Standardization of male genital tract colour-Doppler ultrasound parameters in healthy, fertile men.

Colour-Doppler ultrasound evaluation

All patients should undergo scrotal and, when possible, transrectal colour-Doppler ultrasonography (CDU), before and after ejaculation.

All the CDU parameters considered should be evaluated.

A standardized schedule is attached and must be entirely filled in **(Appendix C).**

Sexual abstinence must be reported and expressed in “days”.

In order to standardize the method, we suggest 4 days of sexual abstinence.

1.Scrotal CDU

- 1.1 Testis
- 1.2 Epididymis and proximal vas deferens
- 1.3 Pampiniform plexus

2.Transrectal CDU

- 2.1 Prostate
- 2.2 Ejaculatory ducts and deferential ampullas
- 2.3 Seminal vesicles

1.Scrotal CDU

Scrotal CDU should be performed systematically in various longitudinal, transverse and oblique scans with the patient lying in a supine position using a 7.5 MHz high-frequency linear probe (6-13 MHz) (Behre et al., 1995; Vicari, 1999; Isidori & Lenzi, 2008; Lotti et al., 2011).

1.1 Testis

Testicular **diameters** must be reported (anterior-posterior and lateral-medial diameters in transverse scan; longitudinal diameter in longitudinal scan) (Fig. 1). Much attention must be paid impressing the right pressure to the ultrasound probe. If testicular longitudinal diameter is longer than the probe, and one or both testis poles not entirely visible, this diameter should be measured with a convex probe. Testicular volume will be evaluated at a later time using different mathematical formulas (ellipsoid, Lambert's formula, prolate spheroid) (Pilatz et al., 2012).

Testicular **homogeneity** should be classified on a 4 point scale (0. Homogeneity; 1. Mild inhomogeneity/little hypoechoic areas or thin hypoechoic striae; 2. Moderate inhomogeneity/thick hypoechoic striae; 3.Severe inhomogeneity / netting or geographical map appearance) (Fig. 2).

Testicular **echogenicity** should be classified on a 3 point scale (0. Normoechoic; 1. Mainly hypoechoic; 2. Mainly hyperechoic) (Fig. 3).

The presence of testicular **calcifications** must be reported, and their size measured. The maximum diameter of the major calcification should be reported. Macro-calcifications are defined as calcifications with size > 3 mm, according to previous reports (Dagash & MacKinnon, 2007; Isidori & Lenzi, 2008). The "maximum number of calcifications/ultrasound field" should be reported (Fig. 4A). When observed, a "diffuse pattern" should be reported (Fig. 4B). The localization of the calcifications should be reported, dividing the testis in three virtual areas: upper third, middle third, lower third (Fig. 4C).

The presence of testicular **cysts** must be reported, as well as their localization, and the maximum diameter of the major cyst should be measured (Fig. 5A).

Dilated rete testis should be reported, and three diameters measured (anterior-posterior and lateral-lateral in transverse scan; longitudinal in longitudinal scan) (Fig. 5B).

The presence of testicular **nodules** must be reported, and three diameters measured (anterior-posterior and lateral-lateral in transverse scan; longitudinal in longitudinal scan). Nodules homogeneity must be reported (0. Homogeneous, 1. Inhomogeneous/with cysts), as well as echogenicity (0. Normal echogenicity, 1. Mainly hypoechoic, 2. Mainly hyperechoic), calcifications (present/absent), shape (regular/irregular) and vascularization (0. Absent, 1.peripheral/"basket", 2.intranodular) (Fig. 6).

Testicular **vascularization** should be reported. Arterial peak systolic velocity, acceleration, resistive index (RI) and pulsatility index (PI) should be considered A. in the spermatic cord (testicular artery) about two cm before entrance in the gonadal hilum (Pilatz et al., 2012) and B. in at least two Doppler spots into the testicular parenchyma (Fig. 7). Attention should be paid not to measure subtunical vessels.

The presence of testis **appendices** should be reported, and one diameter measured (Fig. 8A).

The presence of **extratesticular calcifications** should be reported, and one diameter measured (Fig. 8B).

The presence of **hydrocele** should be reported, and 3 diameters measured. A convex probe should be used if the hydrocele is bulky (Fig. 8C).

1.2 Epididymis and proximal vas deferens

Epididymal CDU features should be evaluated, in order to avoid sexual abstinence interference and to observe indirect signs of subobstruction.

Longitudinal diameter of the epididymal **head** should be reported (Fig. 9A) (see also Pezzella et al., 2012).

Epididymal **head homogeneity** should be classified on a 2 point scale (0. Homogeneous; 1. Inhomogeneous) (Fig. 9B)

Epididymal **head echogenicity** should be classified on a 3 point scale (0. Normal echogenicity; 1. Mainly hypoechoic; 2. Mainly hyperechoic) (Fig. 9C).

The number of **cysts** of the epididymal head should be reported. The maximum diameter of the

major cyst should be reported (Fig. 10A). “Polycystic pattern”, when detected, should be reported Fig. 10B). The presence of **appendices** of the head (or of body, tail or vas deferens) should be reported (Fig. 10C) and measured (1 diameter).

The anterior-posterior diameter of the epididymal **body** should be measured (Fig. 11A) (see also Pezzella et al., 2012).

The anterior-posterior diameter of the **epididymal-deferential handle** should be measured as illustrated in Fig. 11B, as well as **epididymal tail and vas deferens size**.

Epididymal **tail homogeneity** should be classified on a 2 point scale (0. Homogeneous; 1.Inhomogeneity) (Fig. 12A) (see Lotti et al., 2011). Report **coarse calcifications** (Fig. 12A).

Epididymal **tail echogenicity** should be classified on a 3 point scale (0. Normal echogenicity; 1. Mainly hypoechoic; 2. Mainly hyperechoic) (Fig. 12B) (see Lotti et al., 2011).

Cysts of the body, tail or vas deferens mast be reported, and the maximum diameter measured (Fig. 13A).

Epididymal **vascularization** should be reported. The presence of “hyperaemia” (“diffuse vascularization”) should be indicated. Arterial peak systolic velocity, acceleration, resistive index (RI) and pulsatility index (PI) should be measured in a Doppler spot of the epididymal head and tail, when it is possible to detect it (Fig. 14).

1.3 Pampiniform plexus

Pampiniform plexus should be studied bilaterally, both with the patient supine and standing. Subinguinal (between inguinal ligament and upper pole of the testis, according to Orda et al., 1987) evaluation of the “diameter of the internal spermatic vein” must be performed in grey scale, both with the patient supine and standing (Fig. 15A).

The presence of a “retrograde venous flow” should be assessed with the patient standing by colour or power-Doppler, and classified on a 3 point scale (0.Absent/no detectable, 1. Intermittent/fluctuating with breath 2. Continuous) (Fig. 15B).

When retrograde venous flow is detected, venous blood flow velocity should be measured.

Then, Valsalva manoeuvre is required, and the changes during Valsalva’s manoeuvre (flow a.

reducing/stopping or b. increasing) has to be reported (Fig. 15C).

2. Transrectal CDU

Prostate-vesicular region should be studied at rectal ultrasonography through transverse, longitudinal and oblique scans with patients placed in the left lateral decubitus (Behre et al., 1995; Vicari, 1999; Lotti et al., 2011, 2012a, 2012b).

An “end fire” probe (“end fire” transducer 6.5 MHz, field of view 50°-200°) or a transrectal biplanar probe (i.e. linear transducer 7.5 MHz; convex transducer 6.5 MHz) with an “end fire” transducer are suggested, the end fire transducer useful to better investigate seminal vesicles and deferential ampullas (Older & Watson, 1996).

2.1 Prostate

Prostate **volume** should be measured using the planimetric method as previously reported (Behre et al., 1995; Vicari, 1999; Lotti et al., 2011). The three maximum diameters (lateral-lateral, anterior-posterior and longitudinal) of the prostate should be reported (see Fig. 16A), and prostate volume should be expressed using the mathematical formula of the ellipsoid (diameter 1 x diameter 2 x diameter 3 x 4/3 x π). **Transitional zone** should be measured too, reporting at least two diameters (lateral-lateral and anterior-posterior diameters in transversal scan) (Fig. 16B).

Prostate **symmetry** should be evaluated, and classified as 0. Symmetric, 1. Asymmetric. In the latter case, which is the bigger lobe should be reported (Fig. 17).

Prostate **homogeneity** should be evaluated in 2 zones: 1) transitional and 2) peripheral, and classified as a dichotomous variable (0.homogeneous;1.inhomogeneous) (Fig. 18).

Prostate **echogenicity** should be classified on a Likert scale as: 0. normal echogenicity, 1.diffuse hypoechoic/with hypoechoic areas, 2.diffuse hyperechoic/with hyperechoic areas; 3. areas of hypo- and hyper-echogenicity (Fig. 19).

Prostate **calcifications** should be reported, and their size measured. Macro-calcifications are defined as calcifications with size > 3 mm, according to previous reports (Danhert et al., 1986). It should be specified a. the type of calcification (micro- or macro-), b. their localization (0. Transitional zone/peri-urethral 1. Peri-transitional/surgical capsule 2. Right lobe, 3. Left lobe, 4. Peripheral) and

if they are a group or isolated. The major calcification should be measured (three diameters: lateral-lateral, anterior-posterior and longitudinal) (Fig. 20).

The presence of an **utricular / mullerian cyst** should be reported, and 3 diameters measured (Fig. 21).

Prostate **vascularization** should be evaluated both before and after ejaculation (Fig. 22). At least 3 Doppler spots should be evaluated, in three different zones: 1. Transitional zone, 2. Right lobe, 3. Left lobe. **Hyperaemia** should be considered in the presence of at least ≥ 15 Doppler spots, according to Cho et al. (2000), and described as: 0. Absent/normal vascularization 1. Focal 2. Diffuse. If there is "**focal hyperaemia**", it should be reported, as well as its localization in the prostate and if detected in a zone with normal, hypo- or hyper echogenicity. In addition, the related Doppler spot should be evaluated too. Arterial prostatic peak systolic velocity (cm/sec) and Resistance Index (RI) should be calculated for every Doppler spot.

Prostatic **venous plexus** should be studied, measuring the maximum anterior-posterior diameter in longitudinal scan, and the basal blood flow velocity (cm/sec) (Fig. 23).

2.2 Ejaculatory ducts and deferential ampullas

Ejaculatory ducts characteristics should be evaluated after ejaculation, in order to better emphasize indirect ultrasound signs of subobstruction (Colpi et al., 1997; La Vignera et al., 2008, Lotti et al., 2011, 2012a).

Ejaculatory duct abnormalities should be reported (Fig. 24A), and classified as 0.absent, 1.unilateral or 2.bilateral:

-dilatation

-calcifications

-cysts

When dilated, ejaculatory duct anterior-posterior diameter should be measured (Fig. 24B).

Deferential ampullas should be studied after ejaculation. Their 0.presence or 1.absence must be reported. Their anterior-posterior diameter must be measured (Fig. 25A). When deferential ampulla is difficult to detect, distal vas deferens diameter should be measured (Fig. 25B).

2.3 Seminal vesicles (SV)

SV should be studied, before and after ejaculation, with a standard sexual abstinence of 4 days.

Diameters and Volume: the maximum longitudinal diameter (from the “SV pole” to the insertion in the prostate), the maximum anterior-posterior diameter (fundus) and the anterior-posterior diameter of the body (see Lotti et al., 2012; La Vignera et al., 2011) should be measured, as reported in Fig. 26A. SV volume, before and after ejaculation, should be calculated using the “ellipsoid/prolate (d1 > d2 = d3) spheroid” mathematical formula (d1 x d2 x d3 x 4/3 x π, considering d1 = maximum SV longitudinal diameter, d2 maximum SV anterior-posterior diameter, and d3 assumed = d2) (Fig. 26B).

SV **homogeneity** should be classified as a dichotomous variable (0. homogeneous; 1. inhomogeneous) (Fig. 27).

SV **echogenicity** should be classified on a Likert scale as: 0. Normal echogenicity, 1.mainly hypoechoic/with hypoechoic areas, 2.mainly hyperechoic/with hyperechoic areas; 3. areas of hypo- and hyper-echogenicity (Fig. 28).

SV **vascularization** should be evaluated both before and after ejaculation (Fig. 29). Arterial prostatic peak systolic velocity (cm/sec) and Resistance Index (RI) should be evaluated in at least a Doppler spot. The presence of “hyperaemia” (diffuse vascularization) should be reported.

SV ultrasound **abnormalities** should be reported (Fig. 30) and classified as 0.absent, 1.unilateral or 2.bilateral:

-areas of endocapsulation/ roundish anechoic areas (Colpi et al., 1997; Vicari, 1999)

-presence of wall thickening and septa (measure septum thickness)

-presence of calcifications

-presence of giant cyst.

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