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TESTICULAR ASYMMETRY IN CONTRAST WITH HORMONES AND SEMEN PARAMETERS IN IDIOPATHIC SEVERE OLIGOASTHENOTERATOZOOSPERMIA SYNDROME

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Abstract

Introduction. Large testicular differences have been linked to abnormal semen characteristics, including total sperm concentration and total motile sperm count in some studies, although this has not been supported by other research.
The objective of the study was to analyze the result of scrotal ultrasonography in contrast to the hormonal background in patients with idiopathic severe changes in semen quality.
Materials and methods. After excluding the patients in whom a possible cause of infertility was identified, the study group included 106 patients in whom the following parameters were evaluated: testicular volume, Testicular Asymmetry Ratio (TAR), parenchyma echotexture, echogenicity, the presence of testicular calcifications, solid lesions and epididymal cysts, the semen results and hormonal evaluation.
Results. From the studied group, 56 patients (52.83%) had TAR<1, meaning low right testicular volume, 32

Résumé

Asymétrie testiculaire en contraste avec les paramètres hormonaux et séminaux dans le syndrome idiopathique d'oligoasthénotératozoospermie sévère

Introduction. De grandes différences testiculaires ont été liées à des caractéristiques anormales de sperme, notamment la concentration totale de spermatozoïdes et le nombre total de spermatozoïdes mobiles dans certaines études, bien que cela n'ait pas été soutenu par d'autres recherches.
L'objectif de l'étude était d'analyser le résultat d'une échographie scrotale contrairement au contexte hormonal chez les patients présentant des changements graves idiopathiques de la qualité du sperme.
Matériels et méthodes. Après avoir exclu les patients chez qui une possible cause d'infertilité a été identifiée, le groupe d'étude comprenait 106 patients chez lesquels les paramètres suivants ont été évalués: volume testiculaire, rapport d'asymétrie testiculaire

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men (30.18%) with TAR=1-1.2 were considered with normal testicular volume difference, and 18 patients (16.99%) with TAR>1.2 were considered with a significant lower left testicular volume. There was a significant correlation between the volumes of the two testicles ($r = 0.586$; $p < 0.001$). Homogeneous testicular echotexture was found in 67% of patients on the right testis (RT) and 76.4% on the left testis (LT). Regarding testicular echogenicity, 69.8% presented a normo-echoic testicular parenchyma on the RT and 75.5% on the LT. A positive correlation between luteinizing hormone (LH) and follicle stimulating hormone (FSH) was observed ($r = 0.671$; $p < 0.001$) and negative correlation between LH and FSH and testicular volume.

Conclusions. Larger studies are needed to see whether testicular asymmetry is an important factor in idiopathic oligoasthenoteratozoospermia syndrome. TAR could be a valuable instrument in testicular asymmetry assessment. Considering that patients with RT asymmetry have higher testicular volume than patients with pathologic LT asymmetry, we can conclude that that lower RT volume can have a greater impact on overall fertility than lower LT volume.

Keywords: male infertility, testicular asymmetry ratio, testicular volume, right testicular asymmetry.

List of abbreviations:
TAR – Testicular Asymmetry Ratio
RT – right testis
LT – left testis
EAU – European Association of Urology
AUA – American Urology Association
TAI – Testicular Atrophy Index
TVD – testicular volume difference
OAT – oligoasthenoteratozoospermia
BTV – bilateral testicular volume
FSH – follicle stimulating hormone
LH – luteinizing hormone
SHBG – sex hormone binding globulin

(TAR), échotexture du parenchyme, échogénicité, présence de calcifications testiculaires, lésions et kystes épididymaires, les résultats du sperme et l'évaluation hormonale.

Résultats. D'après le groupe étudié, 56 patients (52,83%) avaient du TAR <1, ce qui signifie un volume testiculaire droit faible, 32 hommes (30,18%) avaient du TAR = 1-1,2 et ont été considérés avec une différence de volume testiculaire normal et 18 patients (16,99%) avaient du TAR > 1,2, à qui le volume testiculaire gauche est significatif plus bas. Il y avait une corrélation significative entre les volumes des deux testicules ($r = 0,586$; $p < 0,001$). L'échotéxture testiculaire homogène a été trouvée chez 67% des patients sur le testicule droit (RT) et 76,4% sur le testicule gauche (LT). En ce qui concerne l'échogénicité testiculaire, 69,8% ont présenté un parenchyme testiculaire normo-échogène sur le RT et 75,5% sur le LT. Une corrélation positive entre l'hormone lutéinisante (LH) et l'hormone stimulante des follicules (FSH) a été observée ($r = 0,671$; $p < 0,001$) et une corrélation négative entre LH et FSH et volume testiculaire (VT).

Conclusions. Des études plus importantes sont nécessaires pour voir si l'asymétrie testiculaire est un facteur important dans le syndrome idiopathique oligoasthénoteratozoospermie. Le TAR pourrait être un instrument précieux dans l'évaluation de l'asymétrie testiculaire. Étant donné que les patients atteints d'asymétrie RT ont une VT plus élevée que les patients atteints d'asymétrie LT pathologique, nous pouvons conclure que le volume du RT plus faible peut avoir un impact plus important sur la fertilité globale que le volume inférieur du LT.

Mots-clés: infertilité masculine, rapport d'asymétrie testiculaire, volume testiculaire, asymétrie testiculaire droite

Introduction

Male infertility can be caused by urogenital abnormalities that are either congenital or acquired, also urogenital tract infections, elevated scrotal temperature due to varicocele, endocrine disorders, genetic abnormalities, immunological factors, lifestyle habits (such as obesity, smoking, and gonadotoxic use), systemic diseases, erectile dysfunction, and improper coital habits. Just half of the patients can have their infertility clearly explained after a full workup that includes history, physical examination, semen analysis, and laboratory tests[1]. Moreover, approximately 5% of couples remain unwillingly childless despite multiple interventions[2]. The patients are classified as having male infertility of unknown origin when there is no identifiable etiology. This classification is further divided into idiopathic versus unexplained. The prevalence of idiopathic male infertility is three times higher than unexplained male infertility (33% versus 11%)[2]. With a decline in semen quality, patients with idiopathic male infertility often undergo normal endocrine and physical examinations[3]. Patients with unexplained male infertility, however, will have a normal semen analysis.

The main imaging technique for the scrotum is ultrasound. It is crucial to comprehend the clinical (palpatory) and ultrasonography findings of the scrotum. Male sexual and reproductive function is frequently assessed using testicular ultrasound, and both grayscale and color Doppler ultrasound imaging can clarify the structure and function of the testicles[4]. Ultrasound is considered the gold standard for measuring testicular volume, which Prader's orchidometer frequently overestimates[5]. A lower testicular volume is considered as being less than 12 mL[6], and is linked with poor sperm qualities, reduced fertility, and hypogonadism[7]. Like the echogenicity of the normal thyroid gland, the normal adult testis displays a homogeneous fine echotexture, formed of medium level echoes with uniform distribution[8]. Also, decreased echogenicity has been linked to abnormal interstitial proliferation and decreased spermatogenesis[9]. Homogeneously distributed medium-level echoes, also known as homogeneous echotexture, make up the normal testicular echotexture. Testicular inhomogeneity has been defined as the presence of zones of altered echogenicity, typically hypoechoic, which can be modest, focal ill-defined, or diffuse. Inhomogeneity has been linked histologically to spermatogenic arrest, tubular sclerosis or fibrosis and more recently it has been suggested that inhomogeneity can be used as a valid predictor of male fertility.

Testicular asymmetry, also known as the volumetric predominance of one testis over the other, has been proposed as a prognostic factor for varicocele repair, with a cutoff for significance of 20%, and for the evaluation of the functional capacity of small testes, particularly in patients with a history of cryptorchidism. European Association of Urology (EAU) and American Urology Association (AUA) both state that varicocele in association with a small testis is one of the indication criteria for varicocelectomy in children and adolescents. The EAU states a small testis is present when the Testicular Atrophy Index (TAI) is > 20% or the testicular volume difference (TVD) is ≥ 2 mL[10]. A difference greater than 20% will be considered a delay in development or atrophy of the left testicle. At the same time, we frequently meet patients in whom the right testicle (RT) is smaller than the left testicle (LT) to a different degree. Based on the observations of men with the volume of the RT smaller than the LT, they are often associated with severe changes in the semen quality, including changes in echotexture and echogenicity. Occasionally, it has been observed that the volume of the LT predominates even in patients with non-obstructive azoospermia compared to the volume of the RT, a fact that deserves attention[11]. Theoretically, a reduced volume of the RT normally indicates the delay in its development or atrophy, unlike the reduced volume of the LT compared to the RT, which can be determined by a multitude of factors.

The objective of the study was to analyze the result of scrotal ultrasonography in contrast to the hormonal background in patients with severe changes in the semen quality, in whom a possible cause that would explain infertility was not identified.

Materials and methods

239 patients with severe oligoasthenoteratozoospermia (OAT) syndrome were included in the study, between September 2022 and November 2023. The patients with numerical chromosomal anomalies (Klinefelter syndrome), congenital hypogonadism, Y chromosome microdeletions, varicocele of different degrees, following mumps orchitis, torsion, anamnesis of orchoepididymitis, cryptorchidism or post-orchidopexy were excluded from the initial group. After excluding the patients in whom a possible cause of infertility was identified, the study group included 106 patients in whom the following parameters were evaluated: testicular volume, testicular asymmetry ratio, parenchyma echotexture, echogenicity, presence of testicular calcifications, solid lesions and epididymal cysts, the semen results and hormonal evaluation. The study was approved by the Institutional Ethics Committee of "Nicolae Testemitanu" State University of Medicine and Pharmacy, Chisinau, Republic of Moldova (decision no 3 from 26.02.2021).

Ultrasound evaluation

A GE Logiq E9 with a 7–15 MHz wideband linear transducer was used for ultrasonography. Axial and transverse examinations were included in the standard protocol. The LT and RT volume, echotexture, echogenicity, and presence of testicular calcification, solid lesions and epididymal cysts were evaluated during all ultrasonographic examinations. Testicular volume was measured using the ellipsoid formula (height [cm] x width [cm] x 0.523]), and it was expressed in mL as either left or right testicular volume, bilateral testicular volume, and testicular asymmetry ratio (TAR) = RT volume/LT volume. Testicular echotexture was characterized as either homogenous or inhomogeneous following exploratory data analysis. Testicular echotexture homogeneity was evaluated according to the classification of the European Academy of Andrology ultrasound consortium: homogeneity; mild (grade 1) inhomogeneity: presence of small hypoechoic foci (arrowheads)/thin hypoechoic striae (arrows); moderate (grade 2) inhomogeneity: presence of thick hypoechoic striae

(arrows); severe (grade 3) inhomogeneity: diffuse inhomogeneity with "netting"/"geographical map" appearance. Testicular echogenicity was rated according to echogenicity classification of the European Academy of Andrology ultrasound consortium: normo-echoic; mainly hypoechoic; mainly hyperechoic. Testicular calcifications: isolated macrocalcifications (size > 3 mm); isolated microcalcifications (small [1-3 mm] bright echogenic foci with no acoustic shadow; limited and "clusters" (white circle) testicular microlithiasis (presence of ≥ 5 microcalcifications in a single ultrasound scan); diffuse ("starry sky" appearance) testicular microlithiasis[12].

Hormone evaluation

Morning baseline blood samples (07:30-10:00 hours) were obtained from all subjects by antecubital venous puncture after an overnight fast. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol were double-measured with electrochemiluminescence technology (Cobas® e 801 Module from Roche Diagnostics); prolactin, total testosterone, and sex hormone binding globulin (SHBG) were double-measured with enzyme-amplified chemoluminescence immunoassay technology (IMMULITE 2000 XPi – Immunoassay – Siemens Healthineers). The laboratory reference ranges for adult men were as follows: 1.5-12.4 mIU/mL for FSH; 1.7-8.6 mIU/mL for LH; 53.0-360.0 mIU/L for prolactin; 250-1200 ng/dL for total testosterone; 11.3-43.2 pg/mL for estradiol and 10-57 nmol/L for SHBG. We calculated the absolute values of free testosterone (fT) by using the Vermeulen equation, based on SHBG levels and assuming a fixed albumin concentration of 4.3 g/dL.

Semen assessment

Semen samples were collected by masturbation directly into a sterile plastic container after 2-7 days of sexual abstinence and examined by optical microscopy according to World Health Organization criteria, 5th edition. The following variables were assessed: sperm concentration (n × 106/mL), total sperm number (n × 106/ejaculate), total motility (%) and morphology (% normal forms). Severe OAT syndrome was considered when sperm concentration was < 5 mln/ml or total sperm number < 10 mln/ml, total motility < 30% and normal morphology < 4%.

Sperm concentration assessment

Semen aliquot to be diluted for sperm concentration assessment was taken with a positive displacement pipette using a recommended diluent. Only standard dilutions were used. Sperm concentration was assessed using hemocytometers with improved Neubauer ruling. Hemocytometers were allowed to rest for 10–15 min in a humid chamber to allow sedimentation of the suspended spermatozoa onto the counting grid before counting. Sperm counting was done using phase contrast microscope optics (200–400). Comparisons were made between duplicate counts, and counts were re-done when the difference exceeded the acceptance limits. Typically, at least 200 spermatozoa were counted in each of the duplicate assessments[13].

Semen motility assessment

Motility assessments were performed at 37^{0}C. Motility assessments were initiated within 30–60 min after sample collection. Motility assessments were performed using phase contrast microscope optics (200–400). Sperm motility was classified using a 3-category scheme: progressive, non-progressive, and immobile. Motility assessments were done in duplicate and compared; counts were re-done on new preparations when the difference between duplicates exceeded the acceptance limits. At least 200 spermatozoa were assessed in each duplicate motility count. At least five microscope fields of view were examined in each duplicate count[13].

Sperm morphology assessment

Tygerberg Strict Criteria were used for the evaluation of human sperm morphology. Abnormalities were recorded for the four defined regions of the spermatozoon (head, neck/midpiece, tail and cytoplasmic residue). The Papanicolaou staining method adapted for the assessment of human sperm morphology was used. At least 200 spermatozoa were assessed in each ejaculate. Assessments were done under high magnification (1000– 1250) using a 100 high resolution oil immersion objective and bright field microscope optics (Kohler illumination)[13].

Statistical analysis

The distributions of scale variables were checked for normality and reported as mean ± standard deviation or median [25th – 75th percentile], if appropriate. Pearson correlation statistics and Kendall rank correlation coefficient were examined. All statistical significance levels are set at $p < 0.05$. Data was analyzed using IBM SPSS Statistics 23.

Results

The data obtained from ultrasound assessment, hormonal evaluation and semen results were summarized in Tables 1, 2 and 3. The mean age of the participants was 30.7±6.2 years. The main ultrasound findings on testicular structure are represented in Figure 1.

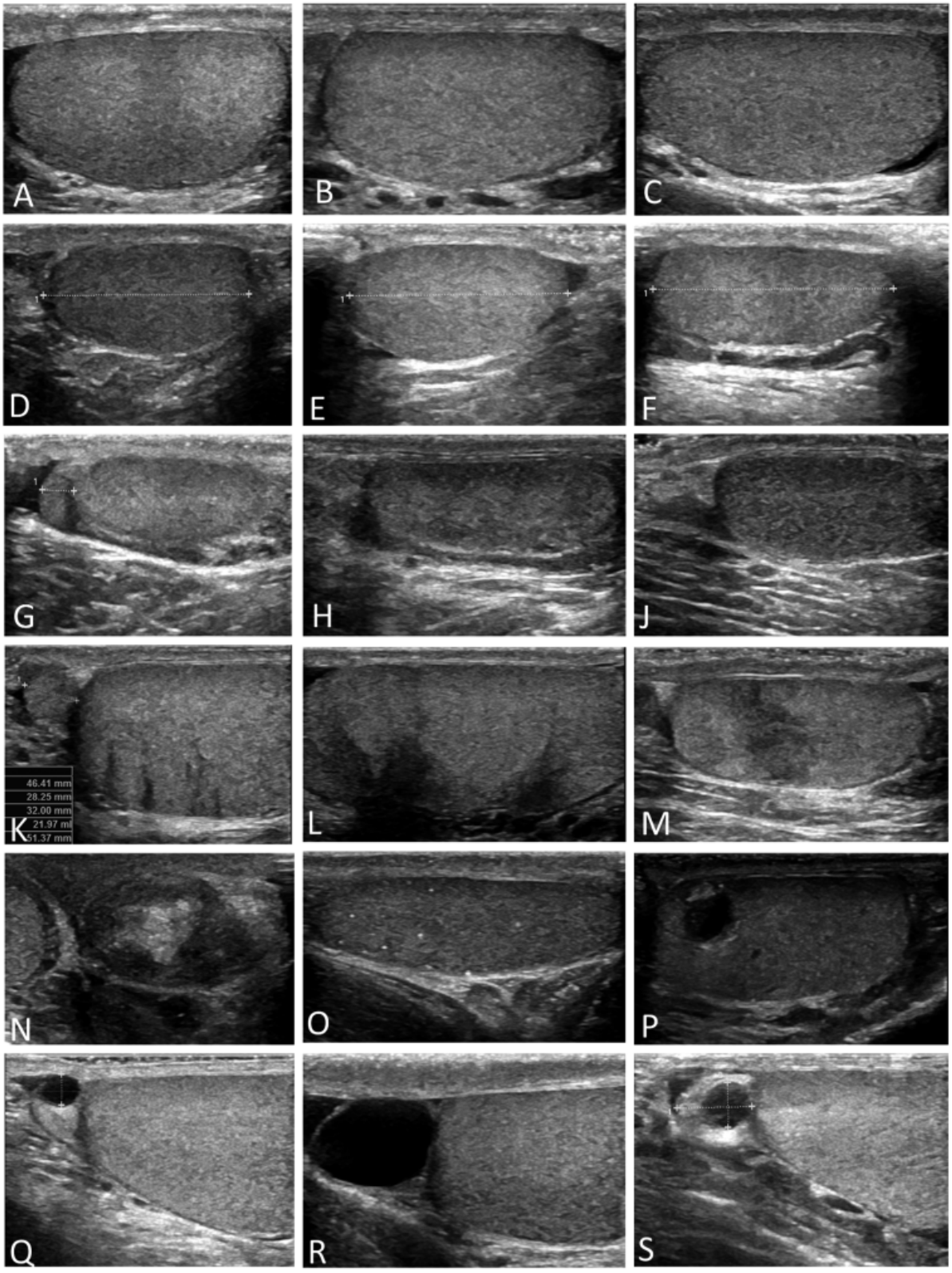

Figure 1. Testicular ultrasound findings
A, B, C – normal testicular size, echotexture and echogenicity; D – reduced testicular size, hypoechoic;
E, F – reduced testicular size normo-echoic; G, H, J – reduced testicular size, inhomogeneity gr. 1;
K, L, M – inhomogeneity gr. 2; N, P – testicular lesion; O – testicular microlithiasis; Q, R, S – epididymal cysts.

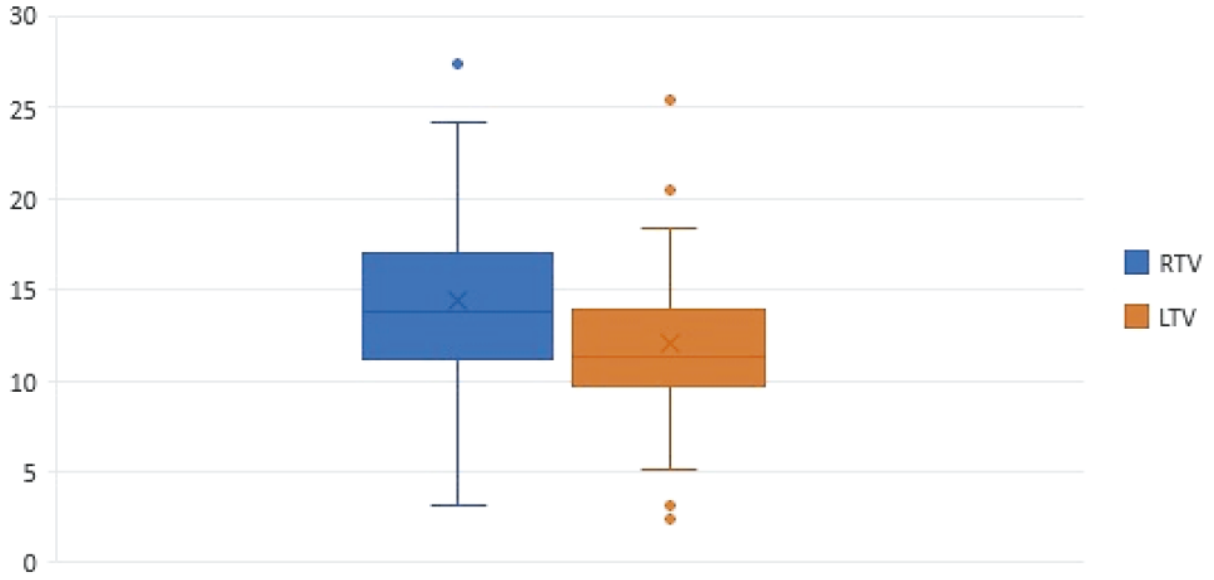

Figure 2. Testicular volumes distribution

Table 1. TAR correlation with testicular volumes

	Testicular asymmetry ratio (TAR)			
	<1	*1-1.2*	*>1.2*	*Total*
Mean value of RT volume	12.05±4.34	14.29±4.69	14.50±5.91	13.14±4.84
Mean value of LT volume	15.62±5.55	13.05±4.22	10.26±4.57	13.93±5.37
Mean value of BTV	27.67±8.84	27.34±8.87	24.76±10.38	27.07±9.09

Legend. RT – right testicle, LT – left testicle; BTV – bilateral testicular volume.

Initially, the ratio between the volume of the RT and the volume of the LT was calculated, this ratio was empirically called the testicular asymmetry ratio (TAR). The TAR values were grouped into 3 categories: < 1, between 1 and 1.2 and > 1.2. The logic of this distribution was related to the normal values of the testicular atrophy index found in the literature, more frequently used in studies on adolescents and children with varicocele and cryptorchidism. The reason for not using this index here is the fact that for adult men, at reproductive age, the reduced volume of the testicle can be both due to atrophy from a larger volume, as well as developmental delay for known or less known reasons. So, if the testicular atrophy index is considered normal up to 20%, then the ratio between the volume of the RT and LT should normally have values between 1 and 1.2, respectively values lower than 1 will indicate a reduced volume of the RT and values greater than 1.2 will indicate a pathological volume of the LT. From this perspective, we tried to organize the results to assess associations depending on these 3 intervals of the TAR calculated for each patient.

Thus, after the analysis of the group of 106 patients with severe OAT syndrome of unknown etiology, 56 (52,83%) were identified with TAR < 1, 32 (30.18%) with TAR=1-1.2, respectively 18 (16.99%) patients with TAR > 1.2. The median value of TAR < 1.0 was 0.85 [0.72-0.92], TAR=1-1.2 was 1.11 [1.05-1.14] and TAR > 1.2 with 1.38 [1.29-1.57].

There was a significant correlation between the volumes (Figure 2) of the two testicles (r = .586; p < 0.001), the mean volume of the RT and LT was 13.14±4.48 and 13.93±5.37, respectively. The bilateral testicular volume median was 26.74 [20.79-32.33] and was positively corelated with RT volume and LT volume (r=.878 and r=.902 respectively; p < 0.001). If considering TAR intervals, the mean testicular volume for TAR < 1.0 was 12.05±4.34 for the RT and 15.62±5.55 for LT. For normal range TAR interval (1.0 – 1.2), the mean testicular volume was 14.29±4.69 for the RT and 13.05±4.22 for the LT. In patients with pathological reduced LT volume and TAR higher than 1.2, the mean testicular volume was 14.50±5.91 for RT and 10.26±4.57 for LT (Table 1). In case of lower RT volume associated with TAR <1.0, the mean volume was higher than mean LT volume with TAR > 1.2, showing that patients with significant RT pathologic asymmetry have higher testicular volume than

Table 2. TAR correlation with ultrasound findings.

Ultrasound parameters		*Testicular asymmetry ratio*							
		<1		*1-1.2*		*>1.2*		*Total*	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Right testicular Echotexture	homogenous	36	64.3	24	75.0	11	61.1	71	67.0
	inhomogeneity grade 1	15	26.8	5	15.6	5	27.8	25	23.6
	inhomogeneity grade 2	1	1.8	2	6.3	1	5.6	4	3.8
	inhomogeneity grade 3	4	7.1	1	3.1	1	5.6	6	5.7
	Total	56	100.0	32	100.0	18	100.0	106	100.0
Right testicular Echogenicity	normal echogenicity	36	64.3	27	84.4	11	61.1	74	69.8
	hypoechoic	16	28.6	5	15.6	4	22.2	25	23.6
	hyperechoic	4	7.1	0	0.0	3	16.7	7	6.6
	Total	56	100.0	32	100.0	18	100.0	106	100.0
Right testicular calcifications	absent	54	96.4	32	100.0	18	100.0	104	98.1
	Isolated microcalcification	1	1.8	0	0.0	0	0.0	1	0.9
	≥ 5 microcalcifications	1	1.8	0	0.0	0	0.0	1	0.9
	Total	56	100.0	32	100.0	18	100.0	106	100.0
Right testicular lesions	absent	56	100.0	32	100.0	18	100.0	106	100.0
	Total	56	100.0	32	100.0	18	100.0	106	100.0
Right testicular epididymal cyst	absent	50	89.3	31	96.9	18	100.0	99	93.4
	present	6	10.7	1	3.1	0	0.0	7	6.6
	Total	56	100.0	32	100.0	18	100.0	106	100.0
Left testicular Echotexture	homogenous	46	82.1	25	78.1	10	55.6	81	76.4
	inhomogeneity grade 1	10	17.9	4	12.5	5	27.8	19	17.9
	inhomogeneity grade 2	0	0.0	2	6.3	0	0.0	2	1.9
	inhomogeneity grade 3	0	0.0	1	3.1	3	16.7	4	3.8
	Total	56	100.0	32	100.0	18	100.0	106	100.0
Left testicular Echogenicity	normal echogenicity	43	76.8	27	84.4	10	55.6	80	75.5
	hypoechoic	10	17.9	5	15.6	5	27.8	20	18.9
	hyperechoic	3	5.4	0	0.0	3	16.7	6	5.7
	Total	56	100.0	32	100.0	18	100.0	106	100.0
Left testicular calcifications	absent	56	100.0	32	100.0	17	94.4	105	99.1
	Macro calcinates > 3 mm	0	0.0	0	0.0	1	5.6	1	0.9
	Total	56	100.0	32	100.0	18	100.0	106	100.0
Left testicular lesions	absent	56	100.0	32	100.0	17	94.4	105	99.1
	present	0	0.0	0	0.0	1	5.6	1	0.9
	Total	56	100.0	32	100.0	18	100.0	106	100.0
Left testicular epididymal cyst	absent	54	96.4	30	93.8	18	100.0	102	96.2
	present	2	3.6	2	6.3	0	0.0	4	3.8
	Total	56	100.0	32	100.0	18	100.0	106	100.0

Ultrasound findings on echotexture, echogenicity, calcifications, lesions and epididymal cysts distributed accordingly with the testicular asymmetry ratio.

patients with significant LT pathologic asymmetry. Considering that all patients had almost same semen results, we can conclude that that lower RTV volume can have greater impact on overall fertility than lower LTV, but of course larger studies should be done.

Homogeneous testicular echotexture was found in 67% of patients on the RT and 76.4% on the LT. If compared with TAR, higher homogeneity in the RT (75%) was found when TAR > 1-1.2, respectively higher left homogeneity (82.1%) was associated with TAR < 1.0 (Table 2). In the group with TAR < 1.0, a higher number was with RT inhomogeneity (35.7%) compared with the LT inhomogeneity (17.9%). RT inhomogeneity was less frequently found (25%) in the

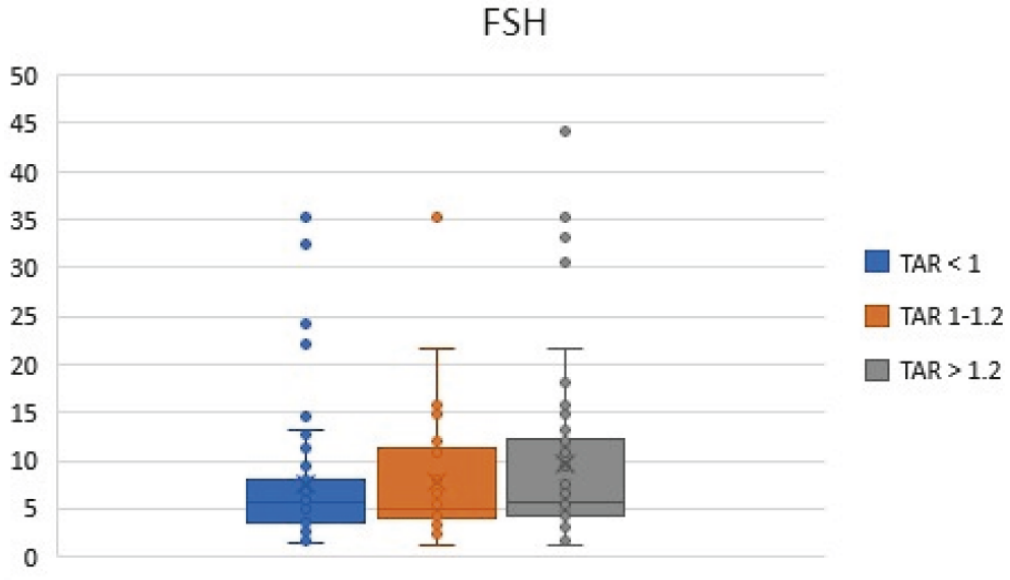

Figure 3. FSH values distribution according to TAR reference range

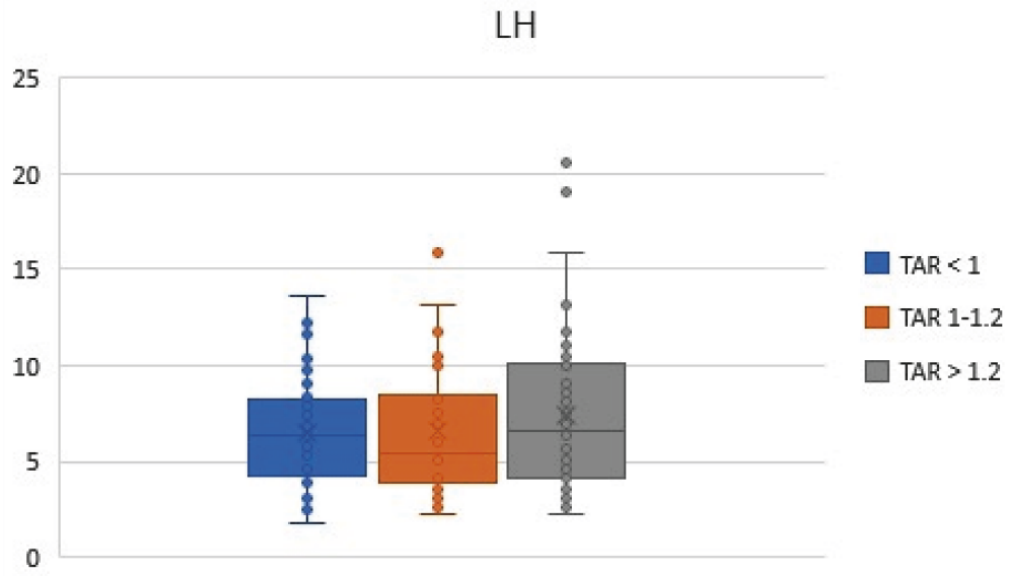

Figure 4. FSH values distribution according to TAR reference range

group with normal TAR (1.0-1.2) compared with the group with abnormal TAR (>1.2), in whom inhomogeneity was higher (39%). LT inhomogeneity was 21.9% in those with TAR between 1.0-1.2 and 44.5% for those with TAR >1.2. Related to echotexture, a positive association of inhomogeneity with higher pathologic testicular asymmetry was observed.

Regarding testicular echogenicity, 69.8% presented a normoechoic testicular parenchyma of the RT and 75.5% of the LT. A higher percentage with normal echogenicity was found in patients with normal TAR, on both sides was 84.4%, and a lower percentage with normal echogenicity was on the RT (61.1%) and on the LT (55.6%) when TAR > 1.2 (Table 2). From these data, it results that normal echogenicity was negatively associated with pathologic testicular asymmetry.

Testicular calcifications were found in the group with RT pathologic asymmetry and TAR < 1.0 and LT pathologic asymmetry and TAR > 1.2 with 3.6% and 5.6%, respectively. No calcifications were found in the group with normal asymmetry and TAR between 1.0-1.2 (Table 2). No significant findings regarding testicular lesions were observed within the studied group. Testicular calcifications were positively associated with pathologic testicular asymmetry.

The relationship between ultrasound characteristics and hormonal values was seen only for FSH and

Table 3. The median values for hormones and semen parameters.

	Testicular asymmetry ratio (TAR)			
	<1	*1-1.2*	*>1.2*	*Total*
TAR	0.85 [0.72-0.92]	1.11 [1.05-1.14]	1.38 [1.29-1.57]	0.98 [0.85-1.13]
FSH	5.64 [3.66-7.90]	5.08 [4.06-11.25]	7.46 [4.53-18.00]	5.64 [3.86-11.40]
LH	6.40 [4.25-8.25]	5.40 [3.97-8.40]	8.45 [5.10-11.10]	6.40 [4.20-8.50]
PRL	239.50 [154.00-311.50]	211.00 [175.50-240.50]	199.00 [135.00-346.00]	218.00 [156.00-288.00]
TT	309.50 [247.00-398.00]	339.00 [267.00-412.00]	282.50 [216.00-400.00]	315.00 [247.00-400.00]
E	24.30 [18.10-29.05]	25.55 [22.10-32.30]	22.60 [18.30-26.90]	24.45 [18.70-29.10]
SHBG	28.10 [22.05-37.95]	32.30 [24.80-41.95]	28.55 [24.60-35.80]	30.50 [23.80-38.40]
Free T	6.10 [5.29-8.15]	6.62 [5.91-7.78]	5.92 [5.02-7.50]	6.19 [5.30-7.98]
Sperm concentration	4.00 [2.00-5.00]	4.00 [2.00-5.00]	3.00 [1.00-4.00]	4.00 [2.00-5.00]
Sperm total number	8.50 [5.00-10.00]	8.00 [4.25-10.00]	7.50 [3.00-9.00]	8.00 [4.00-10.00]
Sperm motility	13.00 [5.00-18.50]	14.00 [7.50-17.50]	9.00 [3.00-15.00]	13.00 [5.00-18.00]
Sperm morphology	0.00 [0.00-1.00]	0.00 [0.00-1.00]	0.00 [0.00-0.00]	0.00 [0.00-1.00]

Median values and 25%-75% interquartile ranges for hormones and semen parameters distributed accordingly with testicular asymmetry ratio range.

LH, other parameters did not show any significant result. A positive correlation between LH and FSH was observed ($r = 0.671$; $p < 0.001$) and a negative correlation between LH, FSH and testicular volume: RT volume and FSH ($r = -.456$; $p < 0.001$); RT volume and LH ($r = -0.335$; $p < 0.001$); LT volume and FSH ($r = -0.465$; $p < 0.001$); RT volume and FSH ($r = -0.293$; $p < 0.002$). The median values of FSH were higher in the group with pathologic testicular asymmetry: right – 5.64 [3.66-7.90] and left – 7.46 [4.53-18.00] compared to those with normal testicular asymmetry – 5.08 [4.06-11.25] (Figure 3). The same observation for LH, with median values on the right pathologic testicular asymmetry – 6.40 [4.25-8.25] and left – 8.45 [5.10-11.10] compared to normal asymmetry median values 5.40 [3.97-8.40] (Figure 4). Based on these data, we can conclude that the patients with pathologic testicular asymmetry have higher gonadotropin hormones, in most of the cases in normal range limits (Table 3).

Discussion

The testicular volume and TVD are mostly investigated in adolescent males with varicocele and cryptorchidism, as these are frequent pathologies in children and pubertal boys[14]. Large testicular volume differences have been linked to abnormal semen characteristics, including total sperm concentration and total motile sperm count in some studies, although this has not been supported by other research[15]. Testicular asymmetry is frequently the result of temporary asymmetrical growth during puberty, hence the evidence about TAI in adolescents with varicocele is conflicting and inconsistent. Many young boys with varicoceles and TAI under 20% show a catch-up growth[16]. In addition, many adolescents without varicoceles also have a TAI of 20% or less. This supports the idea that testicular asymmetries are a natural feature of pubertal development.

In one research, 105 cryptorchid boys aged between 1 and 15 years (average age 4.8 years) who underwent unilateral orchiopexy were examined to determine the value of the TAI in the evaluation of undescended testicles. Scrotal ultrasound was used to evaluate the testes' size and volume, and TAI was calculated before and a year after surgery. Thirty-five boys were separated into five age-dependent groups, and pre- and postoperative scrotal ultrasound measurements were examined. TAI levels prior to surgery varied from 27.1% to 52.8%. Boys aged between 4 and 10 years showed the greatest decline in testicular volume (35.4% to 52.8%). TAI measurements taken a year after orchiopexy were lower than those taken before surgery. The boys aged between 2 and 10 years showed a significant variation in TAI values, ranging from 18.16% to 36.43% ($p<0.001$). The difference between the youngest (0–2 years old) and oldest males (> 10 years old) was not statistically significant. TAI has proven to be a useful and accurate technique for determining which patients with undescended testes should undergo surgery and for evaluating the efficacy of the procedure. Boys with retractile testes

should be considered candidates for surgery if TAI has a value of 20% or above[17].

An observational cross-sectional study involving 539 adolescent boys aged 11 to 16 years was undertaken from April 2015 to December 2016, to evaluate the presence of testicular asymmetry and the current threshold values in varicocele treatment in a healthy adolescent population. A clinical examination was conducted, and the size of the testicles was measured using ultrasound. The Lambert formula (length x breadth x height x 0.71) was used to determine the testicular volume. The second Tanner stage, then the third Tanner stage, was where most boys were. Overall, 142 (41.16%) and 203 (58.84%) boys had smaller RT and LT, respectively. The study concluded that a smaller ipsilateral testis in conjunction with a TAI of > 20% and/or TVD of >2 mL demands a careful interpretation in left-sided unilateral inguinoscrotal disease, and repeated testicular volume measurements should always be taken[18].

Testicular asymmetry is almost exclusively approached from the perspective of the classic model, whereby the LT is normally smaller than the RT, respectively the role of asymmetry in male infertility has been less studied. The curent study highlighted, first of all, that testicular asymmetry can have value in idiophatic male infertility. We consider important to study the possible mechanisms that lead to physiological and pathological asymmetry. By comparison, the testicular asymmetry has been more studied in birds than in humans, possibly because of the lack of recognition of the importance of the subject. Considering the valuable conclusions regarding testicular asymmetry formulated on animal models, we will refer to some conclusions found in these studies.

Animals' studies on testicular asymmetry

Considering that sperm quality and quantity are correlated with testes' relative size and that polygynous bird species have larger testes, it follows that in species with asymmetric testes, functional differences may be conferred by the relative sizes of each testis[19]. Testes asymmetry is especially evident in birds, where the LT is typically larger than the RT[20]. So far, it is still unclear what causes testes asymmetry and what are the effects on reproduction. The functional ovary exclusively grows from the left gonad in females, while the right gonad regresses during avian development, while the testes in males develop on both sides[21]. Yet, the presence of right-bias testes asymmetry in several species suggests that additional causes are likely to be significant. Furthermore, avian testes asymmetry may originate from selection on asymmetric development of female gonad morphology[20]. According to the compensation concept, a relatively smaller testicle acts as a backup by keeping the function if the larger one becomes disabled. Research has also revealed that castrating one testis can induce the other one to grow[22]. Increasing the relative size of testes has been linked to reducing testes asymmetry across bird species, but only in those with a larger LT[20]. After it was discovered that the direction of testes asymmetry varied among Maluridae species, it was observed that those with smaller testes were left-biased and those with larger testes were right-biased[22]. Moreover, some species changed from having more symmetrical testes to developing ones that are biased to the right in response to strong sperm competition[22]. Although Maluridae appear to have no variation in the quantity of seminiferous tissue within or among species displaying divergent testes asymmetry, which indicates a functional equivalent between asymmetric testes, testes may not always confer differences in functioning[22].

Conclusions

Larger studies are needed to determine whether testicular asymmetry is an important factor in idiopathic OAT syndrome. TAR could be a valuable instrument in testicular asymmetry assessment. Practical important conclusions about asymmetry trends can be made using the values of TAR distribution. A pathologic testicular asymmetry was positively associated with testis parenchyma inhomogeneity and testicular calcification, but negatively with normal testis echogenicity. Considering that patients with RT asymmetry have a higher testicular volume than patients with pathologic LT asymmetry, we can conclude that lower RT volume can have greater impact on overall fertility than lower LT volume. Based on study data, pathologic testicular asymmetry is associated with higher gonadotropin hormones compared with normal asymmetry, in most of the cases within the normal range.

It would be interesting to evaluate whether morphological adaptations of asymmetric testes in humans translate into variation in sperm quantity and quality, for a better understanding of these processes.

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Conceptualization, I.A. and I.D.; methodology, I.A., I.D., V.G., A.G., A.B., E.C.; software, I.A.; validation, I.D., E.C.; formal analysis, V.G.; investigation, I.A., V.G., A.G., A.B.; resources, A.B.; data curation, I.A. and I.D.; writing–original draft preparation, I.A.; writing–review and editing, I.D., V.G., A.G., A.B., E.C.; visualization, A.B.; supervision, E.C.; project administration, I.A. and I.D. All the authors have read and agreed with the final version of the article.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest."

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