Received: 22 July 2022 | Revised: 29 July 2022 | Accepted: 29 July 2022

DOI: 10.1111/andr.13260

ANDROLOGY WILEY

SUPPLEMENT ARTICLE

The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: An overview on male genital tract ultrasound reference ranges

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Abstract

Background: So far, male genital tract color-Doppler ultrasound (MGT-CDUS) was not standardized. Recently, the European Academy of Andrology (EAA) published the

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[Correction added on 25 November 2022, after first online publication: CRUI-CARE funding statement has been added.]

Funding information
European Academy of Andrology; Ministry of University and Scientific Research (Scientific Independence of young Researchers [SIR] project to Francesco Lotti), Grant/Award Number: RBSI14LFMQ

results of a multicenter study assessing the CDUS characteristics of healthy-fertile men (HFM) to obtain normative parameters.

Objectives: To report the EAA US study (i) standard operating procedures (SOPs) for assessing MGT-CDUS, (ii) main MGT-CDUS normative parameters, and (iii) compare the EAA and previously published "normal" CDUS values.

Methods: A cohort of 248 HFM (35.3 ± 5.9 years) was studied, evaluating MGT-CDUS before and after ejaculation following SOPs.

Results: SOPs for MGT-CDUS assessment are summarized here. All subjects underwent scrotal CDUS and 188 men underwent transrectal ultrasound before and after ejaculation. The main CDUS reference ranges and characteristics of the HFM-MGT are reported here. The mean testicular volume was ~17 mL. The lower limit for right and left testis was 12 and 11 mL, defining testicular hypotrophy. The upper limit for epididymal head, body, tail, and vas deferens was 11.5, 5, 6, and 4.5 mm, respectively. Testicular and epididymal arterial reference ranges are reported. The EAA varicocoele classification is reported. CDUS-varicocoele was detected in ~37% of men. Prostate mean volume was ~25 mL, while lower and upper limits were 15 and 35 mL, defining hypotrophy and enlargement, respectively. Prostate arterial reference ranges are reported. Prostate calcifications and inhomogeneity were frequent; midline prostatic cysts were rare and small. Ejaculatory duct abnormalities were absent. The upper limit for periprostatic venous plexus was 4.5 mm. Lower and upper limits of seminal vesicles (SV) anterior–posterior diameter were 6 and 16 mm, defining hypotrophy or dilation, respectively. Seminal vesicle volume and ejection fraction reference ranges are reported. SV-US abnormalities were rare. Deferential ampullas upper limit was 6 mm. A discussion on the EAA and previously published "normal" CDUS values is reported here.

Conclusions: The EAA findings will help in reproductive and general male health management.

KEYWORDS
healthy, fertile men, scrotal and transrectal ultrasound, male genital tract ultrasound, scrotal organs reference ranges and normative parameters, prostate and seminal vesicles reference ranges and normative parameters

1 | INTRODUCTION

To date, imaging of the male genital tract (MGT) represents an essential diagnostic tool in andrology, allowing physicians to complete the diagnostic work-up of the andrologic patient, especially when anamnesis, physical, and biochemical examinations do not provide sufficient information for adequate patient management.[1] In particular, ultrasound (US) represents the gold standard method for scrotal investigation,[1,2] and a useful tool to evaluate the prostate-vesicular region.[1] Using high-frequency sound waves, US is a simple, rapid, and harmless diagnostic tool able to provide live images of the MGT organs and, among imaging techniques, is the least expensive.[1,2] The high-resolution grey-scale mode associated with color- and power-Doppler examination allow sonographers to investigate size, echotexture, and vascular features of the scrotal and prostate-vesicular organs, and to detect their abnormalities.[1,2] So far, scrotal US has shown a relevant impact both on reproductive and general male health, assessing scrotal features related to reproductive health, scrotal pain, masses, and trauma.[1–7] In addition, transrectal US (TRUS) application has assumed a growing relevance especially in infertility and chronic pelvic pain assessment.[1,8–14]

Although US has been widely used to explore the MGT organs, until very recently the method used to assess several qualitative and quantitative US parameters had not been standardized, and normative parameters and thresholds to distinguish normal and pathologic features were often not evidence-based.[1] In the last few years, thanks to the efforts of different radiological, urological, and andrological societies,[3,5–10,15–21] some standards in MGT US have been achieved. However, only very recently the European Academy of

Andrology (EAA) published the results[22–24] of an international multicenter study entitled "Standardization of the MGT color-Doppler ultrasound (CDUS) parameters in healthy, fertile men" (shortened to "EAA US study"; see http://www.andrologyacademy.net/studies),[22] aimed at establishing the reference ranges and characteristics of the organs of the scrotal and prostate-vesicular regions in healthy, fertile men. The EAA US study provided the standard operating procedures (SOPs) for the assessment of MGT-CDUS qualitative and quantitative parameters and defined MGT-CDUS normative parameters.[23–25] In addition, the EAA US study evaluated and reported the associations between MGT-CDUS parameters and clinical, seminal, and biochemical characteristics of healthy, fertile men.[23–25]

We report here an overview on the EAA US study-derived (i) SOPs for the assessment of MGT-CDUS qualitative and quantitative parameters, (ii) main MGT-CDUS normative parameters, and (iii) discuss the comparison between the EAA US study and previously published "normal values," focusing on clinical implications.

2 | METHODS

The EAA US study was designed as a multicenter, international, observational study.[23] Eleven EAA centers (Ancona, Italy; Barcelona, Spain; Cairo, Egypt; Catania, Italy; Florence, Italy; Giessen, Germany; Halle, Germany; L'Aquila, Italy; Muenster, Germany; Rome, Italy; Tartu, Estonia) joined the project and enrolled 248 healthy, fertile men from February 2016 to February 2019.[23] The definition of "healthy, fertile men" established by the EAA US consortium has been reported and discussed in a previous study.[23] In particular, the inclusion criteria of the EAA US study were: 1. healthy, fertile men (see below); 2. age ≥ 18 years; 3. capacity to give consent for study participation. "Fertile men" were defined as (i) partners of a pregnant woman in the second or third trimester of pregnancy or (ii) men with a child less than one year old, achieved through natural conception.[23] "Healthy men" were defined as subjects with no personal history of previous or current systemic diseases or treatments with a recognized negative effect on semen parameters.[23] All subjects were asked to undergo a standardized protocol performed entirely on the same day, including scrotal and transrectal CDUS before and after ejaculation.[23] The SOPs for the assessment of MGT-CDUS qualitative and quantitative parameters and the intra- and inter-operator comparability of the MGT-CDUS parameters among different operators have been defined during investigator meetings organized before starting the enrollment of healthy, fertile men, as previously reported,[23] and have been described in previous studies.[24,25] Below we summarize the EAA US study SOPs for the assessment of the main MGT-CDUS parameters (see "Results" section).

2.1 | Clinical, biochemical, and seminal parameters

The methods related to the clinical, seminal, and biochemical parameters of the cohort studied have been reported and discussed in a previous study.[23]

2.2 | SOPs to assess MGT-CDUS parameters

The MGT-CDUS parameters to be analyzed and the methods used to evaluate them were standardized and reported at http://www.andrologyacademy.net/studies.[22] In addition, exemplary figures reporting (a) how to measure quantitative parameters and (b) classifications of qualitative characteristics—using Likert scales—of the organs of the scrotal and prostate-vesicular regions were reported on the EAA website,[22] and the most relevant figures have been reported in previous studies.[24,25] Finally, standardized schedules to report parameters detected before and after ejaculation in each EAA center were uploaded and made available at http://www.andrologyacademy.net/studies.[22]

2.3 | Scrotal CDUS and TRUS

Scrotal CDUS and TRUS have been performed systematically on the subjects studied, scanning the organs at various longitudinal, transverse, and oblique scans using a high-frequency linear probe (7–15 MHz)[24] and a transrectal probe (3–13 MHz),[25] respectively. To make the study results applicable to the clinical reality of any sonographer, the EAA US consortium approved the use of any US equipment present in the different centers instead of using a standard US console. The US equipment used by the different EAA centers have been reported in previous studies.[24,25]

2.4 | Intra- and inter-operator comparability of MGT-CDUS parameters

Intra- and inter-operator comparability of the MGT-CDUS parameters were assessed on seven males of infertile couples.[23–25] Intra-operator comparability was assessed for the main quantitative and qualitative MGT-CDUS parameters considering the results of three evaluations for each parameter. Inter-operator comparability was derived from the measures and observations obtained by six different sonographists (Francesco Lotti; Francesca Frizza; Olev Poolamets; Gianmaria Salvio; Elisa Maseroli; Sarah Cipriani) for the main quantitative and qualitative parameters, respectively. The comparability of quantitative and qualitative parameters was expressed using the coefficient of variation (CV) ([standard deviation (σ) / mean (μ)] × 100) and the concordance rate (CR) ([number of concordant observations/number of operators] × 100), respectively.[24–26] A CV < 10 is considered acceptable.[24,25,27]

2.5 | Statistical analysis

Statistical analysis used in the EAA US study has been extensively discussed in previous studies.[23–25] Of note, the reference range for MGT-CDUS organs was estimated according to the Clinical and Laboratory Standard Institute (CLSI) guidelines,[28] as the 5th and

TABLE 1 EAA standard operating procedures (SOPs) to assess scrotal CDUS

Testis
Testicular volume
Evaluate the three maximum diameters of each testis (anterior–posterior [height] and transverse [width] diameters in transverse scan; longitudinal diameter [length] in longitudinal scan)
Calculate TV using the ellipsoid formula (length × height × width × 0.52)
Testicular homogeneity
Use a four point-Likert scale:
0. Homogeneity
1. Mild (grade 1) inhomogeneity [presence of small hypoechoic foci/thin hypoechoic striae]
2. Moderate (grade 2) inhomogeneity [presence of thick hypoechoic striae]
3. Severe (grade 3) inhomogeneity [diffuse inhomogeneity with "netting"/"geographical map" appearance])
Testicular echogenicity
Use a three point-Likert scale: 0. Normoechoic 1. Mainly hypoechoic 2. Mainly hyperechoic
Calcifications and microlithiasis
Macrocalcifications: calcifications with a size >3 mm
Microcalcifications: small (1–3 mm) bright echogenic foci with no acoustic shadowing
Microlithiasis: presence of ≥5 microcalcifications in a single US scan, classified as: 1, limited; 2, "clusters"; or 3, diffuse ("starry sky" appearance). Report localization in the upper, middle, and lower third of the testis
Testicular nodules
Evaluate the three diameters and characteristics (0, cystic; 1, mixed; 2, solid), shape (0, regular; 1, irregular), homogeneity (0, homogeneous; 1, inhomogeneous), echogenicity (0, normal echogenicity; 1, mainly hypoechoic; 2, mainly hyperechoic), calcifications and/or cysts (0, absent; 1, present) and vascularization (0, absent; 1, peripheral; 2, intranodular)
Testicular vascularization
Qualitative assessment: normal, reduced, enhanced (in the entire testis and/or focal areas); compare the two testes
Quantitative assessment: evaluate arterial PSV, acceleration, RI, and PI in the testicular artery—in the spermatic cord, 2 cm before the gonadal hilum—and the intratesticular arteries (recurrent rami of the centripetal arteries).
Other findings
Evaluate and measure dilated rete testis (three diameters).
Evaluate and measure parenchymal cysts (major diameter).
Evaluate and measure testis appendices (longitudinal diameter).
Evaluate and measure (major diameter) extratesticular calcifications (including scrotoliths).
Evaluate and measure hydrocele (three diameters and volume); use convex probe when bulky.
Epididymis and vas deferens
Evaluate the CDUS features of the three epididymal segments (head, body, and tail) and vas deferens
Size (diameters)
Head: measure the longitudinal diameter from the top to the base of the triangle
Body and tail: measure the anterior-posterior diameters in a single longitudinal scan (if possible, including the proximal vas deferens)
Vas deferens: evaluate presence or absence. Measure the anterior-posterior diameter (if possible, in the same longitudinal scan with epididymal body and tail)
Homogeneity/inhomogeneity
Report it as a dummy variable (0, homogeneous; 1, inhomogeneous),
Echogenicity
Use a three-point Likert scale (0, normal echogenicity; 1, mainly hypoechoic; 2, mainly hyperechoic)
Vascularization
Qualitative assessment: normal, reduced, enhanced; compare the two epididymis

(Continues)

TABLE 1 (Continued)

Epididymis and vas deferens
Quantitative assessment: evaluate arterial PSV, acceleration, RI, and PI at the level of the head (branch of the testicular artery) and of the tail (branch of the deferential artery)
Other findings
Evaluate the presence of nodules (in the same way of "testicular nodules")
Evaluate the presence and number of cysts and the three diameters of the major cyst for each segment
Evaluate and measure epididymal calcifications (major diameter).
Evaluate and measure epididymal appendices (longitudinal diameter).
Pampiniform plexus/varicocoele
Measure the largest vein, irrespective of location, with the patient standing, at rest, bilaterally.
Evaluate the extension of the largest vein to the funicular region, upper or lower pole of the testis.
Evaluate the presence of a retrograde venous flow the patient standing, at rest, using CDUS, and classify it as a dummy variable (0: absent or intermittent/fluctuating during spontaneous breath; 1: continuous).
Then evaluate the presence of a retrograde venous flow during Valsalva maneuver.
CDUS varicocoele is defined in presence of venous vessels >3 mm at rest, with retrograde venous flow detected at least during Valsalva maneuver.
Use Sarteschi et al./Liguori et al. classifications for grading varicocoele.
"Severe" varicocoele: venous vessels dilation (>3 mm) characterized by a continuous venous reflux at rest, increasing or not during a Valsalva maneuver (consistent with grade 4 and 5 of Sarteschi et al./Liguori et al. classifications)
Subclinical varicocoele: venous reflux detected by CDUS but not clinically evident
EAA classification of varicocoele
• Grade 1: venous vessels dilation (>3 mm) at rest at the funicular region with retrograde venous flow absent/intermittent at rest and enhanced during Valsalva maneuver. • Grade 2: venous vessels dilation (>3 mm) at rest at the upper pole of the testis with retrograde venous flow absent/intermittent at rest and enhanced during Valsalva maneuver. • Grade 3: venous vessels dilation (>3 mm) at rest at the lower pole of the testis with retrograde venous flow absent/intermittent at rest and enhanced during Valsalva maneuver. • Grade 4: venous vessels dilation (>3 mm) at rest (irrespective of location, but usually extending to the peritesticular region) with retrograde venous flow *continuous* at rest and enhanced during Valsalva maneuver.
Possible testicular hypotrophy
• Grade 5: venous vessels dilation (>3 mm) at rest (irrespective of location, but usually extending to the peritesticular region) with retrograde venous flow *continuous* at rest and not increasing during Valsalva maneuver.
Possible intratesticular varices and/or testicular hypotrophy

The EAA SOPs are derived from the EAA scrotal US study.[24] See also https://www.andrologyacademy.net/eaa-studies.[22]
Abbreviations: PSV, peak systolic velocity; RI, resistive index; PI, pulsatility index.
Source: Adapted from reference [2].

the 95th percentiles of its distribution.[24,25,28] All statistical analysis was performed on SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) for Windows 26.0.

3 | RESULTS

Overall, 248 healthy, fertile men (35.3 ± 5.9 years; range 23–53) were enrolled in the EAA US study from February 2016 to February 2019.[23] All subjects underwent scrotal CDUS[24] and 188 men underwent TRUS[25] before and after ejaculation.

The socio-demographic, clinical, seminal, and biochemical characteristics of the sample have been extensively reported in previous studies.[23,25] In particular, the cohort studied showed semen parameters consistent with those reported by the World Health Organization (WHO, 2010),[29] representing a valid reference point for assessing MGT-CDUS normative parameters.[23]

3.1 | SOPs to assess MGT-CDUS parameters

Tables 1 and 2 summarize the SOPs used to assess scrotal (Table 1) and transrectal (Table 2) CDUS qualitative and quantitative parameters.

3.2 | Intra- and inter-operator comparability of MGT-CDUS parameters

The intra- and inter-operator comparability of the main MGT-CDUS parameters have been reported in previous studies.[24,25] The

TABLE 2 EAA standard operating procedures (SOPs) to assess prostate-vesicular CDUS

Prostate
Prostate volume
Evaluate the three maximum diameters (anterior–posterior and transverse diameters in transverse scan; longitudinal diameter in longitudinal scan) of the prostate and, if present, of enlarged transitional zone or adenoma
Calculate prostate (and adenoma) volume using the ellipsoid formula (length × height × width × 0.52)
Report whether adenoma lifts the bladder floor (median lobe) and by how much
Prostate symmetry
Prostate symmetry should be evaluated, and classified as 0. symmetric, 1. asymmetric (report the biggest lobe)
Prostate homogeneity
It should be classified as: 0. homogeneity; 1: mild (grade 1) inhomogeneity [presence of small hypo- or hyper-echoic foci]; 2: moderate (grade 2) inhomogeneity [presence of large hypo- or hyper-echoic areas]; 3: severe (grade 3) inhomogeneity [diffuse inhomogeneity with "geographical map" appearance]
Prostate echogenicity
It should be classified as: 0: normal echogenicity; 1: mainly hypoechoic/presence of large hypoechoic areas; 2: mainly hyperechoic/presence of large hyperechoic areas; 3: mixed [diffuse hypo- and hyper-echoic areas]
Calcifications
Macrocalcifications: calcifications with a size >3 mm; microcalcifications: small (<3 mm)
Report a. the type of calcification (micro- or macro-), b. their localization (transitional zone/peri-urethral; peri-transitional/surgical capsule; right lobe; left lobe; peripheral) and if they are a cluster or isolated. The major calcification should be measured (three diameters: lateral–lateral, anterior–posterior, and longitudinal)
Midline prostatic cyst
Evaluate the three diameters and volume (ellipsoid formula)
Prostate arterial vascularization
It should be evaluated before ejaculation, to avoid the bias because of its increase after ejaculation
Qualitative assessment: normal, reduced, enhanced (hyperemia)
Quantitative assessment: evaluate arterial PSV, acceleration, RI, and PI in the transitional zone (or adenoma)
Prostatic venous plexus
Measure the maximum anterior–posterior diameter in longitudinal scan, and eventually blood flow velocity
Prostate peripheral nodules/equivocal areas
Evaluate, measure, and report the presence of peripheral nodules / equivocal areas
Other findings
Evaluate and measure parenchymal cysts (major diameter).
Ejaculatory ducts (EDs)
EDs characteristics should be evaluated after ejaculation, to better emphasize indirect US signs of obstruction
EDs abnormalities (dilation, calcifications, cysts) should be reported and classified as: 0, absent; 1, uni-; or 2, bilateral
When dilated, ED anterior–posterior diameter should be measured
Seminal vesicles (SV)
Absence and/or abnormalities of one or both SV must be reported. In subjects with oligo/azoospermia and/or low seminal volume (and pH), evaluation of SV before and after ejaculation (with a standard sexual abstinence of 4 days) can be useful to evaluate indirect US signs of obstruction (see below "SVEF"). SV should be studied after ejaculation to better emphasize indirect US signs of obstruction.
Diameters and volume
The maximum longitudinal diameter (from the "SV pole" to the insertion in the prostate) and the maximum anterior–posterior diameter of the fundus should be measured, SV volume, before and after ejaculation, should be calculated using the "ellipsoid/prolate (d1 > d2 = d3) spheroid" mathematical formula (d1 × d2 × d3 × 0.52, considering d1 = 1/2 the maximum SV-longitudinal diameter, d2 = 1/2 the maximum anterior–posterior diameter and d3 = d2). Report SV dilation or hypoplasia, according to reference ranges.
SV ejection fraction (SVEF)
SVEF can be calculated as "[(total SV volume before ejaculation−total SV volume after ejaculation)/total SV volume before ejaculation] × 100". A SVEF < 21.6% suggests distal partial or complete obstruction

(Continues)

TABLE 2 (Continued)

Seminal vesicles (SV)
SV homogeneity/inhomogeneity
SV homogeneity should be classified as a dichotomous variable (0. homogeneous; 1. inhomogeneous)
SV echogenicity
SV echogenicity should be classified as: 0, normal echogenicity; 1, mainly hypoechoic/with hypoechoic areas; 2, mainly hyperechoic/with hyperechoic areas; 3, mixed: areas of hypo- and hyper-echogenicity
SV vascularization
Qualitative (report hyperemia) and quantitative (arterial PSV, acceleration, RI, PI in peripheral Doppler spots) study
SV abnormal findings
SV US abnormalities should be reported and classified as: 0, absent; 1, uni-; or 2, bilateral: areas of endocapsulation/roundish anechoic areas; wall thickening and septa; calcifications; giant cyst.
Deferential ampullas/ distal vas deferens
Their 0, presence or 1, absence must be reported. Their anterior–posterior diameter must be measured. Report dilation.

The EAA SOPs are derived from the EAA TRUS study.[25] See also https://www.andrologyacademy.net/eaa-studies.[22]
Abbreviations: PSV, peak systolic velocity; RI, resistive index; PI, pulsatility index.

coefficient of variation for quantitative parameters was < 10 for all parameters and the concordance rate between operators for qualitative parameters was 83%–100%.[24,25]

3.3 | Reference ranges of MGT-CDUS parameters

Tables 3 and 4 show the reference ranges of the main scrotal (Table 3) and transrectal (Table 4) CDUS quantitative parameters as well as the prevalence of the main MGT-CDUS echotexture abnormalities.

3.4 | Comparison of previously published and EAA US study-derived normal values, cut-off, and classifications of the main MGT-CDUS parameters

Table 5 summarizes the previously published and EAA US study-derived normal values, cut-off, and classifications of the main MGT-CDUS parameters / characteristics.

4 | DISCUSSION

The EAA US study is the first study which standardized the SOPs to evaluate by CDUS the entire human MGT and assessed the reference range, the echotexture, and vascular characteristics of the organs of the scrotal and prostate-vesicular regions in a reference cohort of healthy, fertile men.[23–25]

The EAA SOPs for the assessment of MGT-CDUS parameters[23–25] are summarized here. The careful methodological workout and the agreement reached by the different EAA centers led to high inter- and intra-operator comparability,[24,25] expressed by a low coefficient of variation (<10)[27] and a high concordance rate for quantitative and qualitative MGT-CDUS parameters, respectively, according to the National Association of Testing Authorities.[26] Following the EAA SOPs in clinical practice will help in reducing the operator-dependent differences among sonographers. In addition, the use of different US equipment in different EAA centers makes the study results applicable to the clinical reality of any sonographer.

The EAA US study reported the reference range of US-derived testicular volume (TV) according to different mathematical formulas.[24] Using the ellipsoid formula, a mean TV of ~17 mL was found in healthy, fertile men. Previous studies using the ellipsoid formula reported a median TV of ~14 mL in healthy men[30–32] and a mean TV of ~15 mL and ~19 mL in young[33] and fertile[12,34,35] men, respectively. Conversely, infertile patients have a lower US-TV, ranging from ~10 mL to ~15 mL.[12,21,34,36] In the EAA US study, the lower limit for right and left testis was 12 and 11 mL, respectively, allowing to define "testicular hypotrophy" in an evidence-based way. Previous studies defined testicular hypotrophy for a TV < 12 mL[37,38] or < 10 mL,[39,40] however, with no evidence. TV reflects the sperm production and hormonal status of the subject as well as the presence of previous or current testicular or systemic disorders.[1,41,42] Hence, the availability of evidence-based US-TV thresholds represents an essential tool in andrological clinical practice.

In the EAA US study, most of the subjects evaluated showed testicular echotexture homogeneity, and when inhomogeneity was found it was of mild degree.[24] On the other hand, infertile men frequently show testicular inhomogeneity (TI).[1,2] TI has been associated with testicular function impairment[31,43–46] and several pathological conditions.[1] The fact that TI is virtually absent in healthy, fertile men points out that it is an US characteristic associated with testicular dysfunction, and ennobles US as a diagnostic tool useful to find out infertility-related findings not detectable with physical examination. TI was previously classified on a five-point scale by Lenz et al.[31] and Westlander et al.[47] The EAA US consortium proposed a new, four-point scale classification, easy to use in clinical practice.[2,24]

TABLE 3 Reference range and mean/median values and percentages of the scrotal organs color-Doppler ultrasound (CDUS) parameters in healthy, fertile men

Testis main CDUS parameters ($n = 248$)	Mean/median values and percentages	Reference range
Testicular volume (mL) ("ellipsoid" mathematical formula)		
Mean	17.2 ± 4.1	11.8–24.4
Right	17.9 ± 4.4	12.0–25.7
Left	16.5 ± 4.1	11.0–24.1
Testicular homogeneity (%)		
Homogeneous (grade 0)	97.2	
Mild inhomogeneity (grade 1)	2.8	
Moderate inhomogeneity (grade 2)	0.0	
Severe inhomogeneity (grade 3)	0.0	
Testis echogenicity (%)		
Normoechoic	97.2	
Hypoechoic	2.8	
Hyperechoic	0.0	
Testicular macro-calcifications (> 3 mm) (%)	1.2	
Testicular micro-calcifications (1-3 mm) (%) ^	16.8	
Testicular microlithiasis (%)	0.0	
Dilated rete testis (%)	2.0	
Parenchymal cysts (%)	1.2	
Hypoechoic micronodular lesion (spermatocele) (%)	0.4	
Testicular artery mean PSV (cm/s)	9.0 ± 3.0	3.0–11.0
Intratesticular arteries mean PSV (cm/s)	5.7 ± 1.1	3.7–7.0
Epididymal and vas deferens main CDUS parameters		
Epididymal head diameter (including men with cysts) (mm)	9.5 ± 1.5	6.9–12.0
Epididymal head diameter (excluding men with cysts) (mm)	9.0 ± 1.5	7.0–11.5
Epididymal body diameter (mm)	3.8 ± 0.8	2.5–5.0
Epididymal tail diameter (mm)	4.8 ± 0.7	4.0–6.0
Vas deferens diameter (mm)	3.5 ± 0.7	2.3–4.5
Epididymal head echotexture inhomogeneity (%)	25.0	
Epididymal head cysts (%)	30.0	
Epididymal tail echotexture inhomogeneity (%)	19.6	
Epididymal body or tail, or vas deferens cysts (%)	0.0	
Epididymal head mean PSV (cm/s)	4.2 ± 0.6	3.1–4.6
Epididymal tail mean PSV (cm/s)	5.5 ± 1.6	1.8–8.0
Epididymal hyperaemia (%)	0.8	
Varicocoele / pampiniform plexus main CDUS parameters		
Left side varicocoele (%)	37.2	
Grade I	0.0	
Grade II	12.8	
Grade III	6.0	
Grade IV	16.0	
Grade V	2.4	
Right side varicocoele (%)	3.2	
Bilateral varicocoele (%)	3.2	
"Severe" varicocoele * (grade IV and V **) (%)	18.4	
Continuous venous reflux velocity (cm/s) in "severe" varicocoele at rest	4.7 ± 2.2	2.0–10.0

Data are expressed as mean ± SD when normally distributed, as medians (quartiles) for parameters with non-normal distribution, and as percentages when categorical. The reference range of each testicular parameter has been estimated according to the CLSI Guidelines[28] as the 5th and the 95th percentiles of its distribution.

Abbreviations: US, ultrasound; PSV, peak systolic velocity.

^ Hyperemia was defined as a "diffuse enhanced vascularization."

*"Severe" varicocoele was defined as venous vessel dilation (> 3 mm) characterized by a continuous venous reflux at rest, increasing or not during a Valsalva maneuver.[2,24.]

**Grade IV and V CDUS varicocoele according to Sarteschi et al./Liguori et al. classifications.[2,24]

Source: Adapted from reference [24].

The EAA US study did not reveal testicular microlithiasis or nodular lesions in healthy, fertile men. Currently, the association between infertility and testicular microlithiasis is debated,[19,48–50] while that with testicular malignancy is consolidating over time.[2] In this scenery, the EAA US study suggests a possible relationship between male infertility and testicular microlithiasis (the latter to be considered, at least, an epiphenomenon of spermatogenesis derangement), and supports an increased risk of testicular tumor in infertile men.[51,52]

The EAA US study reported, for the first time, the reference range of several blood flow parameters related to testicular arteries. Increased testicular vascularization represents a qualitative sign suggestive of orchitis[53–55] or some hematological tumors.[55] On the other hand, the research in the US field is trying to find out testicular

TABLE 4 Reference range and mean or median values and percentages of the TRUS-related color-Doppler ultrasound (CDUS) parameters in healthy, fertile men

Prostate CDUS parameters	Mean or median values and percentages	Reference range
Volume (mL)	25.0 ± 6.3	15.0–35.0
Diameters (mm)		
Transversal (td)	45.0 ± 4.4	38.0–52.5
Anterior–posterior (apd)	25.5 ± 3.7	18.0–31.0
Longitudinal (ld)	42.0 ± 4.3	34.0–49.0
Asymmetry (%)	0.0	
Homogeneity (%)		
Homogeneous (grade 0)	65.4	
Mild inhomogeneity (grade 1)	29.8	
Moderate inhomogeneity (grade 2)	4.8	
Severe inhomogeneity (grade 3)	0.0	
Echogenicity (%)		
Normoechoic	87.8	
Mainly hypoechoic	6.4	
Mainly hyperechoic	0.5	
Mixed	5.3	
Calcifications (%)	42.5	
Micro-calcifications (1–3 mm) (%)	9.0	
Macro-calcifications (>3 mm)** (%)	33.5	
Major calcification diameter (mm)	7.5 [4.2–12.0]	3.0–18.0
Midline prostatic cyst (%)	5.0	
Transversal diameter (mm)	4.0 [3.25–4.75]	3.0–5.0
Anterior-posterior diameter (mm)	3.0 [2.25–4.75]	2.0–6.0
Longitudinal diameter (mm)	6.0 [4.0–7.5]	4.0–9.0
Volume (mL)	0.038 [0.026–0.069]	0.012–0.117
Parenchymal cysts (%)	3.2	
Ejaculatory ducts		
Dilation (>2 mm)	0.5	
Cysts	0.0	
Micro-calcifications	0.0	
Peripheral nodules	0.0	
Transitional arteries mean PSV (cm/s) before ejaculation	8.3 ± 1.8	5.0–11.0
Hyperaemia (%) before ejaculation	0.5	
Periprostatic venous plexus size (mm) before ejaculation	2.9 ± 0.9	1.5–4.5
Periprostatic venous plexus flux velocity (cm/s) before ejaculation	3.8 ± 1.4	2.0–7.0
Transitional arteries mean PSV (cm/s) after ejaculation	9.8 ± 1.9	6.5–13.0
Hyperaemia (%) after ejaculation	0.5	
Periprostatic venous plexus size (mm) after ejaculation	3.0 ± 0.9	1.7–4.6
Periprostatic venous plexus flux velocity (cm/s) after ejaculation	5.0 ± 1.4	3.0–8.0
Seminal vesicles (SV) CDUS parameters		
Before ejaculation		
Mean SV ld (mm)	48.1 ± 5.4	40.0–56.0
Mean SV apd (mm)	12.5 ± 3.5	8.0–18.0
Median SV volume (mL)	3.4 [2.1–5.8]	1.4–9.0
Total SV volume (mL)	6.7 [4.3–11.6]	3.0–18.0
SV arteries mean PSV (cm/s)	6.4 ± 1.3	4.0–9.0
After ejaculation		
Mean SV ld (mm)	44.9 ± 5.4	37.0–53.0
Mean SV apd (mm)	9.8 ± 3.3	6.0–16.0
Median SV volume (mL)	1.9 [1.1–3.5]	0.6–6.0
Total SV volume (mL)	3.8 [2.3–7.0]	1.2–12.0
SV arteries mean PSV (cm/s)	6.6 ± 1.3	4.4–9.5
Delta SV and SVEF		
Delta SV ld (mm)	3.3 ± 1.4	2.0–6.3
Delta SV apd (mm)	2.7 ± 1.0	2.0–4.8
Delta SV total volume (DSTV) (mL)	3.1 [2.0–4.4]	1.3–6.4
SV total ejection fraction (SVEF) (%)	43.2 [35.0–52.0]	20.0–58.0
Echotexture abnormalities		
Inhomogeneity (%)		
Before ejaculation	34.2	
After ejaculation	16.7	
Roundish anechoic areas/areas of endocapsulation		
Before ejaculation	16.4	
After ejaculation	8.3	
Wall/thickened septa (%)	3.6	
Calcifications (%)	0.0	

(Continues)

TABLE 4 (Continued)

Prostate CDUS parameters	Mean or median values and percentages	Reference range
Giant cysts (%)	0.0	
Deferential ampullas mean size (mm)	4.4 ± 0.6	3.5–6.0

Data are expressed as mean ± SD when normally distributed, as medians (quartiles) for parameters with non-normal distribution, and as percentages when categorical. The reference range of each parameter has been estimated according to the CLSI guidelines[28] as the 5th and the 95th percentiles of its distribution.

Abbreviations: PSV, peak systolic velocity; SV, seminal vesicles; ld, longitudinal diameter; apd, anterior–posterior diameter.

Source: Adapted from reference [25].

vascular predictors of positive surgical sperm retrieval in men with non-obstructive azoospermia.[1,2] In this scenery, the availability of normative quantitative testicular vascular parameters could help to better define differential diagnosis between normal and pathological conditions and, maybe, in surgical sperm retrieval prognosis.

The EAA US study investigated the reference range of epididymal segments and vas deferens, reporting an upper limit for epididymal head, body, and tail of 11.5, 5, and 6 mm, respectively, and for proximal vas deferens of 4.5 mm. Previously, some authors proposed as abnormal an epididymal head > ~11–12 mm[56–58] and a tail > 6 mm,[57,58] considered suggestive of inflammation and/or obstruction, while vas deferens thresholds have never been reported. Epididymis and vas deferens US reference ranges can increase the diagnostic accuracy of proximal or distal obstruction (along with other prostate-vesicular US signs) and of epididymal inflammation.[1,2] In addition, the EAA US study reports the reference range for epididymal arterial-related parameters. Previously, some authors[53,55] reported that epididymal hyperemia, a qualitative parameter, could indicate inflammation. Now, the availability of EAA quantitative vascular normative values can help in ameliorating the diagnosis of epididymal inflammation. Finally, the EAA US study found epididymal cysts or inhomogeneity in one out of four fertile men, suggesting their scanty role in male infertility.[1,2]

The EAA US study reported the reference range of pampiniform plexus and the prevalence and characteristics of varicocoele in fertile men.[24] The study found a high frequency of varicocoele (~37%) in fertile men, similar to that of men with primary infertility,[3,4] suggesting its scanty effect on male fertility and prompting to limit varicocoele surgical correction to highly selected populations. Accordingly, the European Association of Urology[8] supports specific indications for varicocoele treatment. Of note, recently the EAA published its own varicocoele classification,[2] resembling that of the European Society of Urogenital Radiology.[3]

Regarding TRUS, the EAA US study reported a mean prostate volume (PV) of ~25 mL, with a lower and upper limit of 15 and 35 mL, respectively,[25] defining, in men of reproductive age, a small (< 15 mL) or enlarged (> 35 mL) prostate. Previous studies suggested a PV > 30 mL[59] and >60 mL[60] as indicative of an initial and severe prostate enlargement, respectively. In addition, the EAA US study derived a mathematical formula (1/3 age + 15) to calculate, in young-adult men, the age-adjusted normative mean PV,[25] useful in clinical practice to derive the expected average PV by age.

Regarding prostate arterial vascular parameters, the EAA study found in healthy, fertile men an upper limit of prostatic arterial peak systolic velocity (PSV) of 11 cm/s.[25] Previous studies found that a PSV > 11 cm/s identifies men with prostatitis-like symptoms, indicating current prostate inflammation.[13,61] Hence, in young-adult men, a prostatic arterial PSV < 11 cm/s can be considered "normal," while higher values indicate current prostate inflammation. Of note, to standardize the use of prostatic arterial PSV to assess inflammation, it must be measured before ejaculation. In fact, the EAA US study found that PSV increases significantly after ejaculation,[25] in line with a previous report.[62]

Evaluating prostate US abnormalities, calcifications and inhomogeneity were found in ~43% and ~33% of subjects, respectively.[25] Previous studies attributed these findings to chronic prostate inflammation or inflammatory outcomes.[1] Some authors suggested that prostate inflammation could be associated with poor seminal parameters[63] and male infertility,[64] however this issue is controversial.[13,63,65] So far, the detection of the aforementioned prostate US abnormalities plays a modest role in the clinical management of male infertility.[1] The EAA US study, reporting a high frequency of prostate calcifications and inhomogeneity in healthy, fertile men, supports the latter vision, suggesting that these findings have a marginal impact on male fertility.

In the EAA US study, no ejaculatory duct abnormalities were found in fertile men, supporting their negative role on male fertility.[66–68] Furthermore, midline prostatic cysts (MPC) were rare (5%) and small (volume < 0.117 mL and transversal diameter < 5 mm).[25] A previous study[12] reported in men with a severe infertility factor a higher prevalence of MPC (up to 15%) and a larger size than those observed in fertile men. In particular, a MPC volume > 0.117 mL identified men with severe oligo- or azoospermia with good accuracy, and almost half of these patients had a volume > 0.250 mL (transversal diameter > 1 cm).[12] Hence, small MPC (< 0.117 mL) seem to exert no negative impact on male fertility,[25] while larger MPC, frequent in infertile men, can lead to oligo- or azoospermia.[12] MPC must be investigated during infertility work-up as they represent a treatable cause of obstructive infertility. In fact, in males of infertile couples with oligo-/azoospermia and low semen volume, the transrectal aspiration of MPC, especially when large and when FSH is in the normal range (< 8 U/L), can restore a good semen quality and eventually lead to natural pregnancy.[12]

The EAA US study reported, for the first time, the reference range of the periprostatic venous plexus, identifying an evidence-based upper limit of 4.5 mm. Some authors previously suggested to define periprostatic venous plexus dilation as > 3 mm[69] or >4 mm,[70] however with no evidence.

Evaluating the seminal vesicles (SV), the EAA US study reported, for the first time, evidence-based upper and lower limits of their diameters and volume before and after ejaculation, and criteria to define SV asymmetry.[25] Regarding SV diameters, the upper and lower limits of

TABLE 5 EAA US study derived and previously published normal values, cut-off and classifications of the main MGT-CDUS parameters/characteristics

	Previously proposed normal values, cut-off and classifications at CDUS	EAA US study normal values, cut-off and classifications at CDUS
Testis		
Mean TV (ellipsoid)	From 14 to 19 mL in different studies	17 mL
Right TV	≥12 mL	≥12 mL (when < 12 mL: hypotrophy)
Left TV	≥12 mL	≥11 mL (when < 11 mL: hypotrophy)
TI classification	*Lenz et al. (1993)*[31]	*EAA US study*
	1. Very uniform pattern	0. Homogeneity
	2. Slightly irregular pattern	1. Mild inhomogeneity (presence of small hypoechoic foci/thin hypoechoic striae)
	3. Moderately irregular pattern or small echogenic points	2. Moderate inhomogeneity (presence of thick hypoechoic striae)
	4. Very irregular pattern or bright echogenic spots	3. Severe inhomogeneity (diffuse TI with "netting"/"geographical map" appearance)
	5. Tumor suspected (demarcated area)	
	Westander et al. (2001)[47]	
	1. Homogeneous	
	2. Homogeneous with some hyperechogenic foci	
	3. Heterogeneous with spread hyperechogenicity	
	4. Heterogeneous with both hyperechogenic and cystic (hypoechogenic) parenchyma	
	5. Post-operative intratesticular lesion	
TML (most used definitions)	≥5 Microcalcifications per field of view	≥5 Microcalcifications per field of view
	≥5 Microcalcifications in the whole testis	
Vascularization	Normal, reduced or enhanced	Testicular artery PSV^: 3–11 cm/s
		Intratesticular arteries PSV^: 3.7–7 cm/s
Epididymis and vas deferens		
Head diameter	≤12 mm	≤12 mm and ≤11.5 mm in men with and w/o cysts
Body diameter	≤4 mm	≤5 mm
Tail diameter	≤6 mm	≤6 mm
Vas deferens diameter	Not reported	≤4.5 mm
	Higher values suggestive of inflammation/obstruction	Higher values suggestive of inflammation/obstruction
Inhomogeneity	Homogeneous or inhomogeneous	EAA US study
		0. Homogeneity
		1. Inhomogeneity
Echogenicity	Normoechoic, hypoechoic, hyperechoic	EAA US study
		0. Normal echogenicity
		1. Mainly hypoechoic
		2. Mainly hyperechoic
Vascularization	Normal or enhanced	Head artery PSV^: 3.1–4.6 cm/s
		Tail artery PSV^: 1.8–8.0 cm/s
Varicocoele	Several classifications*	Venous vessels >3 mm at rest, irrespective of location, with retrograde venous flow detected at least during Valsalva maneuver, with grading according to Sarteschi et al. /Liguori et al.

(Continues)

TABLE 5 (Continued)

	Previously proposed normal values, cut-off and classifications at CDUS	EAA US study normal values, cut-off and classifications at CDUS
Prostate		
Volume	Normal: 20–25 mL. Enlarged: > 30 mL Severe enlargement: > 60 mL	Normal: reference range 15–35 mL (when <15 mL or >35 mL suggestive of hypertrophy or hyperplasia, respectively)
Inflammation	Hyperemia: qualitative Hyperemia: semiquantitative[1]	Normal arterial prostatic PSV^ < 11 cm/s (young adults) (when higher suggestive of inflammation)
Midline prostatic cyst	No cut-off for EDs obstruction	Normal volume < 0.117 (when higher, especially when >0.250 mL with normal FSH, suggestive of partial and complete obstruction)
Ejaculatory ducts (EDs)	Dilated: >2 mm	Normal: <2 mm (when >2 mm suggestive of obstruction)
Periprostatic venous plexus	Dilated: >3 or 4 mm (not evidence based)	Normal: reference range 1.5–4.5 mm
Seminal vesicles (SV)		
Diameters	Dilation: SV apd > 15 mm	Before ejaculation • apd: reference range 8–18 mm • ld: reference range 40–56 mm
	Hypoplasia: SV apd < 5 or < 7 mm and/or SV longitudinal d < 25 mm	After ejaculation • apd: reference range 6–16 mm (< 6 mm suggestive of hypoplasia; > 16 mm suggestive of dilation) • ld: reference range 37–53 mm
Volume	No cut-off for dilation or hypoplasia	Before ejaculation: reference range 1.4–9 mL After ejaculation: reference range 0.6–6 mL
SVEF	SVEF < 21.6% suggestive of distal obstruction	Normal SVEF > 20.0% (when < 20.0% suggestive of partial and complete obstruction)
Deferential ampullas		
Diameter	Normal apd < 6 mm	apd: reference range 3.5–6.0 mm (when > 6 mm suggestive of partial and complete obstruction)

Of note, we report here the main findings of the EAA US study. In the original articles[24,25] normative values/reference ranges of all the scrotal organs CDUS parameters have been reported extensively.

^Along with peak systolic velocity (PSV) reference range, the EAA US study reports normative values for acceleration, pulsatility and resistive index in different MGT organs. For a detailed description of "previously published normal values," see the main text.

Abbreviations: TV, testicular volume; TI, testicular inhomogeneity; SVEF, seminal vesicles ejection fraction; ld, longitudinal diameter; apd, anterior–posterior diameter.

the mean SV anterior-posterior diameter, often used in literature to define the cut-off for SV dilation[57,67,68] or hypotrophy,[57,71,72] respectively, were 16 and 6 mm after ejaculation. Previous studies proposed an SV anterior–posterior diameter > 14 mm[57] or >15 mm[67,68] to indicate SV dilation, suggestive of partial or complete ejaculatory duct obstruction. On the other hand, some authors proposed a SV anterior-posterior diameter < 7 mm[57] or <5 mm[71] to indicate SV hypotrophy, or a longitudinal diameter < 25 mm.[72] Conversely, the EAA US study identified a longitudinal diameter threshold of 36 mm.[25] Regarding SV volume, the upper and lower limits of a single SV "after ejaculation" were 0.6 and 6 mL, thresholds that could be used to define SV hypotrophy or dilation, respectively.[25]

Evaluating the SV before and after ejaculation, the normative "delta SV total volume" (SV total volume before ejaculation – SV total volume after ejaculation) was reported, and the lower limit of "delta" SV longitudinal and anterior–posterior diameters was 2 mm. Hence, the normal SV emptying with ejaculation can be defined by a reduction in the SV diameters of at least 2 mm, introducing an evidence-based, easy to use, parameter in US clinical practice. In addition, the EAA US study evaluated another parameter, the "SV ejection fraction" (SVEF).[25] Previous studies[1,14] reported, in infertile men, that a SVEF < 21.6% identifies subjects with reduced seminal volume (< 1.5 mL) and pH (< 7.2), representing a useful indicator of ejaculatory ducts sub-obstruction. The lower SVEF limit observed in the EAA US study was 20%,[25] similar to that reported above in infertile men with distal sub-obstruction.[1,14] The EAA US study reported also a deferential ampulla upper limit of 6 mm, in line with previous reports,[1,14] suggesting that a larger size can represent another indicator of distal sub-obstruction.

The EAA US study evaluated also SV-US abnormalities. The study found[25] that the detection of "roundish anechoic areas,"[1] observed before ejaculation in one out of six men, was halved after ejaculation. Similar figures were previously observed in infertile men,[14] which,

however, showed a frequency of these areas double[14] than that of fertile men.[25] The EAA and previous studies suggest that these findings represent liquid areas expelled from the SV with ejaculation, and that when present in the SV after ejaculation may indicate incomplete SV emptying.[25] Previous studies reported that anechoic areas can indicate SV stasis[14,66] and/or chronic inflammation.[14,57,58] The EAA US study agrees with this vision, however suggesting to asses these areas after ejaculation, to avoid their overestimation and an excessive diagnosis of pathology. The EAA US study[25] also observed, in healthy, fertile men, rare SV thickened septa, usually associated with chronic SV inflammation[14,57,58] and no SV giant cysts, which conversely are frequent in men with genitourinary abnormalities.[1,14]

5 | CONCLUSIONS

The EAA US study, for the first time, standardized the SOPs to evaluate with US the organs of the entire human MGT and assessed, in a multinational cohort of healthy, fertile men, the reference ranges and characteristics of scrotal and transrectal CDUS parameters.[24,25] The findings of the EAA US study represent a new milestone in the uro-andrological and radiological fields, and can help in clinical practice to reduce the operator-dependent differences among sonographers, define normal and pathologic CDUS characteristics, better understanding the significance attributed to specific MGT-CDUS findings, and the relationship between abnormal CDUS parameters and male reproductive and general health.

ACKNOWLEDGMENTS

Open Access Funding provided by Universita degli Studi di Firenze within the CRUI-CARE Agreement.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Francesco Lotti made substantial contribution to the conception and design of the manuscript, analysis and interpretation of data, and drafting the manuscript and the tables. All the authors revised the manuscript for intellectual content. All the authors gave final approval of the submitted version of the manuscript.

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How to cite this article: Lotti F, Frizza F, Balercia G, et al. The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: An overview on male genital tract ultrasound reference ranges. *Andrology*. 2022;10(Suppl. 2):118–132. https://doi.org/10.1111/andr.13260