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Is thyroid hormones evaluation of clinical value in the work-up of males of infertile couples?

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STUDY QUESTION: Is thyroid hormones (TH) evaluation of clinical value in the work-up of males of infertile couples?

STUDY ANSWER: Our results suggest that TH evaluation is not mandatory in the work-up of male infertility.

WHAT IS KNOWN ALREADY: A few previous studies performed on a limited series of subjects reported a negative impact of hyper- and hypo-thyroidism on semen volume, sperm concentration, progressive motility and normal morphology. No previous study has systematically evaluated associations between TH variation, semen parameters and ultrasound characteristics of the male genital tract.

STUDY DESIGN, SIZE AND DURATION: Cross-sectional analysis of a consecutive series of 172 subjects seeking medical care for couple infertility from September 2010 to November 2014.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Of the entire cohort, 163 men (age 38.9 $\pm$ 8.0 years) free of genetic abnormalities were studied. All subjects underwent a complete andrological and physical examination, biochemical and hormonal assessment, scrotal and transrectal colour-Doppler ultrasound (CDUS) and semen analysis (including seminal interleukin 8 levels, sIL-8) evaluation within the same day.

MAIN RESULTS AND THE ROLE OF CHANCE: Among the patients studied, 145 (88.9%) showed euthyroidism, 6 (3.7%) subclinical hyper- and 12 (7.4%) subclinical hypo-thyroidism. No subjects showed overt hyper- or hypo-thyroidism. At univariate analysis, no associations among thyroid-stimulating hormone (TSH) or TH levels and sperm parameters were observed. Conversely, we observed positive associations among free triiodothyronine (fT3) and free thyroxine (fT4) levels, ejaculate volume and seminal fructose levels. In a multivariate model, after adjusting for confounders such as age, body mass index, smoking habit, sexual abstinence, calculated free testosterone, prolactin and sIL-8 levels, only the associations found for fT3 levels were confirmed. When CDUS features were investigated, using the same multivariate model, we found positive associations between fT3 levels and seminal vesicles (SV) volume, both before and after ejaculation (adj. $r = 0.354$ and adj. $r = 0.318$, both $P < 0.0001$), as well as with SV emptying (ΔSV volume; adj. $r = 0.346$, $P < 0.0001$) and echo-texture inhomogeneity. In addition, after adjusting for confounders, negative associations between fT4 levels and epididymal body and tail diameters were found. No significant associations between TSH or TH levels and CDUS features of other organs of the male genital tract, including testis and prostate, were found. Finally, when the features of subjects with euthyroidism, subclinical hypo- and hyper-thyroidism were compared, no significant differences in seminal or hormonal parameters were found. Conversely, evaluating CDUS parameters, subjects with subclinical hyperthyroidism showed a higher difference between the SV longitudinal diameters measured before and after ejaculation when compared with that of subclinical hypothyroid men, even after adjusting for confounders ($P < 0.007$). All the other male genital tract CDUS characteristics did not differ among groups.

LIMITATIONS, REASONS FOR CAUTION: First, the number of patients investigated is relatively small and those with (subclinical) thyroid dysfunctions are an even smaller number; hence, it is therefore difficult to draw firm conclusions. Moreover, the present results are derived from patients consulting an Italian Andrology Clinic for couple infertility, and could have different characteristics from the male general population or from those males consulting general practitioners for reasons other than couple infertility. Finally, due to the cross-sectional nature of the study, neither a causality hypothesis nor mechanistic models can be inferred.

WIDER IMPLICATIONS OF THE FINDINGS: Although no associations between TH and sperm parameters were observed, present data support a positive effect of TH on SV size and a permissive role on the ejaculatory machinery, likely through an action on SV and epididymal

contractility. This is the first study reporting such evidence. However, in contrast with the view that TH assessment is important for female fertility, our results do not support a systematic evaluation of thyroid function in males of infertile couples. How TH abnormalities impact 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: thyroid hormones / infertile men / ejaculate volume / fructose / seminal vesicles ultrasound

Introduction

Thyroid diseases are the most common endocrine conditions affecting women of reproductive age (Jefferys *et al.*, 2015). Hyperthyroidism is found in 2.3% of women presenting with fertility problems (Poppe *et al.*, 2002; Jefferys *et al.*, 2015), hypothyroidism in about 2–4% of women of reproductive age (Wang and Crapo, 1997; Jefferys *et al.*, 2015), whereas subclinical hypothyroidism is found in up to 11% of those with ovulation disorders (Strickland *et al.*, 1990). Changes in thyroid function negatively impact female reproductive health before, during and after conception (Jefferys *et al.*, 2015). Accordingly, both hypo- and hyper-thyroidism have been associated with menstrual disturbances (Krassas *et al.*, 1994; Kakuno *et al.*, 2010; Jefferys *et al.*, 2015). In addition, thyroid diseases have long been considered as a risk factor for miscarriage (Anselmo *et al.*, 2004; Thangaratinam *et al.*, 2011; van den Boogard *et al.*, 2011; Carp *et al.*, 2012; Twig *et al.*, 2012; Jefferys *et al.*, 2015). Finally, thyroid diseases can also exert adverse effects on pregnancy outcomes and perinatal mortality (van den Boogard *et al.*, 2011; Cignini *et al.*, 2012; Khandelwal and Tandon, 2012). Hence, according to a recent review (Jefferys *et al.*, 2015) - also supported by the Royal College of Obstetricians & Gynecologists (https://www.rcog.org.uk/en/news/) - the screening for thyroid disease is suggested in women presenting with fertility problems, recurrent pregnancy loss and at the beginning of any pregnancy.

In contrast with the view that thyroid hormones (TH) are important for female fertility, their impact on male reproductive function is largely unknown and scarcely addressed (see, for review, Krassas *et al.*, 2010). This may be due to the lower prevalence of thyroid dysfunctions in the male (Vanderpump *et al.*, 1995; Hollowell *et al.*, 2002; Krassas and Perros, 2003) and/or to their lower clinical impact on reproductive function compared with their well-known systemic effects (Krassas *et al.*, 2010). The relationship between thyroid function and spermatogenesis in adult men and how TH act on testicular function is still controversial (see, for review, Rajender *et al.*, 2011). Previous studies reported a negative impact of hypo- and hyper-thyroidism on conventional semen parameters (see, for review, Jannini *et al.*, 1995; Krassas *et al.*, 2010; Rajender *et al.*, 2011; Krajewska-Kulak and Sengupta, 2013). In particular, a negative association between hyperthyroidism and sperm count (Clyde *et al.*, 1976; Kidd *et al.*, 1979; Hudson and Edwards, 1992; Krassas *et al.*, 2002) and motility (Clyde *et al.*, 1976; Hudson and Edwards, 1992; Krassas and Perros, 2003) has been reported. On the other hand, hypothyroidism has been shown to negatively affect sperm motility (Corrales Hernandez *et al.*, 1990; Krassas *et al.*, 2008), morphology (Krassas *et al.*, 2008) and ejaculate volume (Corrales Hernandez *et al.*, 1990). In addition, upon appropriate therapy of hyper- (Clyde *et al.*, 1976; Hudson and Edwards, 1992; Krassas *et al.*, 2002, 2003) or hypo-thyroidism (Corrales Hernandez *et al.*, 1990; Krassas *et al.*, 2008), correction of semen abnormalities has been also reported. TH-induced changes in sex hormone-binding globulin (SHBG) and sex steroid levels were advocated to justify these results (see Krajewska-Kulak and Sengupta, 2013). However, it should be noted that available studies focusing on thyroid function and male fertility have been performed on a limited series of subjects, ranging from 3 to 35 patients (see, for review, Krassas *et al.*, 2010; Rajender *et al.*, 2011). In addition, the primary aim of the aforementioned studies was to investigate semen parameters in men with thyroid dysfunctions, whereas it is presently unknown whether TH levels are altered in men seeking medical care for infertility and whether TH variations are related to semen and/or male genital tract characteristics. The key question of whether or not TH should be assessed in male infertility work-up presently remains unanswered.

The primary aim of this study was to evaluate associations between thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4) and clinical, seminal and male genital tract ultrasound characteristics in men attending an Andrology Clinic for couple infertility. The secondary aim was to assess the prevalence of thyroid dysfunctions in a cohort of males of infertile couples and to investigate differences in the fertility-related parameters comparing subjects with normal and abnormal thyroid function.

Materials and Methods

Ethical approval

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). 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.

Patients

We studied a consecutive series of 172 male patients (mean age 38.4 $\pm$ 7.7 years, range 18–59) attending our Outpatient Clinic for the first time from September 2010 to November 2014, 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 >12 months, according to the World Health Organization (WHO, 2000). Subjects with karyotype abnormalities ($n = 2$), chromosome Y micro-deletions ($n = 1$) or the absence of at least one vas deferens and/or one seminal vesicle ($n = 6$) were excluded from the analysis.

All patients were evaluated before beginning any treatment. 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 (CDUS) evaluation and semen analysis.

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, weight and waist circumference. Weight and height were used to calculate body mass index (BMI, kg/m^2).

Quantification of smoking

Self-reported data on smoking habits were collected using a structured interview. Smoking habit duration, measured in years, as well as the total number of cigarettes smoked per day were assessed. 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; Lotti *et al.*, 2015).

Colour-Doppler ultrasound

All patients underwent scrotal and transrectal 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), which is 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, for review, 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 (Lotti *et al.*, 2014a; see Lotti and Maggi, 2015). SV longitudinal and anterior–posterior diameters were measured, both before and after ejaculation. 'SV volume' was calculated using the 'ellipsoid/prolate ($d1 > d2 = d3$) spheroid' formula ($d1*d2*d3*4/3*\pi$, 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, 2015). SV total volume, both before and after ejaculation, was calculated as 'right SV volume + left SV volume'. SV emptying with ejaculation was estimated by calculating 'SV total volume before ejaculation − SV total volume after ejaculation', and defined as 'Δ SV volume'. SV echo-texture features were defined according to previous studies (see Lotti and Maggi, 2015).

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 evaluated after ejaculation and defined as previously reported (Lotti *et al.*, 2012b, 2013b; Lotti and Maggi, 2015).

Semen analysis, determination of seminal interleukin 8 and semen fructose levels

All patients underwent, during the same ultrasound session, semen analysis, performed according to the WHO criteria (2010). Semen samples were obtained by masturbation following a period of 2–7 days of sexual abstinence. Patients were asked to report days of sexual abstinence.

Fructose concentration (mg/ml) was measured in seminal fluid after centrifugation at 500*g* for 10 min. The samples were purified with zinc sulphate and sodium hydroxide after reacting with alcoholic resorcinol and 30% HCl for 15 min at 90°C (Jequier and Crich, 1986). 'Seminal fructose levels' were calculated by multiplying seminal fructose concentration (mg/ml) * ejaculate volume.

Furthermore, a quantification of seminal interleukin 8 (sIL-8), a reliable surrogate marker of male genital tract inflammation (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 (assay sensitivity: 0.35 nmol/l), LH (assay sensitivity: 0.2 IU/l), FSH (assay sensitivity: 0.2 IU/l), prolactin (PRL; assay sensitivity: 4.24 mU/l), TSH (assay sensitivity: 0.005 mU/l), fT3 (assay sensitivity: 0.77 pmol/l), fT4 (assay sensitivity: 1.3 pmol/l) and prostate-specific antigen (PSA; assay sensitivity: 0.03 ng/ml) by electrochemiluminescent method (Modular Roche, Milan, Italy), SHBG (assay sensitivity: 0.35 nmol/l) by modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany), blood glucose (by glucose oxidase method; Aeroset Abbott, Rome, Italy; assay sensitivity: 0.06 mmol/l), total cholesterol (assay sensitivity: 1.29 mmol/l), high-density lipoprotein cholesterol (assay sensitivity: 0.08 mmol/l) and triglycerides (by automated enzymatic colorimetric method, Aeroset Abbott, Rome, Italy; assay sensitivity: 0.02 mmol/l). Calculated free testosterone was derived according to Vermeulen's formula (available at http://www.issam.ch/freetesto.htm; Vermeulen *et al.*, 1999).

Definition of thyroid dysfunctions

The reference ranges of TSH, fT4 and fT3 of the Florence Central Lab were 0.4–4.0 mU/l, 11.5–21.0 pmol/l and 3.5–6.4 pmol/l, respectively, according to previous studies (Corona *et al.*, 2012a; Maseroli *et al.*, 2015). Subclinical hypothyroidism was defined as TSH levels between 4.5 and 10.0 mU/l and TH levels within the normal range, and subclinical hyperthyroidism as TSH < 0.4 mU/l and TH levels within the normal range, according to the Consensus Statement of the American Association of Clinical Endocrinologists, the American Thyroid Association and the Endocrine Society (Gharib *et al.*, 2005). Overt hypothyroidism was defined as TSH > 10 mU/l and fT4 < 11.5 pmol/l, and overt hyperthyroidism was defined as TSH < 0.1 mU/l and fT4 > 21 pmol/l.

Statistical 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. Stepwise multiple linear or logistic binary regressions were applied for multivariate analyses whenever appropriate. When distribution of non-normal parameters could be normalized through logarithmic transformation, as in the case of TSH, sperm concentration and total count, progressive motility and normal morphology, SV total volume before and after ejaculation, ΔSV volume, PRL and sIL-8, the same tests were applied to logarithmically transformed data. For continuous

parameters, comparison among more than two groups was performed with Kruskal–Wallis test. When significant differences among groups were found overall, Mann–Whitney *U*-test and analysis of covariance (ANCOVA) was used for comparisons between two groups, in a univariate and multivariate setting, respectively. When performing Mann–Whitney *U*-test or ANCOVA, Bonferroni correction was applied, considering as significant a *P*-value of <0.017 (0.05/3, since comparison of three groups was performed at Kruskal–Wallis test). Relative risk and 95% confidence interval were calculated for the association of categorical parameters, and χ^2 test was used for comparisons.

To evaluate if the observed prevalence of subclinical hypo- and hyperthyroidism in our cohort was significantly different from that expected in the general population, *z*-test for one proportion was performed, by using as the 'null hypothesis value' the frequency of subclinical thyroid diseases derived from the NHANES III study (Hollowell *et al.*, 2002).

Since prostate and SV characteristics (Parsons *et al.*, 2006, 2013 for prostate; Kim *et al.*, 2009 and Lotti *et al.*, 2012a for SV), as well as semen quality (see Zitzmann, 2013 and Smith and Walker, 2014), are related to age and testosterone levels, data have been adjusted for age and calculated free 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 BMI, unless otherwise specified. Since ejaculate (see WHO, 2010 and Lotti *et al.*, 2012a, 2013a, 2015) and SV (see Lotti *et al.*, 2012a, 2013a, 2015) volume are affected by duration of sexual abstinence, PRL levels and smoking habit (pack-years of smoking), these confounders have also been included in the multivariate analyses, when specified. Finally, since inflammation affects male genital tract ultrasound characteristics, sIL-8 levels, a surrogate marker of male genital tract inflammation (see Lotti and Maggi, 2013), were included as a confounder in multivariate analyses.

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

Results

Among the patients studied ($n = 163$, mean age 38.9 ± 8.0 years), 145 (88.9%) showed euthyroidism, 6 (3.7%) subclinical hyperthyroidism (TSH < 0.4 mU/l and normal TH levels) and 12 (7.4%) subclinical hypothyroidism (TSH between 4.5 and 10.0 mU/l and normal TH levels), while no subjects showed overt hyper- or hypo-thyroidism. At *z*-test for one proportion, using the prevalence of subclinical hypo- and hyperthyroidism observed in the general male population in the NHANES III study (3.4 and 1.8%, respectively) as the 'null hypothesis value', we found that, in our cohort, the frequency of subclinical hyper-thyroidism was not significantly different from what was expected ($P = 0.07$). Conversely, the prevalence of subclinical hypo-thyroidism was higher than expected ($P = 0.005$).

Table I reports the clinical, biochemical and seminal parameters and Table II the CDUS characteristics of the whole sample and of euthyroid, subclinical hyper- and hypo-thyroid subjects.

1) Associations among TSH, fT3, fT4 levels and clinical characteristics

In the whole sample, fT4 levels showed a negative association with age and BMI ($r = -0.178$, $P = 0.023$ and $r = -0.166$, $P = 0.034$, respectively) and fT3 with waist circumference and BMI ($r = -0.210$, $P = 0.007$ and $r = -0.234$, $P = 0.003$, respectively). Hence, all the following analyses were adjusted for age and BMI. No association of TSH with the aforementioned parameters was observed (not shown). In addition, we found no association of the three hormones with other clinical parameters, including mean testis volume, as assessed by Prader orchidometer (not shown).

TSH levels were positively associated with PRL levels ($r = 0.212$, $P = 0.007$), whereas fT4 levels were related to total and calculated free testosterone ($r = 0.178$, $P = 0.024$ and $r = 0.181$, $P = 0.022$, respectively). After adjusting for age and BMI, only the former association was confirmed (adj. $r = 0.253$, $P < 0.0001$). No other correlations between the three hormones and other biochemical parameters (lipid and glucose profile, PSA) were observed (not shown).

2) Associations among TSH, fT3, fT4 and seminal parameters

Table III shows correlations between circulating TSH, fT3 and fT4 levels and standard semen parameters obtained on the same morning, as observed in the whole sample. At univariate analysis, we observed positive associations among fT3 and fT4 levels, seminal volume and seminal fructose levels (Table III and Fig. 1). In a multivariate model, after adjusting for age, BMI and other confounders such as smoking habit (pack-years), sexual abstinence (days), calculated free testosterone, PRL and sIL-8 levels, only the associations for fT3 were confirmed (Table III). TSH levels were also positively associated with seminal fructose levels, but this relationship was not confirmed in the fully adjusted model (Table III). Conversely, no associations among TSH, fT3 or fT4 levels and sperm parameters or sIL-8 levels were observed (Table III).

3) Associations between TSH, fT3, fT4 levels and male genital tract CDUS features

In the whole sample, at univariate analysis, we found positive associations between fT3 levels and SV total volume, both before and after ejaculation, as well as with their emptying (ΔSV volume) (Table III and Fig. 2A–C). Similar associations were observed when TSH was considered (Table III). After adjusting for confounders, all the aforementioned associations were confirmed (Table III). Finally, when both TSH and fT3 levels were included in a fully adjusted, multivariate model, only the associations between fT3 levels and SV ultrasound parameters were confirmed (adj. $r = 0.331$, $P < 0.0001$, adj. $r = 0.297$, $P = 0.001$ and adj. $r = 0.314$, $P < 0.0001$, for SV total volume before and after ejaculation and ΔSV volume, respectively). In addition, the risk of SV inhomogeneity was positively associated with fT3 levels, both before and after ejaculation (Table III), and confirmed in a confounders-adjusted binary logistic model (Table III).

At univariate analysis, when relationships among TSH, fT3, fT4 and CDUS features of the testis, pampiniform plexus and prostate gland were evaluated in the whole sample and in subjects with euthyroidism, subclinical hypo- and hyper-thyroidism, no significant associations were found (not shown). Conversely, considering the entire cohort, we observed negative associations between fT4 levels and epididymal body and tail diameters, as well as with deferential diameters (Table III). After adjusting for the aforementioned confounders, the associations between fT4 levels and epididymal body and tail diameters were confirmed (Table III).

Table I Clinical characteristics of the whole sample and of euthyroid, subclinical hyper- and hypo-thyroid subjects.

	All patients (n = 163)	Euthyroid (n = 145)	Subclinical hypothyroid (n = 12)	Subclinical hyperthyroid (n = 6)
Clinical and laboratory parameters				
Age (years)	38.9 ± 8.0	38.9 ± 8.4	39.1 ± 5.5	38.6 ± 3.9
Pack-years of smoking	6.4 ± 3.8	6.5 ± 3.3	5.9 ± 3.2	6.2 ± 3.4
Systolic BP (mmHg)	125.7 ± 17.6	126.8 ± 15.6	112.1 ± 31.6	133.0 ± 16.4
Diastolic BP (mmHg)	79.2 ± 9.8	79.5 ± 9.8	74.2 ± 7.6	85.0 ± 9.4
BMI (kg/m^2)	27.9 ± 6.3	28.0 ± 6.4	25.5 ± 3.6	31.1 ± 8.6
Waist (cm)	99.5 ± 16.6	99.7 ± 16.4	92.6 ± 9.6	111.7 ± 28.9
Glycaemia (mmol/l)	5.06 ± 0.67	5.06 ± 0.67	4.89 ± 0.33	5.11 ± 2.00
Total cholesterol (mmol/l)	5.01 ± 0.94	5.01 ± 0.95	5.03 ± 1.06	5.29 ± 0.71
HDL cholesterol (mmol/l)	1.25 ± 0.32	1.25 ± 0.32	1.16 ± 0.26	1.45 ± 0.48
LDL cholesterol (mmol/l)	3.05 ± 0.83	3.03 ± 0.82	3.17 ± 0.98	3.19 ± 0.86
Triglycerides (mmol/l)	1.32 [0.91–2.03]	1.30 [0.91–2.01]	1.38 [0.96–2.18]	1.47 [0.62–2.18]
FSH (IU/l)	5.00 [3.00–7.30]	4.80 [3.00–7.30]	6.00 [3.43–8.65]	6.25 [1.59–6.83]
LH (IU/l)	3.47 [2.32–4.73]	3.54 [2.03–5.01]	3.54 [2.03–5.07]	3.02 [0.98–4.62]
PRL (mU/l)	182 [127–228]	179 [125–227]	202 [150–214]	248 [59–302]
TSH (mU/l)	1.92 [1.27–2.60]	1.91 [1.28–2.53]♣	5.65 [4.84–6.89]♣	0.05 [0.01–0.19]♣
fT3 (pmol/l)	5.17 ± 0.75	5.18 ± 0.74	5.46 ± 0.80	4.42 ± 0.75
fT4 (pmol/l)	13.48 ± 2.55	13.34 ± 2.40	13.74 ± 3.22	16.64 ± 3.80
Total testosterone (nmol/l)	13.4 ± 5.7	13.2 ± 5.1	12.6 ± 4.8	18.9 ± 15.6
SHBG (nmol/l)	30.9 ± 14.1	30.3 ± 13.9	31.8 ± 7.9	43.2 ± 25.6
Calculated free testosterone (nmol/l)	0.285 ± 0.104	0.285 ± 0.101	0.265 ± 0.113	0.320 ± 0.173
PSA	0.66 [0.40–0.96]	0.66 [0.51–0.95]	0.75 [0.33–1.16]	0.49 [0.42–0.91]
Mean testis volume (Prader) (ml)	19.0 ± 5.0	19.0 ± 5.0	19.3 ± 4.5	16.6 ± 7.0
Seminal parameters				
Sexual abstinence (days)	4.3 ± 2.0	4.2 ± 2.0	4.9 ± 2.0	4.2 ± 0.8
pH	7.6 ± 0.3	7.6 ± 0.3	7.6 ± 0.2	7.6 ± 0.3
Semen volume (ml)	3.6 ± 1.7	3.5 ± 1.7	3.1 ± 1.2	3.7 ± 1.9
Sperm concentration, *10^6/ml	12.3 [3.0–47.0]	13.5 [3.5–48.8]	5.8 [0.0–39.5]	7.4 [0.25–26.7]
Sperm total count, *10^6/ejaculate	44.1 [8.5–145.7]	44.8 [10.2–151.2]	35.3 [0.0–157.2]	31.8 [1.0–100.1]
Sperm progressive motility, %	42.0 [19.0–54.5]	41.0 [18.5–55.0]	38.5 [32.0–55.8]	43.0 [16.8–52.0]
Sperm morphology, % normal forms	3.0 [1.0–6.0]	3.0 [1.0–6.0]	3.0 [2.0–11.8]	2.0 [0.0–6.2]
Seminal interleukin-8 (ng/ml)	3.99 [2.11–7.17]	4.03 [2.00–7.23]	3.59 [2.56–19.80]	4.44 [2.22–5.39]
Seminal fructose (mg/ejaculate)	7.4 [4.8–11.5]	8.1 [4.8–11.8]	5.5 [4.6–10.0]	10.48 [5.40–18.03]
History of infertility				
Duration of infertility (years)	1.3 [1.0–3.0]	1.5 [1.0–3.0]	1.2 [1.0–3.3]	1.4 [1.0–2.8]
Female partner age (years)	34.8 ± 7.7	34.7 ± 7.9	37.4 ± 5.8	32.8 ± 5.0

Data are expressed as mean ± SD or as median (quartiles) when appropriate, and as percentages when categorical. A comparison of the parameters detected in euthyroid, subclinical hyper- and hypo-thyroid subjects has been performed using Kruskal–Wallis analysis.

♣$P < 0.0001$ at Kruskal–Wallis analysis.

BP, blood pressure; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, prolactin; TSH, thyroid-stimulating hormone; fT3, free triiodothyronine; fT4, free thyroxine; SHBG, sex hormone-binding globulin; PSA, prostate-related antigen.

4) Associations and comparison of subjects with euthyroidism, subclinical hypo- and hyper-thyroidism

Supplementary data, Tables SI and SII describe the aforementioned relationships (see paragraphs 1–3) with seminal and ultrasound parameters, respectively, after categorizing patients according to their thyroid status. By stratifying patients in euthyroidism, subclinical hyper- and hypo-thyroidism, we were able to confirm associations only in the euthyroid cohort, most probably due to the reduced representation of the pathological groups (Supplementary data, Tables SI and SII).

When features of subjects with euthyroidism, subclinical hypo- and hyper-thyroidism were compared, at Kruskal–Wallis analysis only a significant difference in TSH levels was found, while no significant

Table II CDUS characteristics of the whole sample and of subclinical euthyroid, hyper- and hypo-thyroid subjects.

	All patients ($n = 163$)	Euthyroid ($n = 145$)	Subclinical hypothyroid ($n = 12$)	Subclinical hyperthyroid ($n = 6$)
CDUS parameters				
Testis				
Mean testis volume (ml)	15.3 ± 5.0	15.4 ± 5.0	14.9 ± 5.0	13.5 ± 8.3
Testicular inhomogeneity (%)	47.7	57.9	50.0	83.3
Testicular microcalcifications (%)	11.7	12.4	8.3	0.0
Epididymis and vas deferens				
Mean size of the head (mm)	9.6 ± 1.6	9.6 ± 1.6	9.5 ± 1.1	9.2 ± 1.9
Mean size of the body (mm)	4.0 ± 0.9	4.0 ± 0.9	4.0 ± 1.3	3.6 ± 0.6
Mean size of the tail (mm)	5.0 ± 1.1	5.0 ± 1.1	4.8 ± 1.8	4.2 ± 0.9
Mean size of the vas deferens (mm)	3.7 ± 0.8	3.7 ± 0.8	3.4 ± 0.5	3.7 ± 4.3
Inhomogeneous head (%)	49.9	70.3	58.3	83.0
Inhomogeneous tail (%)	43.6	45.5	25.0	49.8
Hypoechoic tail (%)	4.9	5.5	0.0	0.0
Hyperechoic tail (%)	5.5	6.2	0.0	0.0
Coarse tail calcifications (%)	1.8	2.1	0.0	0.0
Hyperaemia (%)	1.2	1.4	0.0	0.0
Varicocele (%)[a]	23.3	15.2	16.7	16.7
Prostate				
Prostate volume (ml)	27.1 ± 8.7	27.4 ± 8.6	24.4 ± 9.5	27.8 ± 9.7
Prostate calcifications (%)	51.5	51.0	50.0	66.8
Prostate macro-calcifications (%)[b]	38.3	41.1	33.3	66.8
Major calcification size (mm)	9.0 [5.7–13.9]	8.5 [5.3–13.8]	13.2 [7.4–18.8]	5.0 [4.0–10.5]
Inhomogeneous prostatic texture (%)	66.3	64.8	63.3	66.6
Hypoechoic prostatic texture (%)	6.2	6.9	16.7	33.4
Hyperechoic prostatic texture (%)	12.6	12.1	16.7	33.4
Prostatic hyperaemia (%)	16.0	14.6	25.0	33.4
Mean arterial peak systolic velocity (cm/s)	9.9 ± 3.2	9.9 ± 3.3	9.8 ± 3.7	10.1 ± 2.9
Mean prostatic venous plexus (mm)	4.1 ± 1.6	4.4 ± 1.8	4.0 ± 2.3	4.2 ± 0.9
Ejaculatory duct dilation (%)	3.1	3.4	0.0	0.0
SV				
Total volume before ejaculation (ml)[c]	9.5 [5.9–17.1]	9.7 [5.8–17.1]	11.3 [6.7–17.4]	4.4 [2.6–18.8]
Total volume after ejaculation (ml)[c]	6.4 [3.7–10.9]	6.5 [3.7–10.9]	6.5 [4.1–22.2]	3.1 [1.3–6.3]
ΔSV (ml)[d]	3.1 [1.4–5.2]	3.2 [1.4–5.2]	2.7 [2.1–11.8]	1.2 [0.8–3.1]
ΔSV both longitudinal diameters (mm)	5.3 [2.9–7.9]	5.1 [3.0–7.8]♣	4.1 [1.3–7.0]♣	12 [7.8–14.1]♣
ΔSV both anterior–posterior diameters (mm)	3.9 [2.1–6.3]	3.9 [2.2–6.3]	4.1 [2.5–8.1]	2.9 [1.5–4.7]

Data are expressed as mean ± SD or as median (quartiles) when appropriate, and as percentages when categorical. A comparison of the parameters detected in euthyroid, subclinical hyper- and hypothyroid subjects has been performed using Kruskal–Wallis analysis for continuous variables and χ^2 test for categorical parameters.
♣$P < 0.02$ at Kruskal–Wallis analysis.
[a]Echographic defined severe varicocele = basal venous reflux increasing after Valsalva's manoeuvre at CDUS (according to Lotti and Maggi, 2015).
[b]Calcifications with size >3 mm (according to Lotti and Maggi, 2015).
[c]Calculated using the 'ellipsoid/prolate ($d1 > d2 = d3$) spheroid' formula ($d1*d2*d3*4/3*\pi$, 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]Calculated as (pre-ejaculatory total volume − post-ejaculatory total volume).
CDUS, colour-Doppler ultrasound; SV, seminal vesicles.

differences in hormonal or seminal parameters, including seminal volume or fructose, were observed (Table I). Among the CDUS parameters evaluated, only the difference between the SV longitudinal diameters measured before and after ejaculation (ΔSV longitudinal diameters) resulted as being different among groups ($P = 0.011$; Table II). Mann–Whitney analysis with Bonferroni correction showed a higher reduction of SV longitudinal diameters with ejaculation in subjects with subclinical hyperthyroidism, with respect to those with subclinical hypothyroidism ($P = 0.008$) or to the rest of the sample ($P = 0.007$) (Fig. 2D). At ANCOVA analysis, with Bonferroni correction, after adjusting for

Table III Associations among TSH, fT3, fT4 levels and seminal parameters or significant ultrasound features of the organs of the male genital tract in the whole sample.

	TSH (mU/l)		fT3 (pmol/l)		fT4 (pmol/l)	
	Univariate analysis	**Multivariate analysis**	**Univariate analysis**	**Multivariate analysis**	**Univariate analysis**	**Multivariate analysis**
Seminal parameters						
Semen volume (ml)	$r = 0.070$, $P = 0.377$	—	**$r = 0.163$, $P = 0.028$**	**Adj. $r = 0.167$, $P = 0.041$**	**$r = 0.178$, $P = 0.024$**	$r = 0.081$, $P = 0.319$
pH	$r = -0.001$, $P = 0.991$	—	$r = -0.001$, $P = 0.991$	—	$r = -0.012$, $P = 0.875$	—
Seminal fructose levels (mg/ejaculate)	**$r = 0.201$, $P = 0.034$**	$r = 0.167$, $P = 0.189$	**$r = 0.215$, $P = 0.023$**	**Adj. $r = 0.241$, $P = 0.021$**	**$r = 0.198$, $P = 0.036$**	$r = 0.181$, $P = 0.093$
Sperm concentration, $\times 10^6$/ml	$r = 0.017$, $P = 0.830$	—	$r = -0.079$, $P = 0.316$	—	$r = -0.099$, $P = 0.208$	—
Sperm total count, $\times 10^6$/ejaculate	$r = 0.031$, $P = 0.699$	—	$r = -0.037$, $P = 0.641$	—	$r = -0.028$, $P = 0.722$	—
Sperm progressive motility, %	$r = -0.005$, $P = 0.954$	—	$r = -0.034$, $P = 0.694$	—	$r = -0.080$, $P = 0.356$	—
Sperm morphology, % normal forms	$r = 0.097$, $P = 0.260$	—	$r = -0.113$, $P = 0.186$	—	$r = -0.181$, $P = 0.063$	—
sIL-8 (ng/ml)	$r = 0.083$, $P = 0.308$	—	$r = -0.005$, $P = 0.952$	—	$r = 0.010$, $P = 0.905$	—
Ultrasound parameters						
SV total volume before ejaculation (ml)	**$r = 0.174$, $P = 0.027$**	**Adj. $r = 0.187$, $P = 0.046$**	**$r = 0.327$, $P < 0.0001$**	**Adj. $r = 0.354$, $P < 0.0001$**	$r = 0.046$, $P = 0.560$	—
SV total volume after ejaculation (ml)	**$r = 0.155$, $P = 0.05$**	**Adj. $r = 0.175$, $P = 0.049$**	**$r = 0.301$, $P < 0.0001$**	**Adj. $r = 0.318$, $P < 0.0001$**	$r = 0.069$, $P = 0.387$	—
ΔSV volume (ml)	**$r = 0.208$, $P = 0.009$**	**Adj. $r = 0.225$, $P = 0.018$**	**$r = 0.304$, $P < 0.0001$**	**Adj. $r = 0.346$, $P < 0.0001$**	$r = 0.056$, $P = 0.485$	—
SV inhomogeneity before ejaculation	**RR = 3.60 [1.21–10.71], $P = 0.021$**	**RR = 6.34 [1.48–22.34], $P = 0.013$**	**RR = 2.60 [1.47–4.62], $P = 0.001$**	**OR = 3.18 [1.66–6.07], $P < 0.0001$**	RR = 091 [0.80–1.03], $P = 0.146$	—
SV inhomogeneity after ejaculation	RR = 2.23 [0.82–6.07], $P = 0.118$	—	**RR = 2.33 [1.31–4.15], $P = 0.004$**	**OR = 2.79 [1.48–5.27], $P = 0.002$**	RR = 0.96 [0.84–1.08], $P = 0.498$	—
Epididymal body diameter (mm)	$r = 0.094$, $P = 0.237$	—	$r = -0.031$, $P = 0.698$	—	**$r = -0.213$, $P = 0.007$**	**Adj. $r = -0.191$, $P = 0.028$**
Epididymal tail diameter (mm)	$r = 0.100$, $P = 0.210$	—	$r = 0.003$, $P = 0.967$	—	**$r = -0.217$, $P = 0.006$**	**Adj. $r = -0.208$, $P = 0.018$**
Proximal vas deferens diameter (mm)	$r = -0.077$, $P = 0.335$	—	$r = -0.150$, $P = 0.067$	—	**$r = -0.172$, $P = 0.029$**	Adj. $r = -0.121$, $P = 0.176$

Multivariate analysis has been adjusted for age, body mass index, smoking habit (pack-years), sexual abstinence (days), calculated free testosterone, prolactin and seminal interleukin 8 (sIL-8) levels. RR and OR values are reported for each fT3 unit increment. Significant associations are reported in bold.
TSH, thyroid-stimulating hormone; fT3, free triiodothyronine; fT4, free thyroxine; SV, seminal vesicles; RR, relative risk; OR, odds ratio.

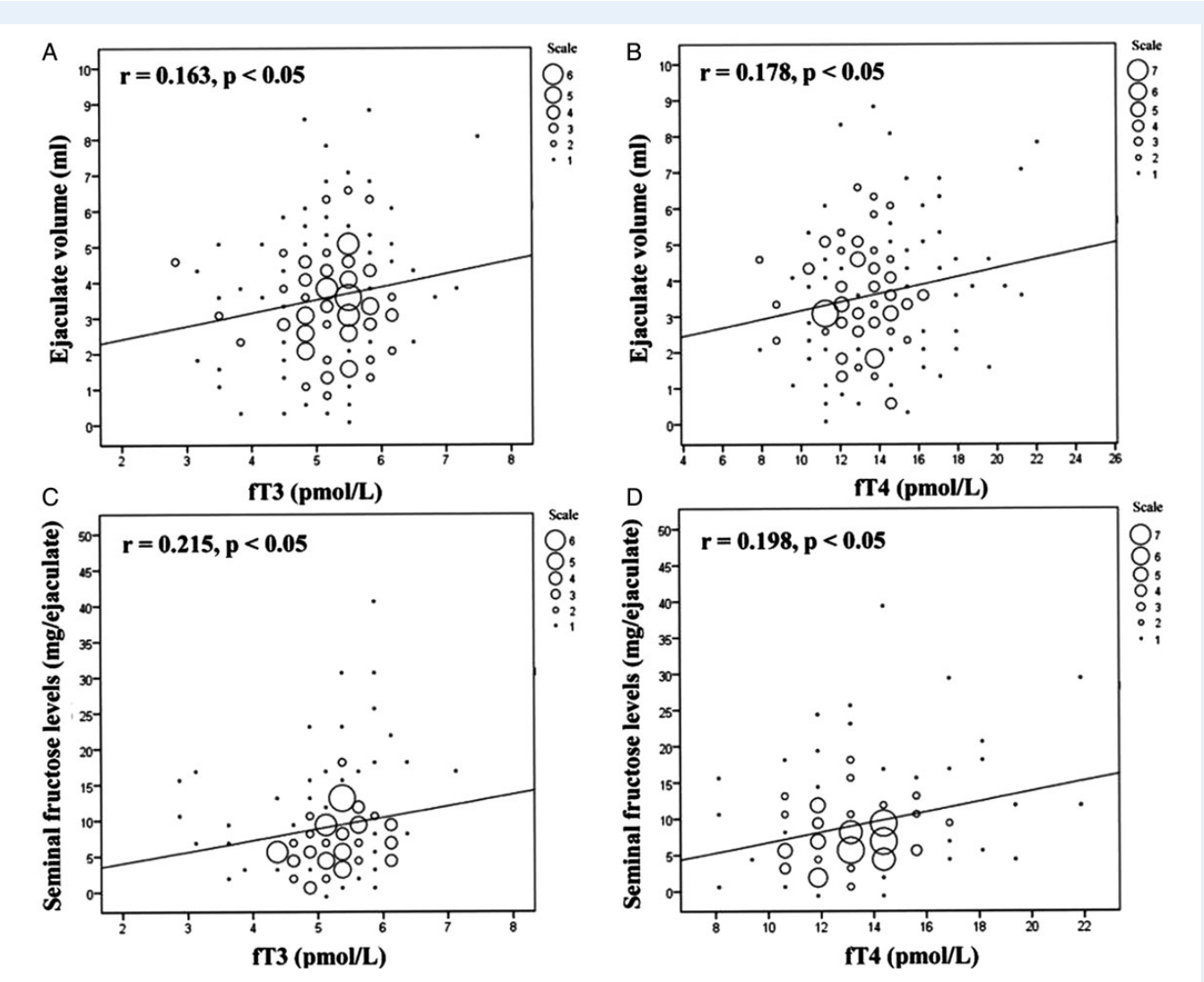

Figure 1 Associations between TH and significant seminal parameters. Associations between fT3 and fT4 levels and ejaculate volume (**A** and **B**, respectively) and seminal fructose levels (**C** and **D**, respectively) are reported. *r* and *P* value of the univariate analysis are reported. For each panel, a scale of circles of increasing size with a number on the right shows the number of subjects represented by each circle.

all the aforementioned confounders, the difference between subclinical hyperthyroidism and hypothyroidism retained significance (adj. difference = 7.0 mm; $P = 0.007$), while the difference with the rest of the sample was attenuated (adj. difference = 4.6 mm; $P = 0.035$). All the other CDUS characteristics of the male genital tract did not differ among groups (not shown).

Discussion

In a cohort of 163 males seeking medical care for couple infertility, fT3 and fT4 levels resulted as being in the normal range in all the subjects evaluated. In contrast with previous studies investigating the effect of thyroid dysfunctions on male reproductive health (see, for review, Jannini *et al.*, 1995; Krassas *et al.*, 2010; Rajender *et al.*, 2011; Krajewska-Kulak and Sengupta, 2013), we here report the associations between physiological TH variations and several seminal and ultrasound features of men of infertile couples. The main finding of our study is the demonstration, for the first time, of a positive association between fT3 levels and SV ultrasound-derived volumes, both before and after ejaculation, along with ejaculation-related SV emptying (ΔSV volume). Accordingly, fT3 levels were positively related to ejaculated volume and fructose levels, both an index of SV secretory function (WHO, 2010). We also observed a positive association between fT4 levels and decreased epididymal body and tail sizes. Overall, these results suggest a permissive role of TH on the ejaculatory machinery and on SV physiological function. Conversely, no associations were found between TSH or TH and other semen parameters or CDUS features of the entire male genital tract, including testis and prostate.

In the present study, a small subset of patients had subclinical hyper- ($n = 6$) and hypo- ($n = 12$) thyroidism. However, according to the reported prevalence in the general population (Hollowell *et al.*, 2002), in our cohort, the frequency of subclinical hyper-thyroidism (3.7%) was in agreement with what was expected (1.8%), while that of

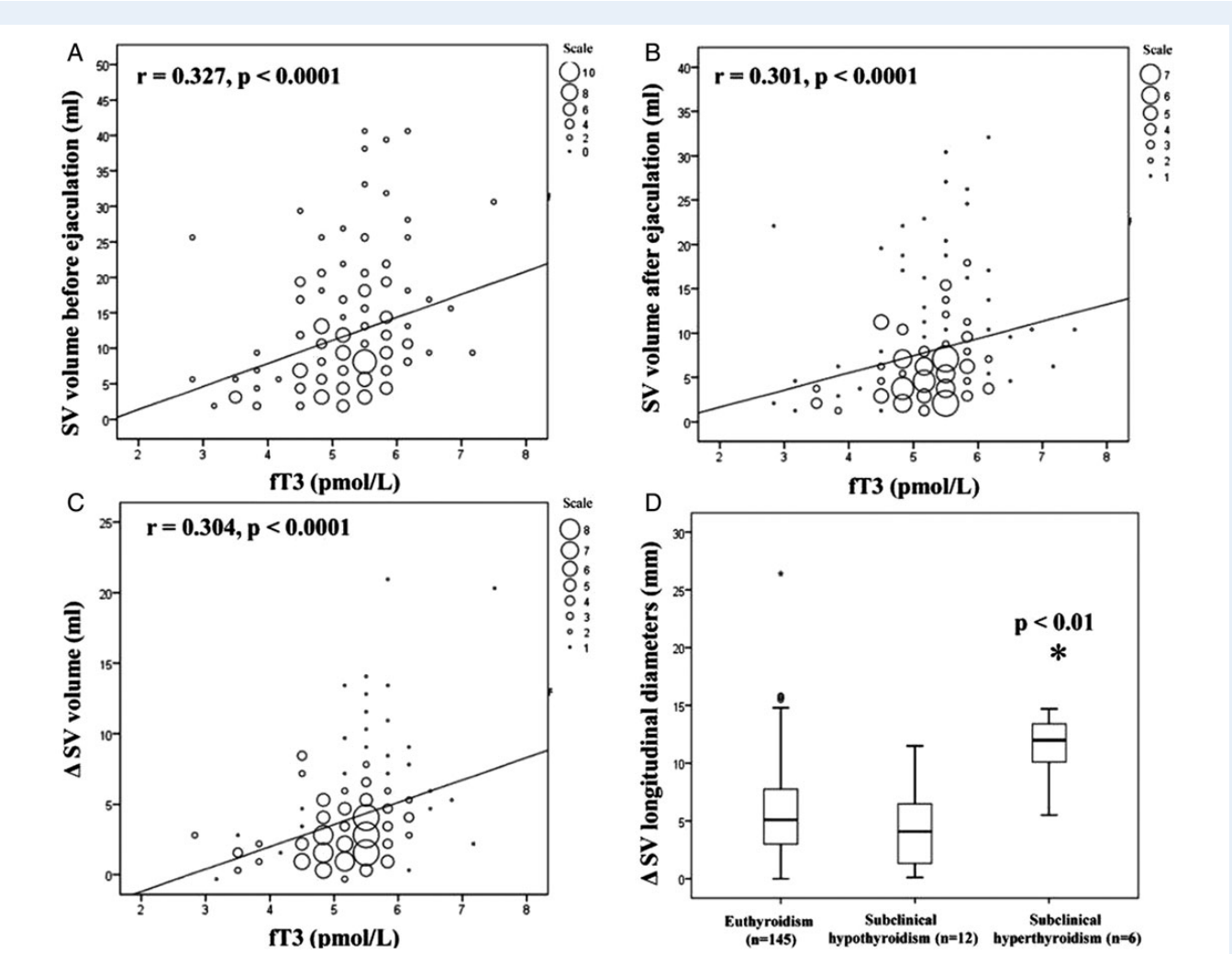

Figure 2 Associations between fT3 or subclinical hyperthyroidism and significant SV ultrasound features. Associations between fT3 levels and SV total volume before (**A**) and after (**B**) ejaculation, and with their difference (Δ SV volume, **C**). *r* and *P* value of the univariate analysis are reported. For each panel, a scale of circles of increasing size with a number on the right shows the number of subjects represented by each circle. (**D**) The difference in reduction (Δ) of SV longitudinal diameters with ejaculation comparing subjects with euthyroidism, subclinical hypo- and hyper-thyroidism. * $P < 0.01$ versus other groups at Mann–Whitney analysis.

subclinical hypothyroidism (7.4%) was significantly higher than expected (3.4%). The latter result might be biased by the small study sample, however, a specific role of subclinical thyroid dysfunction cannot be ruled out, considering the aforementioned association with SV emptying.

SV are paired, androgen-dependent glands, contributing 50–80% of the ejaculate volume (Lotti and Maggi, 2015). SV secretions contain a great variety of substances (Gonzales, 1989), including fructose, which is the main energy source for the spermatozoa (Gonzales, 2001). SV participate in the ejaculation process, contracting and releasing their fructose-rich secretions into the ejaculate (Jeyendran, 2000; Bettocchi *et al.*, 2008; Rowland *et al.*, 2010; Corona *et al.*, 2012b). We here provide evidence of a regulatory role of fT3 levels on SV size and emptying function. In fact, we found that fT3 levels are positively related to SV volume, SV echo-texture inhomogeneity—indicating a possible remodelling effect of this hormone on SV structure—and SV emptying (ΔSV volume), along with the resulting ejaculate volume and seminal fructose levels. Overall, these data suggest that TH regulate SV physiological function within the physiologic range. After adjusting for several potential confounders, the associations between fT3 levels and the aforementioned parameters retained statistical significance, suggesting an independent effect of fT3 on SV size and function. Previous studies in humans (Corrales Hernandez *et al.*, 1990) and in animal models (Maran and Aruldhas, 2002; Jacob *et al.*, 2005; Swapna *et al.*, 2006) are in line with this view. Corrales Hernandez *et al.* (1990) found a lower semen volume in 10 subjects with primary hypothyroidism with respect to 16 fertile controls. Hypothyroidism induced in newborn male rats (Maran and Aruldhas, 2002) and in air-breathing catfish *Clarias gariepinus* (Swapna *et al.*, 2006) was associated with a reduction in SV weight and SV volume, respectively. On the other hand, experimentally induced hyperthyroidism in the latter animal model was associated with a marked reduction of fluid in SV loculi (Jacob *et al.*, 2005), suggesting a massive SV emptying. Overall, all these data suggest that TH levels are involved in the regulation of SV physiology, favouring their functional contribution to the ejaculatory process.

Hyperthyroidism has been associated with a greater propensity to ejaculate in studies involving animal models (Cihan *et al.*, 2009b; Cahangirov *et al.*, 2011) and humans (Corona *et al.*, 2011, 2012b), and in the latter it has been recognized as an acquired cause of premature ejaculation (Corona *et al.*, 2004; Carani *et al.*, 2005; Cihan *et al.*, 2009a; Buvat *et al.*, 2010; Maggi *et al.*, 2013). However, the mechanism by which TH facilitate the ejaculatory process is unknown. Cahangirov *et al.* (2011) speculated that TH excess could act on the supraspinal regions which control the ejaculation process. However, a direct action of TH on sex accessory organs can also be hypothesized. In fact, thyroid hormone nuclear receptors (TR) are located in several portions of the male genital tract (De Paul *et al.*, 2008; Carosa *et al.*, 2010; Rajender *et al.*, 2011). T3 binding sites (Del Rio *et al.*, 2000) and expression of both TRα1 and TRβ1 isoforms (De Paul *et al.*, 2008) have been demonstrated in rat epididymis. We here report that a positive relationship exists between fT4 levels and decreased epididymal body and tail size after ejaculation, suggesting a possible role of TH on emptying of these structures during the emission phase of the ejaculatory process. In addition, in the six subjects with subclinical hyperthyroidism, a higher ΔSV longitudinal diameters (a SV emptying-related parameter) was found, further supporting a permissive role of the hyperthyroid state in SV emptying and, maybe, in the ejaculatory process.

In contrast with the positive association between TH and the ejaculatory machinery, we did not find any relationship between TH levels and other semen parameters, sex hormones or other characteristics of the male genital tract. In particular, neither testis characteristics nor testosterone or SHBG levels were associated with physiological TH variations. Accordingly, stratifying subjects according to their thyroid status (euthyroidism, subclinical hypo- and hyperthyroidism) did not reveal major differences in hormonal and seminal parameters. Overall, our results suggest that evaluation of TH may not be necessary in the work-up of male infertility. In line with the view of a scanty effect of TH on fertility, gene deletion of TRα1, TRα1/α2 and TRβ1/β2 in various animal models was without effect on reproduction capacity, although systemic abnormalities were apparent (see Hsu and Brent, 1998; Flamant and Samarut, 2003; Bochukova *et al.*, 2012). Only $TR\alpha1^{PV/+}$ mutant mice showed, along with dwarfism and high mortality, a reduced fertility, as assessed by a reduction in the pregnancy rate and in the consequent litter size (Kaneshige *et al.*, 2001).

This study has some limitations. First, the number of patients investigated is relatively small, and that of (subclinical) thyroid dysfunctions is even smaller; hence, it is therefore difficult to draw firm conclusions. Moreover, the present results are derived from patients consulting an Italian Andrology Clinic for couple infertility, and could have different characteristics from the male general population or from those males consulting general practitioners for reasons other than couple infertility. Finally, due to the cross-sectional nature of the study, neither a causality hypothesis nor mechanistic models can be inferred.

This study also has several strengths. First, it systematically evaluates several clinical, seminal, laboratory and male genital tract ultrasound parameters in a cohort of 163 male patients with couple infertility. Secondly, we simultaneously evaluated in the same session, before and after ejaculation, the CDUS characteristics of the entire male genital tract. Thirdly, this study considers several possible confounders, such as age, BMI, smoking habit, sexual abstinence, calculated free testosterone, PRL and sIL-8 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 TH.

In contrast with the compelling evidence that TH assessment is important for female fertility, our results do not support a systematic evaluation of thyroid function in males of infertile couples. However, further larger studies are needed in order to make solid recommendations. On the other hand, our data support a positive effect of TH on SV size and suggest a permissive role of these hormones on the ejaculatory machinery, acting on SV emptying and, maybe, on epididymal contraction.

Supplementary data

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

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Authors' roles

F.L. and M.M. made substantial contributions to the conception and design of the manuscript, analysis and interpretation of data and drafting the manuscript. L.B. was involved in analysis and interpretation of data. F.L., M.M. and E.B. revised the manuscript for intellectual content. F.L. performed all scrotal and transrectal ultrasound evaluation. F.L., E.M. and N.F. were involved in acquisition and inclusion of data in a dedicated database. S.D.I and E.B. performed seminal analyses, seminal fructose and interleukin-8 level quantification. All the authors gave final approval of the submitted version of the manuscript.

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Conflict of interest

None of the authors have any conflict of interest to declare.

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