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ORIGINAL ARTICLE *Infertility*

Current smoking is associated with lower seminal vesicles and ejaculate volume, despite higher testosterone levels, in male subjects of infertile couples

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STUDY QUESTION: What is the impact of smoking behaviour on seminal, hormonal and male genital tract ultrasound parameters in subjects seeking medical care for couple infertility?

STUDY ANSWER: In males of infertile couples, current smokers (CS), when compared with non-smokers, show lower ejaculate and ultrasound-derived seminal vesicles (SV) volume, despite higher testosterone levels.

WHAT IS KNOWN ALREADY: Data on the effects of smoking on male fertility are conflicting. A correlation between smoking and reduced semen parameters has been reported, however, with a high heterogeneity among studies. An association between smoking behaviour and higher testosterone levels in men has been described in several, but not all, the previous studies. No study has systematically evaluated the impact of smoking on the male genital tract ultrasound characteristics.

STUDY DESIGN, SIZE AND DURATION: Retrospective cross-sectional analysis of a consecutive series of 426 subjects seeking medical care for couple infertility from January 2010 to July 2013.

PARTICIPANTS/MATERIALS, SETTING, METHODS: From the entire cohort, 394 men (age 36.0 ± 8.0 years) free of genetic abnormalities were selected. All subjects underwent a complete andrological and physical examination, biochemical and hormonal assessment, scrotal and transrectal colour-Doppler ultrasound and semen analysis (including seminal interleukin-8 levels, sIL-8) within the same day.

MAIN RESULTS AND THE ROLE OF CHANCE: Among the patients evaluated, 229 were never smokers (NS), 56 past smokers (PS) and 109 CS. When CS were compared with the rest of the sample (non-smokers, NS + PS), in a multivariate model (analysis of covariance, ANCOVA) adjusted for age, lifestyle (including alcohol, cannabis and physical activity), BMI and sex hormone-binding globulin, significantly higher androgen (total testosterone, $P = 0.001$; calculated free testosterone, $P < 0.005$) and lower FSH ($P < 0.05$) levels were observed in CS. However, when total testosterone was also included in the multivariate model as a further covariate, the difference in FSH levels was not confirmed. In a similar model, a lower ejaculate volume ($P < 0.01$) and a higher prevalence of normal sperm morphology ($P < 0.02$) were also detected in CS in comparison with the rest of the sample. However, when total testosterone was also included in the multivariate model as a further covariate, only the difference in ejaculate volume between CS and non-smokers was confirmed (-0.61 ± 0.23 ml, $P < 0.01$). Finally, CS showed lower total SV volume, before and after ejaculation, even after adjusting for confounders ($P = 0.02$ and <0.01, respectively). Similar results were observed when the reported number of cigarettes smoked or the number of pack-years was considered separately.

LIMITATIONS, REASONS FOR CAUTION: The present results are derived from patients consulting an Andrology Clinic for couple infertility, who could have different characteristics from the general male population or males consulting general practitioners for reasons other than couple infertility. In addition, we did not have a true control group composed of age-matched, apparently healthy, fertile men, and therefore true normative data of sonographic parameters cannot be inferred. Due to the cross-sectional nature of our study, neither a causality hypothesis nor

mechanistic models can be drawn. Finally, this is a retrospective study, and further prospective studies are required.

WIDER IMPLICATIONS OF THE FINDINGS: We report an apparent paradox in CS: lower SV volume despite higher testosterone levels. Our data suggest that smoking may negatively affect SV volume in an independent manner, as the difference between CS and non-smokers retained significance after adjusting for confounders including testosterone. This is the first study reporting such ultrasound evidence. How this new smoking-related alteration, along with low semen volume, impacts male fertility needs to be addressed by further studies.

STUDY FUNDING/COMPETING INTEREST(S): No funding was received for the study. None of the authors have any conflict of interest to declare.

Key words: smoking / infertile men / testosterone / ejaculate volume / seminal vesicles / ultrasound

Introduction

Approximately one out of three subjects of reproductive age in the USA is a current smoker (CS) (see Practice Committee of the American Society for Reproductive Medicine—ASRM, 2012). The deleterious effect of smoking on general health is widely recognized, however, only one out of five women is familiar with the tobacco-related reproductive risk (ASRM, 2012). The current fertility guidelines emphasize the female reproductive risk of a smoking habit (National Collaborating Centre for Women's and Children's Health—NCCWCH, 2004; Dechanet *et al.*, 2011; ASRM, 2012; National Institute of Health and Clinical Excellence—NICE, 2013; O'Flynn, 2014). It has also been suggested that not only maternal but also paternal smoking can adversely affect the success rates of assisted reproduction procedures: smoking in males is associated with a decrease in IVF and ICSI success (NCCWCH, 2004; NICE, 2013). However, the relationship between smoking and male fertility is still under debate (NCCWCH, 2004; NICE, 2013). An association between smoking and altered semen parameters has been recognized (NCCWCH, 2004; NICE, 2013). Accordingly, a recent meta-analysis identified smoking as a risk factor for the conventional semen parameters (semen volume, sperm concentration, total sperm count, percentage of sperm progressive motility and normal sperm morphology) in both infertile and healthy men (Li *et al.*, 2011). However, the effect of smoking on semen volume and sperm concentration varied considerably among countries (subgroup analyses). In addition, heterogeneity among studies included in the meta-analysis was recognized as a limitation (Li *et al.*, 2011).

Smoking habit may affect male reproductive health by way of several different mechanisms (see, for review, Agarwal *et al.*, 2008; Sharma *et al.*, 2013; Barazani *et al.*, 2014). It has been reported that smoking can negatively impact sperm DNA integrity, increasing DNA damage (see Sharma *et al.*, 2013). Smoking can also reduce the mitochondrial activity in spermatozoa, leading to a low fertilization capacity (Calogero *et al.*, 2009). Smoking-related endocrine and testicular dysfunction have also been suggested by some authors (see Pasqualotto *et al.*, 2004; Agarwal *et al.*, 2008; Sharma *et al.*, 2013).

No previous study has systematically evaluated the impact of a smoking habit on the ultrasound characteristics of the organs of the male genital tract. This study was aimed at evaluating the possible correlations between a reported smoking habit and clinical, biochemical, seminal and male genital tract colour-Doppler ultrasound characteristics in males of infertile couples.

Materials and Methods

Patients

We studied a consecutive series of 426 male patients (mean age 36.5 $\pm$ 8.1 years) attending our Outpatient Clinic for the first time from January 2010 to July 2013, seeking medical care for couple infertility. Couple infertility was defined as the inability of a sexually active couple to achieve pregnancy despite unprotected intercourse for a period greater than 12 months, according to the World Health Organization (WHO, 2000). Subjects with karyotype abnormalities ($n = 3$), chromosome Y micro-deletions ($n = 3$) or absence of at least one vas deferens and/or one seminal vesicle ($n = 26$) were excluded from the analysis.

All patients were evaluated before beginning any treatment. The data reported in this study have been collected during routine clinical procedures according to a 'Day Service' standard protocol for males of infertile couples, encoded by PACC L-99 [D/903/110 Azienda Ospedaliera-Universitaria Careggi (AOUC), Florence, Italy] and approved by the Regional Health Care Service (§ DGRT n. 1045; § DGRT n. 722; § DGRT n. 867), as previously described (see Lotti *et al.*, 2014a). According to the PACC L-99 protocol, all patients underwent, within the same day, the following routine procedures: a complete andrological and physical examination, biochemical and hormonal assessment, scrotal and transrectal colour-Doppler ultrasound evaluation and semen analysis. In addition, at the time of the first visit, all patients gave their written informed consent to have their clinical records included in a dedicated database and they were aware that their data, after having been made anonymous, would be used for clinical research purposes.

Andrological and physical examination

A complete andrological examination was performed according to previous reports (see Krausz, 2011; Lotti and Maggi, 2015).

Physical examination included measurement of blood pressure (mean of three measurements 5 min apart, in sitting position, with a standard sphygmomanometer), height and weight. Weight and height were used to calculate BMI (kg/m^2).

Quantification of smoking

Self-reported data on smoking habits were collected during a structured interview. Participants were categorized as current smokers (CS) if their history of smoking had lasted for at least 1 year, past smokers (PS) if they had smoked at least 1 year in their life and were not CS, and never smokers (NS) if they had never smoked or smoked <1 year, according to a previous study (Corona *et al.*, 2005). Smoking habit duration, measured in years, as well as the total number of cigarettes smoked per day were assessed. Subjects were classified according to the number of cigarettes smoked per day on a 0–2 Likert scale (0= 0; 1 = 1–10 and 2 → 10) (see

Wang et al., 2013). In addition, pack-years of smoking were calculated by multiplying the number of packs smoked per day (1 pack = 20 cigarettes) by the number of years smoked (Bernaards et al., 2001).

Evaluation of other lifestyle parameters

Alcohol and cannabis consumption as well as physical activity levels were evaluated using standard questions, and the answer were codified as dummy variables 0–1 (no/yes). In particular, subjects were defined as *regular alcohol users* if they reported the consumption of at least 2 drinks/day. In Italy, a standard alcoholic drink is equal to 10 g/12.7 ml of pure ethanol, or 330 ml of beer; or 100 ml of wine; or 30 ml of straight spirits or liquor like gin, rum, vodka or whiskey (see Boddi et al., 2010). Similarly, patients were defined as *regular cannabis users* if they reported a consumption of at least one cannabis cigarette/week (Corona et al., 2005).

Physical activity was defined according to the WHO definition (http://www.who.int/topics/physical_activity/en/) as any bodily movement produced by skeletal muscles that requires energy expenditure, including walking, cycling or participating in sports. In particular, subjects were considered to practice *regular physical activity* when they declared practicing at least 150 min of moderate-intensity or 75 min of vigorous-intensity aerobic physical activity throughout the week, or an equivalent combination of moderate- and vigorous-intensity activity, according to WHO recommendations for adults aged 18–64 years (see http://www.who.int/topics/physical_activity/en/).

Colour-Doppler ultrasonography

All patients underwent scrotal and transrectal colour-Doppler ultrasonography (CDUS) (see Lotti and Maggi, 2015), performed before and after ejaculation, during the same CDUS session, using the ultrasonographic console Hitachi H21 (Hitachi Medical System, Tokyo, Japan).

Prostate and seminal vesicles (SV) were studied by scanning the organs at 5 mm intervals in various longitudinal, transverse and oblique scans, according to previous studies (Lotti et al., 2014a,b), using a transrectal biplanar probe (linear transducer U533L 7.5 MHz; convex transducer U533C 6.5 MHz), more sensitive for the detection of prostatic features and an 'end fire' probe (V53W 6.5 MHz, field of view 50°–200°) to better investigate SV (Lotti et al., 2012a). Prostate volume was measured using the planimetric method, as previously reported (see Lotti et al., 2014b). Prostate and SV CDUS features were defined as previously reported (see Lotti and Maggi, 2015). In particular, prostate echogenicity and hyperaemia were defined according to previous studies (see Lotti and Maggi, 2015). Prostate vascularization and arterial prostatic peak systolic velocity were evaluated before ejaculation, in order to avoid post-ejaculatory changes in vascular flow pattern, as previously reported (see Lotti and Maggi, 2015; Lotti et al., 2014a). SV volume was calculated using the 'ellipsoid/prolate ($d1 > d2 = d3$) spheroid' formula ($d1 \times d2 \times d3 \times 4/3 \times \pi$, considering $d1 = \frac{1}{2}$ maximum longitudinal diameter of the SV and both $d2$ and $d3 = \frac{1}{2}$ anterior–posterior maximum diameter), according to previous studies (Lotti et al., 2012a, 2013a). SV echo-texture features were defined according to previous studies (see Lotti and Maggi, 2015). Ejaculatory duct CDUS characteristics were evaluated after ejaculation, in order to better emphasize indirect CDUS signs of partial or complete obstruction.

Scrotal CDUS was performed systematically in various longitudinal, transverse and oblique scans, according to previous studies (Lotti et al., 2012b, 2013b) using a 7.5 MHz high-frequency linear probe (L54M 6–13 MHz). Testis, epididymis, vas deferens and venous plexus CDUS features were defined as previously reported (Lotti et al., 2012b, 2013b; Lotti and Maggi, 2015).

Semen analysis and determination of seminal plasma interleukin 8 levels

All patients underwent, during the same ultrasound session, semen analysis, performed according to the WHO criteria (2010). Furthermore, a quantification of seminal plasma interleukin 8 (sIL-8), a reliable surrogate marker of prostatitis (see Lotti and Maggi, 2013) was performed. Seminal plasma aliquots were stored frozen to quantify sIL-8 levels. sIL-8 was quantified by conventional two-site enzyme-linked immunosorbent assay (ELISA) using a human IL-8 ELISA set (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions (Penna et al., 2007). Each seminal plasma sample was diluted from 1:5 to 1:625. Assay sensitivity for sIL-8 was <1 pg/ml.

Biochemical evaluation

Blood samples were drawn in the morning, after an overnight fast, for determination of total testosterone, LH, FSH, prolactin (PRL), thyroid-stimulating hormone (TSH) and prostate-specific antigen (PSA) by electrochemiluminescent method (Modular Roche, Milan, Italy), sex hormone-binding globulin (SHBG) by modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany), blood glucose (by glucose oxidase method; Aeroset Abbott, Rome, Italy), total cholesterol, high-density lipoprotein cholesterol and triglycerides (by automated enzymatic colorimetric method, Aeroset Abbott, Rome, Italy). Calculated free testosterone was derived according to Vermeulen's formula (available at http://www.issam.ch/freetesto.htm) (Vermeulen et al., 1999).

Evaluation of sexual and erectile function

Patients were asked to complete the International Index of Sexual Function-15 (IIEF-15) (Rosen et al., 1997), in its Italian translation, a brief, 15-item, self-reported questionnaire, which addresses the relevant domains of male sexual function (erectile function, orgasmic function, sexual desire, intercourse satisfaction and overall satisfaction). The erectile function domain of the IIEF-15 (IIEF-15-EFD) (Cappelleri et al., 1999) was assessed. A IIEF-15-EFD score <26 indicates erectile dysfunction (ED) (Cappelleri et al., 1999)

Evaluation of premature ejaculation status

Patients were asked to complete the Premature Ejaculation Diagnostic Tool (PEDT) (Symonds et al., 2007), in its Italian translation. PEDT is a brief, multidimensional, psychometrically validated, five-item, self-reported questionnaire for diagnosing ejaculatory status, which provides scores for five subdomains, #1 control, #2 frequency, #3 minimal stimulation, #4 distress and #5 interpersonal difficulty. PEDT score was calculated as the sum of the scores of these domains. A PEDT score of ≤ 8 indicates no-PE (Symonds et al., 2007).

Screening of prostate-related symptoms and lower urinary tract symptoms

Patients were asked to complete the National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI) (Litwin et al., 1999), in its Italian translation, a brief self-reported questionnaire for the screening of prostatitis-like symptoms, which provides scores for pain, voiding symptoms and quality of life (QoL). NIH-CPSI total score was calculated as the sum of the scores of these domains.

Lower urinary tract symptoms (LUTS) were evaluated using the Italian translation of the International Prostate Symptom Score (IPSS), which is a brief self-administered questionnaire for screening symptoms related to benign prostatic hyperplasia and that includes seven questions on symptoms and one question on QoL (Barry et al., 1992).

Screening of psychological traits

Patients were asked to complete the Middlesex Hospital Questionnaire, modified (MHQ) (Crown and Crisp, 1966), a brief self-reported questionnaire for the screening of mental disorders, which provides scores for free-floating anxiety (MHQ-A), phobic anxiety (MHQ-P), somatization (MHQ-S), obsessive–compulsive (MHQ-O), depressive (MHQ-D) and hysterical (MHQ-H) traits and symptoms. MHQ total score was calculated as the sum of the scores of these domains as previously reported (Corona *et al.*, 2009, 2012; Lotti *et al.*, 2012c).

Data analysis

Data were expressed as mean ± SD when normally distributed, as medians (quartiles) for parameters with non-normal distribution, and as percentages when categorical. Correlations were assessed using Spearman's or Pearson's method whenever appropriate. Unpaired two-sided Student's *t*-tests were used for comparisons of means of normally distributed parameters; when distribution could be normalized through logarithmic transformation, as in the case of FSH and sIL-8 levels or SV volume, the same test was applied to logarithmically transformed data. In all other cases, the Mann–Whitney *U*-test was used for comparisons between the groups. Relative risk and 95% confidence interval were calculated for association of categorical parameters, and χ^2 test was used for comparisons. Stepwise multiple linear, logistic binary or ordinal regressions or analysis of covariance (ANCOVA) with the Bonferroni correction were applied for multivariate analyses whenever appropriate.

Since prostate and SV characteristics (Parsons *et al.*, 2006, 2013 for prostate; Kim *et al.*, 2009; Lotti *et al.*, 2012a for SV), as well as semen quality (see Zitzmann, 2013; Smith and Walker, 2014), are related to age and total testosterone, data have been adjusted for age and total testosterone, unless otherwise specified. Furthermore, since BMI affects testosterone levels (Corona *et al.*, 2013), ultrasound characteristics of male genital tract organs (Lotti *et al.*, 2013b, 2014a), and may influence seminal quality (MacDonald *et al.*, 2010; Sermondade *et al.*, 2013), data have been adjusted also for this confounder, unless otherwise specified. Furthermore, since smoking habit was associated with some lifestyle parameters (see the Results section), data have been adjusted also for alcohol and cannabis consumption, as well as physical activity levels. Finally, since ejaculate (see WHO, 2010; Lotti *et al.*, 2012a, 2013a) and SV (see Lotti *et al.*, 2012a, 2013a) volume are modulated by duration of sexual abstinence and PRL levels, also these confounders have been included in the related multivariate analyses, when specified.

All statistical analysis was performed on SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) for Windows 20.0. A *P*-value of <0.05 was considered as significant.

Results

Among the 394 patients studied, 229 (58.1%) were NS, 56 (14.2%) PS and 109 (27.7%) CS. Collectively, 285 (72.3%) subjects were currently non-smokers (NS + PS).

Sociodemographic and clinical parameters

The sociodemographic and clinical characteristics of the sample are summarized in Table I. PS were older when compared with NS and CS, while no age difference was observed between NS and CS patients. After adjusting for age, CS had a lower educational level, a significantly higher prevalence of alcohol or cannabis intake and a lower level of physical activity when compared with NS [adjusted odds ratio and 95% confidence interval = 0.56 (0.37–0.93), $P < 0.05$; 5.79 (3.37–9.95), $P < 0.0001$; 14.5 (3.20–65.6), $P = 0.001$ and 0.65 (0.40–0.97), $P < 0.05$; for educational level, alcohol and cannabis intake and physical activity, respectively].

Biochemical physical parameters

CS showed significantly higher total testosterone, calculated free testosterone and lower FSH levels, when compared either with NS or PS (Fig. 1A–C, respectively; see also Table I). CS did not show any further difference in all other hormonal (including LH), biochemical or physical parameters evaluated, when compared with the rest of the sample (Table I). Conversely, higher triglyceride levels were observed in PS, when compared with NS (Table I). At multivariate analysis (ANCOVA), after adjusting for age, BMI, lifestyle (including alcohol and cannabis intake as well as reported levels of physical activity) and SHBG, total testosterone and calculated free testosterone levels were higher and FSH lower in CS when compared with NS (difference between groups = 1.99 ± 0.71 nmol/l, $P < 0.02$; 0.04 ± 0.02 nmol/l, $P < 0.05$; −0.10 ± 0.05 IU/l, $P < 0.05$; for total testosterone, calculated free testosterone and $\log_{10}$ transformed FSH respectively) or with PS (difference between groups = 2.70 ± 0.91 nmol/l, $P = 0.01$; 0.06 ± 0.02 nmol/l, $P < 0.01$; −0.12 ± 0.05 IU/l, $P < 0.05$; for total testosterone, calculated free testosterone and $\log_{10}$ transformed FSH, respectively), whereas the differences between NS and PS were not statistically significant. However, the difference in FSH levels among groups was not confirmed when total testosterone was also included in the model as a further covariate (not shown). Similarly, no difference among groups was observed when triglyceride levels were considered (not show).

Similar results were observed when CS were compared with the rest of the sample (NS + PS), in a multivariate model (ANCOVA) (Table II). Accordingly, the difference in FSH levels between CS and non-smokers (NS + PS) was no longer confirmed when total testosterone was also included in the multivariate analysis as a further covariate (Table II).

Interestingly, in an ordinal logistic model, the reported number of cigarettes smoked was associated, in a stepwise fashion, with androgen and FSH levels (Fig. 1D–F), even after adjusting for age, BMI, lifestyle and SHBG (Wald = 13.50, $P < 0.0001$; Wald = 13.42, $P < 0.0001$; Wald = 4.39, $P < 0.05$ for TT, cFT and FSH, respectively). However, when total testosterone was also included in the multivariate analysis, the association with FSH levels was not confirmed (not shown).

In line with these data, at univariate analysis, the number of pack-years was positively related to total testosterone and calculated free testosterone levels and negatively to FSH, PRL and TSH levels (all $P < 0.05$). However, after adjusting for age, lifestyle, BMI and SHBG, only the association with androgens retained significance (adj. $r = 0.154$, $P = 0.002$ and adj. $r = 0.189$, $P < 0.005$; for total testosterone and calculated free testosterone, respectively).

Reproductive parameters

CS showed a significantly higher percentage of normal sperm morphology, higher sIL-8 levels and lower ejaculate volume when compared with NS (Fig. 2A–C; see also Table I). No difference in other seminal parameters was observed among groups (Table I). At multivariate analysis (ANCOVA), after adjusting for age, BMI, lifestyle, total testosterone

Table I Sociodemographic/clinical parameters of the whole sample and of NS, CS and PS.

	NS (n = 229)	CS (n = 109)	PS (n = 56)
Sociodemographic parameters			
Age (years)	35.9 ± 7.6	36.4 ± 7.0	**39.7 ± 10.6**$^{**\circ}$
Education			
Not graduated (%)	26.6	**39.4***	31.9
Graduated (high school or university) (%)	73.4	**60.6***	68.1
Employment			
Student (%)	9.4	3.7*	**1.9**$^{**\circ}$
Employed (%)	89.3	91.6	88.9
Unemployed or other (%)	1.3	4.7	**9.3****
Current alcohol consumption (%)	15.9	**49.9***	**33.9***
Current cannabis cigarette consumption (%)	0.4	**9.3******	**3.6**$^{\circ}$
Current physical activity (%)	51.9	**41.1***	50.0
Sexual intercourse frequency (# of sexual intercourses/month)	8.0 (4.0–8.0)	8.0 (4.0–8.0)	8.0 (4.0–8.0)
Clinical parameters			
BMI (kg/m^2)	25.7 ± 4.5	25.9 ± 8.6	26.6 ± 4.6
Systolic BP (mmHg)	124.5 ± 10.8	124.1 ± 16.1	124.6 ± 11.9
Diastolic BP (mmHg)	79.7 ± 7.6	78.3 ± 7.4	80.2 ± 8.1
History of cryptorchidism	8.3	11.0	3.6
History of genito-urinary infections	26.2	32.7	32.1
Mean testis volume (Prader) (ml)	18.4 ± 5.0	19.3 ± 4.9	18.4 ± 5.2
Clinical varicocele (%)[a]	30.6	33.0	23.2
Enlarged prostate at DRE (%)	21.5	21.8	30.4
Laboratory parameters			
Log_{10} [FSH] (IU/l)	0.73 ± 0.29	**0.66 ± 0.33***	**0.78 ± 0.33**$^{\circ}$
Log_{10} [LH] (IU/l)	0.56 ± 0.26	0.57 ± 0.22	0.59 ± 0.19
Log_{10} [PRL] (mU/l)	2.23 ± 0.24	2.19 ± 0.25	2.24 ± 0.22
Log_{10} [TSH] (mIU/l)	0.19 ± 0.35	0.16 ± 0.23	0.21 ± 0.27
Total testosterone (nmol/l)	15.3 ± 5.9	**18.0 ± 6.5*****	**14.1 ± 5.3**$^{\circ\circ}$
SHBG (nmol/l)	30.7 ± 13.1	32.3 ± 12.9	31.1 ± 14.5
Calculated free testosterone (nmol/l)	0.322 ± 0.114	**0.373 ± 0.112*****	**0.294 ± 0.098**$^{\circ\circ}$
PSA (ng/ml)	0.70 (0.45–1.00)	0.66 (0.50–0.91)	0.75 (0.47–1.02)
Glycaemia (mmol/l)	4.94 ± 0.61	5.00 ± 0.50	5.15 ± 0.11
Total cholesterol (mmol/l)	5.05 ± 1.05	5.01 ± 0.94	5.09 ± 1.05
HDL cholesterol (mmol/l)	1.30 ± 0.33	1.29 ± 0.37	1.24 ± 0.33
LDL cholesterol(mmol/l)	3.17 ± 0.95	3.04 ± 0.78	3.17 ± 0.90
Triglycerides (mmol/l)	1.07 (0.77–1.37)	1.28 (0.80–1.71)	**1.49 (0.84–2.01)***
Seminal parameters			
Azoospermic subjects (%)	15.0	17.6	23.6
Sexual abstinence (days)	4.2 ± 1.9	4.1 ± 1.7	4.2 ± 2.3
pH	7.5 ± 0.3	7.6 ± 0.3	7.5 ± 0.3
Semen volume (ml)	3.5 ± 1.7	**2.8 ± 1.4******	3.2 ± 1.6
Sperm concentration ($\times 10^6$/ml)	9.1 (1.0–29.0)	15.0 (1.52–55.5)	13.0 (1.20–47.0)
Spermatozoa per ejaculate ($\times 10^6$)	32.0 (4.25–105.0)	33.0 (5.2–129.3)	35.1 (2.88–145.8)
Sperm progressive motility (%)	37.0 [20.0–52.0]	41.0 (26.5–55.0)	35.0 (18.0–58.0)
Sperm morphology (% normal forms)	3.0 [1.0–7.0]	**5.0 (1.0–12.0)***	4.0 (1.0–8.3)
Leukocytospermia (%)	8.1	8.9	4.3
sIL-8 (ng/ml)	3.3 [1.8–6.0]	**4.6 (1.9–8.3)***	3.4 (1.9–6.2)

Continued

Table I *Continued*

	NS (n = 229)	CS (n = 109)	PS (n = 56)
History of infertility			
Duration of infertility (months)	19.6 ± 17.9	22.3 ± 20.5	**27.9 ± 24.1***
Primary infertility	80.9	82.9	82.6
Secondary infertility	19.1	17.1	17.4
Female partner age (years)	33.4 ± 7.2	33.7 ± 6.7	35.2 ± 10.5
PEDT score (0–20)	3.9 ± 3.7	3.7 ± 3.4	3.7 ± 3.6
IIEF-15 total score (5–75)	64.0 ± 8.5	63.7 ± 10.1	63.0 ± 10.7
IIEF-15-EFD score (1–30)	27.6 ± 4.0	27.0 ± 4.3	27.0 ± 4.9
NIH-CPSI total score (0–43)	5.0 ± 7.9	4.4 ± 6.0	5.2 ± 6.4
IPSS total score (0–40)	4.1 ± 5.4	4.5 ± 4.3	**6.0 ± 6.7***
MHQ total score (0–96)	24.4 ± 13.4	25.4 ± 12.3	26.1 ± 13.5

Data are expressed as mean ± SD or as median (quartiles) when appropriate, and as percentages when categorical.
Iterative comparisons between two groups (CS versus NS; PS versus NS; CS versus PS) are reported here. The statistical analyses have been performed using unpaired two-sided Student's *t*-test or Mann–Whitney *U*-test, whenever appropriate, for linear parameters (see the Materials and Methods section), and χ^2 test for categorical parameters.
BP, blood pressure; DRE, digito-rectal examination; PRL, prolactin; TSH, thyroid-stimulating hormone; PSA, prostate-related antigen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; sIL-8, seminal interleukin 8; SHBG, sex hormone-binding globulin; PEDT, Premature Ejaculation Diagnostic Tool; IIEF-15, International Index of Erectile Function-15; IIEF-15-EFD, International Index of Erectile Function-15 erectile function domain; NIH-CPSI, National Institutes of Health-Chronic Prostatitis Symptom Index; IPSS, International Prostate Symptom Score; MHQ, Middlesex Hospital Questionnaire. Bold characters emphasize parameters significantly different comparing different groups.
[a]Clinical varicocele has been defined according to Dubin and Amilar classification (see Lotti and Maggi, 2015).
PS or CS versus NS: *$P < 0.05$, **$P < 0.005$; ***$P < 0.001$; ****$P < 0.0001$; PS versus CS: °$P < 0.05$; °°$P < 0.0001$.

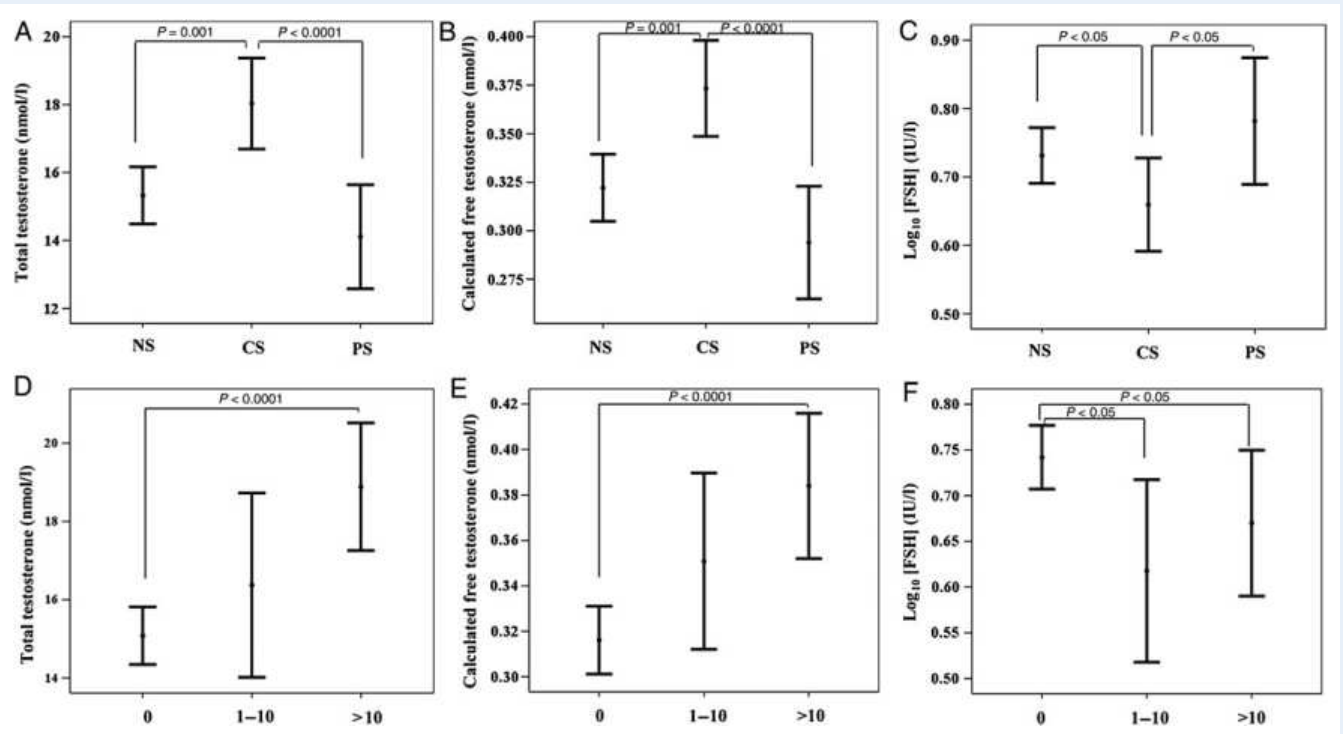

Figure 1 Androgen and FSH levels in relation to smoking habit. (**A–C**) show total testosterone (A), calculated free testosterone (B) and logarithmically transformed FSH (C) levels in NS, PS and CS. (**D–F**) show total testosterone (D), calculated free testosterone (E) and logarithmically transformed FSH (F) in relation to the number of cigarettes smoked per day. Iterative comparisons between two groups (upper panels: CS versus NS; PS versus NS; CS versus PS; lower panels: 0 versus 1–10; 0 versus >10; 1–10 versus >10 number of cigarettes smoked) have been performed using the unpaired two-sided Student's *t*-test. Only the significant *P*-values derived by comparison between groups were reported in each panel.

Table II Differences in laboratory, seminal and colour-Doppler ultrasound parameters comparing CS and non-smokers (NS + PS) using multivariate models.

	Model 1[a]	Model 2[b]
Laboratory parameters		
Total testosterone (nmol/l)	$d = 2.17 \pm 0.68$, $P = 0.001$	—
Calculated free testosterone (nmol/l)	$d = 0.05 \pm 0.02$, $P < 0.005$	—
Log_{10} [FSH] (IU/l)	$d = -0.11 \pm 0.05$, $P < 0.05$	ns
Triglycerides (mmol/l)	ns	ns
Seminal parameters		
Sperm morphology (% normal forms)	$d = 2.54 \pm 1.02$, $P < 0.02$	ns
Log_{10} [sIL-8] (ng/ml)	ns	ns
Semen volume (ml)	$d = -0.59 \pm 0.22$, $P < 0.01$	$d = -0.61 \pm 0.23$, $P < 0.01$
Colour-Doppler ultrasound parameters		
Log_{10} (total SV volume before ejaculation) (ml)	$d = -0.10 \pm 0.04$, $P < 0.02$	$d = -0.10 \pm 0.04$, $P = 0.02$
Log_{10} (total SV volume after ejaculation) (ml)	$d = -0.13 \pm 0.05$, $P < 0.01$	$d = -0.14 \pm 0.05$, $P < 0.01$
Dilated ejaculatory ducts	ns	ns
Prostate calcifications	ns	ns

The multivariate analysis has been performed using ANCOVA for linear variables and binary logistic regression for dummy variables.
Smoking habit has been considered as a dummy variable (CS/non-smoker).
SV, seminal vesicles; *d*, difference; ns, not significant.
[a]Adjusted for age, BMI, current alcohol (no/yes) and cannabis (no/yes) intake, physical activity (no/yes) and SHBG.
[b]Adjusted for Model 1 confounders + total testosterone.

and SHBG, only the difference between CS and NS in ejaculate volume was confirmed (difference $= -0.62 \pm 0.24$ ml, $P < 0.05$).

Similar results were observed when CS were compared with the rest of the sample (NS + PS), in a multivariate model (ANCOVA) (Table II). In particular, the difference between groups in percentage of normal sperm morphology disappeared when total testosterone was introduced into the multivariate model as a further covariate (Table II). Conversely, the difference in ejaculate volume between CS and non-smoker (NS + PS) retained significance even after adjustment for total testosterone levels (Table II). The latter difference was confirmed even after introducing possible further confounders such as sexual abstinence before semen analysis and PRL levels (difference $= -0.56 \pm 0.23$ ml, $P < 0.02$).

Interestingly, smoking habit, independently from the reported number of cigarettes smoked, was negatively associated with ejaculate volume (Fig. 2D). The latter correlation retained significance in an ordinal logistic model even after adjusting for age, BMI and lifestyle, total testosterone and SHBG (Wald $= 7.22$, $P < 0.01$).

Similarly, the number of pack-years was negatively related to ejaculate volume, even after adjusting for confounders (Table III). Finally, PS showed a significantly higher duration of infertility when compared with NS (Table I); however, the association was not confirmed after adjusting for confounders (not shown).

Colour-Doppler ultrasound parameters

Table IV shows the colour-Doppler ultrasound characteristics of patients studied. CS showed a lower total SV volume, either before or after ejaculation, when compared with NS (Fig. 3A and B; see also Table IV). CS showed a higher prevalence of dilated ejaculatory ducts when compared with NS (Table IV). CS and NS showed a lower prevalence of prostate calcifications when compared with PS (Table IV). At multivariate analysis (ANCOVA), after adjusting for age, BMI, lifestyle, total testosterone and SHBG, the difference in total SV volume between CS and NS was confirmed (difference $= -0.12 \pm 0.05$ ml, $P < 0.05$ and -0.16 ± 0.05 ml, $P < 0.01$; for log_{10} transformed total SV volume before and after ejaculation, respectively). After adjusting for confounders, differences among groups in detection of dilated ejaculatory ducts or prostate calcifications were not confirmed (not shown).

Similar results were observed when CS were compared with the rest of the sample (NS + PS), in a multivariate model (ANCOVA) (Table II). These results were confirmed even after introducing possible further confounders such as sexual abstinence before semen analysis and PRL levels (difference $= -0.13 \pm 0.04$ ml, $P = 0.05$ and -0.12 ± 0.05 ml, $P < 0.02$, for log_{10} transformed SV volume before and after ejaculation, respectively). Conversely, in a binary logistic model, after adjusting for the aforementioned confounders, the associations between smoking habit and detection of dilated ejaculatory ducts or prostate calcifications were not confirmed (Table II).

Interestingly, the reported number of cigarettes smoked was associated, in a negative stepwise fashion, with SV volume before and after ejaculation (Fig. 3C and D), even after adjusting for confounders (Wald $= 6.37$, $P < 0.02$ and Wald $= 9.13$, $P < 0.005$, respectively). SV total volume before and after ejaculation was also negatively associated with the number of pack-years (Table III).

Finally, at univariate analysis, we found a positive association between the number of pack-years and mean testis volume, not confirmed in an adjusted model (Table IV).

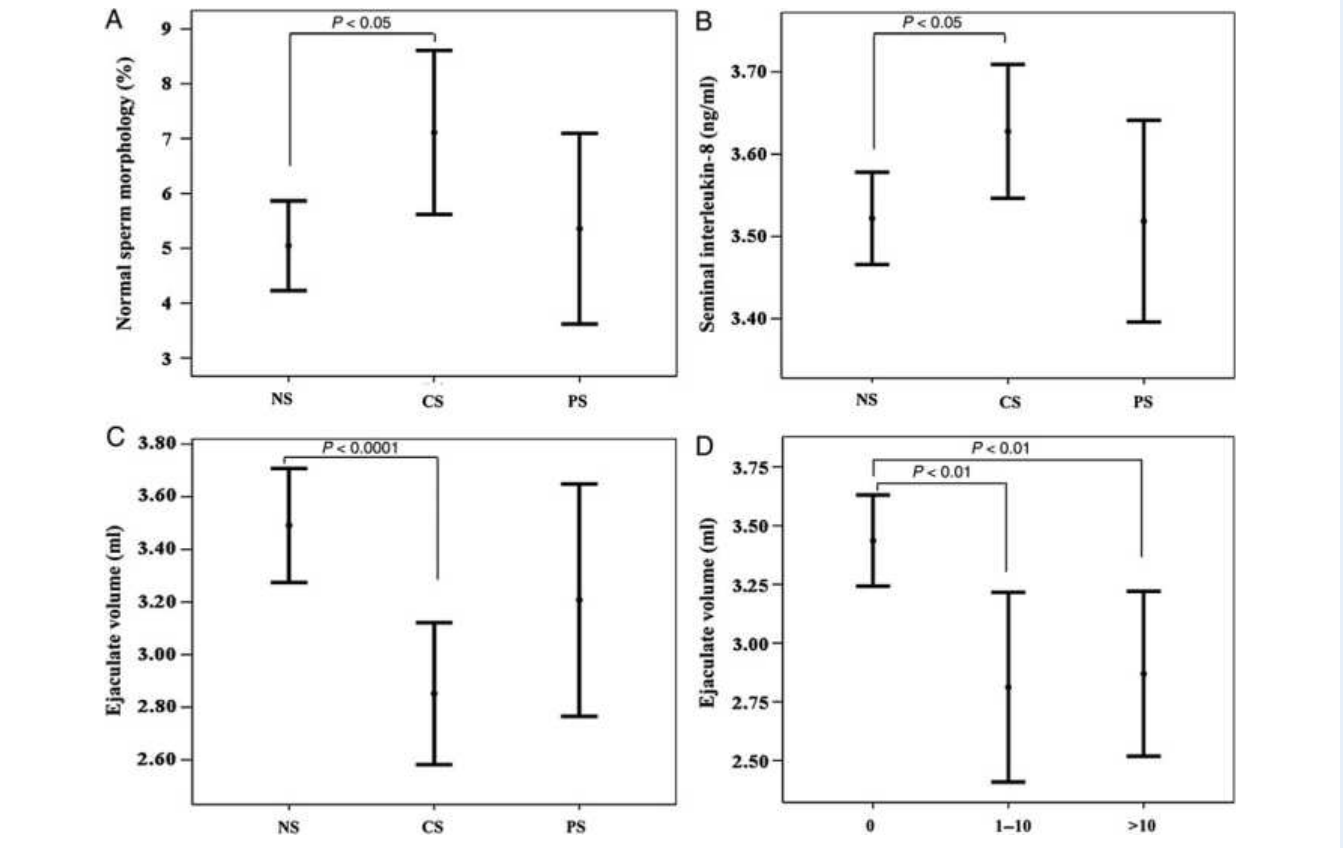

Figure 2 Significant seminal parameters in relation to smoking habit. **A–C** show the percentage of normal sperm morphology (A), seminal interleukin-8 levels (B) and ejaculate volume (C) in NS, PS and CS. **D** shows the ejaculate volume in relation to the number of cigarettes smoked per day. Iterative comparisons between two groups (A–C: CS versus NS; PS versus NS; CS versus PS; panel D: 0 versus 1–10; 0 versus >10; 1–10 versus >10 number of cigarettes smoked) have been performed using unpaired two-sided Student's *t*-test or Mann–Whitney *U*-test, whenever appropriate (see the Materials and Methods section). Only the significant *P*-values derived by comparison between groups were reported in each panel.

No association with other transrectal or scrotal ultrasound parameters was detected (Table IV).

Sexual, genito-urinary and intrapsychic parameters

No difference among groups in either global sexual or erectile function, evaluated by IIEF-15 and IIEF-EFD score, respectively, or in ejaculatory status, as assessed by PEDT score was observed (Table I). Accordingly, no difference in ED or PE prevalence was detected among the groups (Table I). Similarly, no difference in NIH-CPSI total score among groups was detected (Table I).

PS showed a higher IPSS total score when compared with NS subjects (Table I); however, the association was not confirmed after adjusting for confounders (not shown). Finally, no difference in psychological status, as evaluated by MHQ, was observed when comparing the three groups (Table I).

Discussion

This study shows for the first time that, in males of infertile couples, CS have lower ejaculate and ultrasound-derived SV volume, despite higher androgen levels, when compared with non-smoker. After adjusting for testosterone levels, no further associations were found between current smoking and sperm parameters or other male genital tract ultrasound features. Finally, no correlations between current smoking and other clinical, biochemical, sexual and psychological factors were detected.

In the present cohort, the percentage of smokers was comparable with that observed in general male populations of a similar reproductive age in the USA (ASRM, 2012) and in Europe (Corrao *et al.*, 2000), and in other cohorts of infertile men (Trummer *et al.*, 2002; Künzle *et al.*, 2004).

CS had a lower educational level when compared with non-smoker. This finding is in line with previous studies, reporting that smoking has an inverse relationship with socioeconomic status (see Pampel, 2005) and is more concentrated in lower education groups, both in higher- (Pampel, 2005; Pampel and Denney, 2011) and lower-income countries (see Hiscock *et al.*, 2012). This study also confirms that cigarette smokers are greater abusers of recreational substances, such as cannabis (Agrawal *et al.*, 2012) and alcohol (Bonevski *et al.*, 2014), and perform less physical activity (Kaczynski *et al.*, 2008) when compared with non-smokers.

One of the main results of the present study is the positive association between current smoking and testosterone levels. This finding is extensively described in a previous prospective study on infertile men (Trummer *et al.*, 2002) and in several cross-sectional surveys of the

Table III Univariate and multivariate associations between lifetime exposure to cigarette smoking (number of pack-years) and seminal and colour-Doppler ultrasound parameters.

	Univariate analysis	Multivariate analysis
Seminal parameters		
Semen volume (ml)	$r = -0.176$, $P < 0.0001$	**Adj. $r = -0.126$, $P = 0.049$**
Sperm morphology (% normal forms)	$r = 0.111$, $P = 0.046$	Adj. $r = 0.056$, $P = 0.512$
Log_{10} [sIL-8] (ng/ml)	$r = 0.151$, $P = 0.003$	Adj. $r = 0.083$, $P = 0.225$
Colour-Doppler ultrasound parameters		
Mean testis volume (ml)	$r = 0.106$, $P = 0.035$	Adj. $r = 0.081$, $P = 0.236$
Total SV volume before ejaculation (ml)	$r = -0.113$, $P = 0.026$	**Adj. $r = -0.195$, $P = 0.004$**
Total SV volume after ejaculation (ml)	$r = -0.128$, $P = 0.011$	**Adj. $r = -0.219$, $P = 0.001$**

The multivariate analysis has been performed using a stepwise linear regression model, adjusting for total testosterone, SHBG and other confounders, such as age, BMI, current alcohol (no/yes) and cannabis (no/yes) intake and physical activity (no/yes). Bold characters emphasize significant associations.

general population (Svartberg and Jorde, 2007; Wu *et al.*, 2008; Shiels *et al.*, 2009; Wang *et al.*, 2013) or of patients with sexual dysfunction (Corona *et al.*, 2005). However, other studies reported no such correlation (Harman *et al.*, 2001; Richthoff *et al.*, 2008; Halmenschlager *et al.*, 2009). A positive association between current smoking and free testosterone has also been reported by other (Svartberg and Jorde, 2007; Shiels *et al.*, 2009; Wang *et al.*, 2013), but not all (Harman *et al.*, 2001; Wu *et al.*, 2008; Halmenschlager *et al.*, 2009), authors. Interestingly, we observed that PS show lower testosterone levels when compared with CS, in line with other reports (Trummer *et al.*, 2002; Corona *et al.*, 2005; Camacho *et al.*, 2013), where it was suggested that quitting smoking could revert the tobacco-induced testosterone increase. According to previous studies, the difference in testosterone levels between CS and PS retained significance even after adjusting for confounding factors, including age and BMI, which play an independent and crucial role in the control of testosterone plasma levels (Laaksonen *et al.*, 2005; Wu *et al.*, 2008; Tajar *et al.*, 2010; Corona *et al.*, 2013). The mechanism explaining the higher testosterone levels in CS remains unclear. A possible effect of smoking on testosterone levels has been previously suggested. Smoking could act at the hypothalamic–pituitary level or on Leydig cell function (see Svartberg and Jorde, 2007). Chronic and acute mechanisms have been advocated, including GnRH or LH stimulation (Krsmanovic *et al.*, 1998; Mendelson *et al.*, 2003), SHBG increase (Wu *et al.*, 2008) or reduction in the conversion of testosterone to estradiol (Barbieri *et al.*, 1986; Osawa *et al.*, 1990) (see also Shiels *et al.*, 2009; Wang *et al.*, 2013). Another possibility is a smoke-induced reduction in sensitivity to testosterone, both at the central and peripheral levels. Accordingly, no difference in LH levels between CS and non-smoker was observed in the present sample.

Conversely, we observed lower FSH levels in CS when compared with non-smoker. In a previous study on more than 300 military conscripts, Richthoff *et al.* (2008) showed that smoking more than 10 cigarettes a day was associated with reduced FSH levels. However, in our study, the negative relationship between FSH levels and smoking habit was independent of the amount of tobacco smoked and disappeared after adjusting for testosterone levels. Our data suggest that testosterone, rather than smoking *per se*, modulates the negative feedback on the hypothalamic–pituitary region. In particular, it could be speculated that testosterone exerts this effect by promoting spermatogenesis (see Smith and Walker, 2014), which could result in an increase in the levels of Sertoli cell-derived inhibin B and in a subsequent reduction in FSH concentration (see Richthoff *et al.*, 2008). In line with this view, we found that CS had a significantly higher percentage of normal sperm morphology when compared with non-smokers, which was not confirmed when testosterone was included in the multivariate model.

No association between smoking habit and other sperm parameters was observed. Although a recent meta-analysis (Li *et al.*, 2011) identified smoking as a risk factor for conventional sperm parameters, most of the studies considered did not find a clear association, and the relationship between sperm parameters, testosterone levels and smoking habit was not taken into consideration.

In line with the results of a recent meta-analysis (Li *et al.*, 2011), we reported that CS had lower semen volume when compared with non-smokers. Our data suggest that this result could be explained by specific anatomic characteristics of the prostate-vesicular region detected by ultrasound. In particular, CS, when compared with non-smokers, showed significantly lower ultrasound-derived SV volume, either before or after ejaculation. It could be speculated that current smoking leads to low semen volume by exerting a negative effect on SV volume. However, it is well known that SV are androgen-dependent glands (Sasagawa *et al.*, 1989, 1990; Kim *et al.*, 2009; Lotti and Maggi, 2015). Hence, we report here an apparent paradox in CS: smaller SV volume despite higher testosterone levels. Our data suggest that smoking may negatively affect SV volume in an independent manner, as the difference in SV volume between CS and non-smokers retained significance after adjusting for confounders including testosterone. The mechanism mediating the effect of tobacco on SV volume remains unclear. An intriguing working hypothesis is that chronic nicotine exposure negatively affects SV secretion and/or contraction (Ohkawa, 1981; Pakrashi and Chatterjee, 1995), justifying both the reduced SV and semen volume. In fact, nicotine action consists of an initial transient stimulation and then in a more persistent depression of autonomic ganglia or neuromuscular junction (Hibbs and Zambon, 2011), and SV are innerved by adrenergic and cholinergic fibres (Turek, 2012). However, since tobacco contains a multitude of chemicals (Hammond *et al.*, 2006), it is difficult to single out one reproductive toxicant.

This study has some limitations. First, the present results are derived from patients consulting an Italian Andrology Clinic for couple infertility,

Table IV Colour-Doppler ultrasound (CDUS) parameters of the whole sample and of NS, CS and PS.

	NS ($n = 229$)	CS ($n = 109$)	PS ($n = 56$)
Colour-Doppler ultrasound parameters			
Testis			
Mean testis volume (ml)	14.7 ± 5.0	15.8 ± 4.9	14.7 ± 4.5
Testicular inhomogeneity (%)	39.3	35.8	39.3
Testicular hypoechogenicity (%)	19.2	17.0	20.4
Testicular microcalcifications (%)	11.4	13.8	3.6
Epididymis and vas deferens			
Mean size of the head (mm)	9.4 ± 1.9	9.4 ± 1.9	9.9 ± 3.0
Mean size of the tail (mm)	4.6 ± 1.3	4.8 ± 1.4	4.6 ± 1.0
Mean size of the vas deferens (mm)	3.9 ± 0.9	4.0 ± 1.0	3.9 ± 0.9
Inhomogeneous head (%)	43.0	43.5	42.9
Inhomogeneous tail (%)	31.4	30.3	33.9
Hypoechoic tail (%)	11.8	12.8	12.5
Hyperechoic tail (%)	12.7	15.6	12.5
Coarse tail calcifications (%)	6.1	11.9	3.6
Hyperaemia (%)	3.1	4.6	3.6
Varicocele[a]	32.3	26.9	28.6
Prostate			
Prostate volume (ml)	22.9 ± 6.8	23.3 ± 9.4	25.3 ± 10.2
Prostate calcifications (%)	46.3	45.0	**60.7**$^{*\circ}$
Prostate macro-calcifications[b] (%)	27.2	31.5	39.3
Major calcification size (mm)	8.9 ± 6.3	9.5 ± 6.8	8.7 ± 4.6
Inhomogeneous prostatic texture (%)	65.5	67.0	67.9
Hypoechoic prostatic texture (%)	10.8	7.4	11.4
Hyperechoic prostatic texture (%)	23.7	29.5	20.4
Prostatic hyperaemia (%)	22.5	18.9	12.5
Mean arterial peak systolic velocity (cm/s)	9.2 ± 2.4	9.1 ± 3.1	10.0 ± 4.1
Mean prostatic venous plexus (mm)	4.8 ± 1.7	4.8 ± 1.9	4.5 ± 1.5
Dilated ejaculatory ducts (%)	5.2	**14.7***	7.1
Ejaculatory ducts calcifications (%)	3.1	7.3	3.6
Ejaculatory ducts cysts (%)	0.4	1.8	0
Utricular cyst (%)	7.4	4.6	7.1
Seminal vesicles			
Total volume before ejaculation (ml)[c]	11.7 ± 9.6	**9.3 ± 5.8****	10.5 ± 7.7
Total volume after ejaculation (ml)[c]	8.3 ± 8.2	**6.0 ± 4.2****	6.6 ± 5.5
Inhomogeneous texture before ejaculation[d] (%)	38.0	30.3	32.7
Inhomogeneous texture after ejaculation[d] (%)	33.3	28.4	29.1
Areas of endocapsulation before ejaculation[d] (%)	28.3	19.6	22.0
Areas of endocapsulation after ejaculation[d] (%)	20.0	12.1	16.0
Wall thickening and septa[d] (%)	8.3	5.5	7.1
Giant cyst[d] (%)	1.3	0.9	3.6
Deferential ampullas mean diameter (mm)	4.8 ± 0.9	4.8 ± 1.2	5.0 ± 1.2

Iterative comparisons between two groups (CS versus NS; PS versus NS; CS versus PS) are reported here. The statistical analyses have been performed using unpaired two-sided Student's *t*-test or Mann–Whitney *U*-test, whenever appropriate, for linear parameters (see the Materials and Methods section), and χ^2 test for categorical parameters.

[a]CDUS-defined severe varicocele = basal venous reflux increasing or not after Valsalva's manoeuvre at sonography (see Lotti and Maggi, 2015).

[b]Calcifications with size >3 mm (see Lotti and Maggi, 2015).

[c]Calculated using the 'ellipsoid/prolate ($d1 > d2 = d3$) spheroid' formula ($d1 \times d2 \times d3 \times 4/3 \times \pi$, considering $d1 = \frac{1}{2}$ maximum longitudinal diameter of the SV and both $d2$ and $d3 = \frac{1}{2}$ anterior–posterior maximum diameter) (according to Lotti *et al.*, 2012a).

[d]SV ultrasound abnormalities have been defined according to Lotti and Maggi (2015).

PS or CS versus NS: $^{*}P < 0.05$; $^{**}P < 0.005$; PS versus CS: $^{\circ}P < 0.05$.

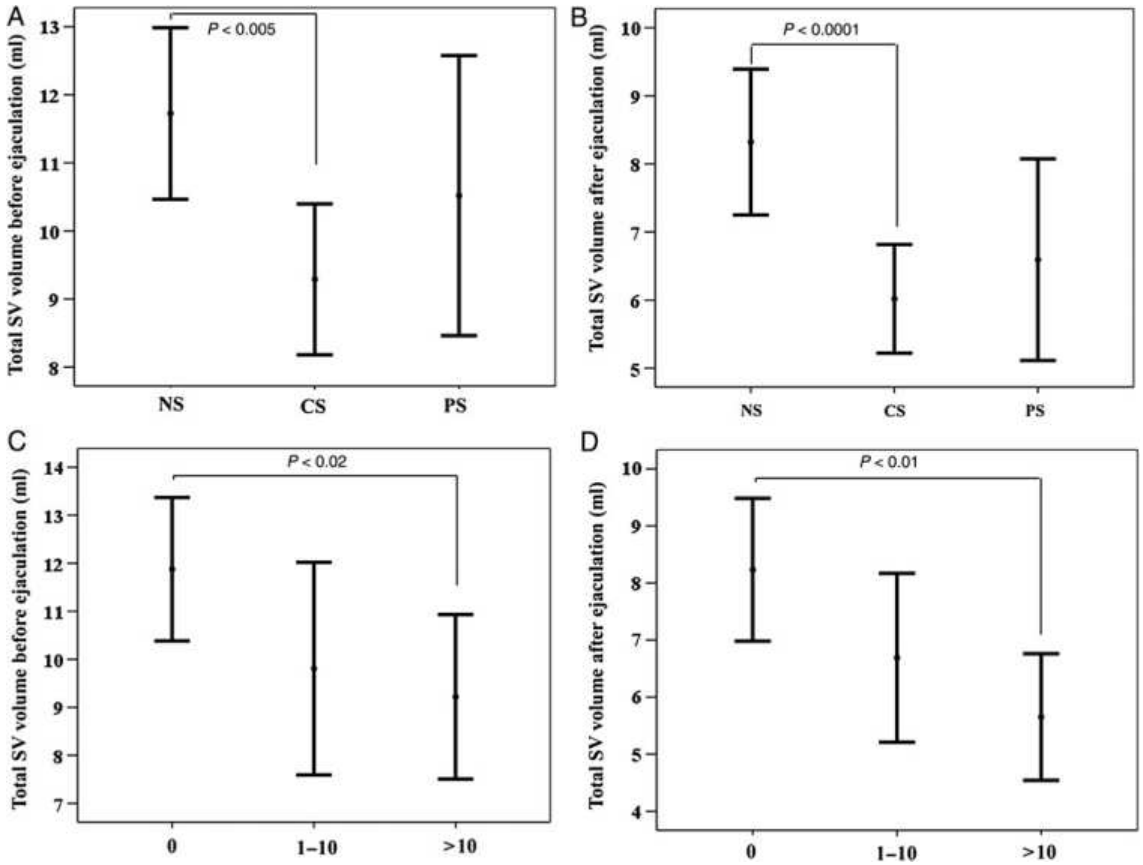

Figure 3 Ultrasound-derived SV volume in relation to smoking habit. **A** and **B** show ultrasound-derived total SV volume, before (A) and after (B) ejaculation, in NS, PS and CS. **C** and **D** show ultrasound-derived total SV volume in relation to the number of cigarettes smoked per day. Iterative comparisons between two groups (upper panels: CS versus NS; PS versus NS; CS versus PS; lower panels: 0 versus 1–10; 0 versus >10; 1–10 versus >10 number of cigarettes smoked) have been performed using the unpaired two-sided Student's *t*-test in relation to log-transformed SV volume. Only the significant *P*-values derived by comparison between groups were reported in each panel.

and could have different characteristics from the general male population or those males consulting general practitioners for reasons other than couple infertility. In addition, we did not have a true control group composed of age-matched, apparently healthy, fertile men and therefore true normative data of sonographic parameters cannot be inferred. Furthermore, due to the cross-sectional nature of our study, neither a causality hypothesis nor mechanistic models can be drawn. Finally, this is a retrospective study, and further prospective studies are required.

This study also has several strengths. First, it systematically evaluates several clinical, seminal, laboratory and male genital tract ultrasound parameters in a cohort of 394 male patients with couple infertility. In addition, we simultaneously evaluated in the same session, before and after ejaculation, the CDUS characteristics of the entire male genital tract. Third, this study considers several possible confounders, such as age, BMI, lifestyle parameters, SHBG and total testosterone levels. Finally, the study simultaneously examined several end-points within the same population, allowing a valid comparison of the co-prevalence of the parameters examined, and supporting their possible association with a smoking habit.

Conclusions

In males of infertile couples, CS, when compared with non-smokers, show lower ejaculate and ultrasound-derived SV volume despite higher testosterone levels. How this new smoking-related alteration impacts male infertility needs to be addressed by further studies.

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Authors' roles

F.L., G.C., P.V. and M.M. made substantial contributions to the conception and design of the manuscript, analysis and interpretation of data, drafting the manuscript and revising it for intellectual content. F.L. performed all scrotal and transrectal ultrasound evaluation. F.L., P.V.,

E.M., M.R. were involved in acquisition and inclusion of data in a dedicated database. M.G.F. performed seminal analyses and seminal interleukin 8 levels quantification. All the authors gave final approval of the submitted version of the manuscript.

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Conflict of interest

None declared.

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