giannetta E & Lenzi A. (2008) Male hypogonadism. *Pituitary* 11, 171–180.

Jenkins TG, Aston KI, Meyer TD, Hotaling JM, Shamsi MB, Johnstone EB, Cox KJ, Stanford JB, Porucznik CA & Carrell DT. (2016) Decreased fecundity and sperm DNA methylation patterns. *Fertil Steril* 105, 51–57. e51–53.

Khabbaz RF, Moseley RR, Steiner RJ, Levitt AM & Bell BP. (2014) Challenges of infectious diseases in the USA. *Lancet* 384, 53–63.

La Vignera S, Condorelli RA, Balercia G, Vicari E & Calogero AE. (2013) Does alcohol have any effect on male reproductive function? A review of literature. *Asian J Androl* 15, 221–225.

Main KM, Skakkebaek NE, Virtanen HE & Toppari J. (2010) Genital anomalies in boys and the environment. *Best Pract Res Clin Endocrinol Metab* 24, 279–289.

Manganaro L, Vinci V, Pozza C, Saldari M, Gianfrilli D, Pofi R, Bernardo S, Cantisani V, Lenzi A, Scialpi M, Catalano C & Isidori AM. (2015) A

prospective study on contrast-enhanced magnetic resonance imaging of testicular lesions: distinctive features of Leydig cell tumours. *Eur Radiol* 25, 3586–3595.
Mulye TP, Park MJ, Nelson CD, Adams SH, Irwin CE Jr & Brindis CD. (2009) Trends in adolescent and young adult health in the United States. *J Adolesc Health* 45, 8–24.
Nieschlag E, Behre HM & Nieschlag S. (2010) *Andrology: Male Reproductive Health and Dysfunction*, 3rd edn. Springer, Heidelberg; New York.
Nigam M, Aschebrook-Kilfoy B, Shikanov S & Eggener S. (2015) Increasing incidence of testicular cancer in the United States and Europe between 1992 and 2009. *World J Urol* 33, 623–631.
Pope HG Jr, Kanayama G, Athey A, Ryan E, Hudson JI & Baggish A. (2014a) The lifetime prevalence of anabolic-androgenic steroid use and dependence in Americans: current best estimates. *Am J Addict* 23, 371–377.
Pope HG Jr, Wood RI, Rogol A, Nyberg F, Bowers L & Bhasin S. (2014b) Adverse health consequences of performance-enhancing drugs: an Endocrine Society scientific statement. *Endocr Rev* 35, 341–375.
Pozza C, Gianfrilli D, Fattorini G, Giannetta E, Barbagallo F, Nicolai E, Cristini C, Di Pierro GB, Franco G, Lenzi A, Sidhu PS, Cantisani V & Isidori AM. (2016) Diagnostic value of qualitative and strain ratio elastography in the differential diagnosis of non-palpable testicular lesions. *Andrology* 4, 1193–1203.
Rachdaoui N & Sarkar DK. (2013) Effects of alcohol on the endocrine system. *Endocrinol Metab Clin North Am* 42, 593–615.
Richiardi L, Pettersson A & Akre O. (2007) Genetic and environmental risk factors for testicular cancer. *Int J Androl* 30, 230–240; discussion 240–231.
Sansone A, Di Dato C, de Angelis C, Menafra D, Pozza C, Pivonello R, Isidori A & Gianfrilli D. (2018a) Smoke, alcohol and drug addiction and male fertility. *Reprod Biol Endocrinol* 16, 3.
Sansone A, Sansone M, Vaamonde D, Sgro P, Salzano C, Romanelli F, Lenzi A & Di Luigi L. (2018b) Sport, doping and male fertility. *Reprod Biol Endocrinol* 16, 114.
Sawyer SM, Afifi RA, Bearinger LH, Blakemore SJ, Dick B, Ezeh AC & Patton GC. (2012) Adolescence: a foundation for future health. *Lancet* 379, 1630–1640.
Schafer JL. (1997) *Analysis of Incomplete Multivariate Data*. CRC Press, London.
Slater C & Robinson AJ. (2014) Sexual health in adolescents. *Clin Dermatol* 32, 189–195.
Stephen EH, Chandra A & King RB. (2016) Supply of and demand for assisted reproductive technologies in the United States: clinic- and population-based data, 1995-2010. *Fertil Steril* 105, 451–458.
Thornton CP. (2016) Best Practice in Teaching Male Adolescents and Young Men to Perform Testicular Self-Examinations: A Review. *J Pediatr Health Care* 30, 518–527.
Townshend JM & Duka T. (2005) Binge drinking, cognitive performance and mood in a population of young social drinkers. *Alcohol Clin Exp Res* 29, 317–325.
U.S. Department of Health and Human Services. (2013) 2020 Topics and Objectives. Available at: https://www.healthypeople.gov/2020/topics-objectives/topic/Adolescent-Health?topicxml:id=2.
Wu FC, Tajar A, Pye SR, Silman AJ, Finn JD, O'Neill TW, Bartfai G, Casanueva F, Forti G, Giwercman A, Huhtaniemi IT, Kula K, Punab M, Boonen S, Vanderschueren D & European Male Aging Study G. (2008) Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. *J Clin Endocrinol Metab* 93, 2737–2745.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Supplementary methods.
Table S1. Data extracted from the YRBS survey matched for sampling period, sex and last year of high-school (12th grade).
Table S2. Supplementary data on drug use in self-reported questionnaires and MD-recorded clinical examinations.
Table S3. Past genitourinary medical history, present sexual complaints and knowledge of andrological conditions in the AAAS-Questionnaires and Clinical Examinations (99% CI) ($^{\S}p < 0.01$: AAAS-Questionnaire vs. Clinical Examination).