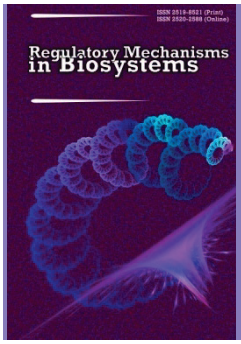

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Testicular ultrasound examination and hemodynamics of patients with azoospermia

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The most difficult form of male infertility to treat is azoospermia. Azoospermia is defined as the complete absence of spermatozoa in the ejaculate. The age of the patients who underwent clinical and diagnostic studies varied between 22 and 45 years. Among 119 examined patients with azoospermia, 58.0% were diagnosed with secretory infertility. In 42.0% patients, the presence of spermatogenesis in excretory-obturational infertility was established. Among 69 patients with secretory infertility, 23 had azoospermia in the absence of spermatozoa and spermatogenic cells, which accounted for 33.3% of all patients with secretory infertility (in particular, 2 with leukocytospermia, which indicated damage to the tubular apparatus as a result of previous orchitis). In 66.6% patients, azoospermia was observed in the absence of spermatozoa, but in the presence of precursor cells of spermatogenesis. 11.5% patients out of 69 (group 1) were diagnosed with concomitant diseases. We see that arterial hypertension, diseases of the gastrointestinal tract, liver, and kidneys occur. Hereditary diseases were not detected in the examined patients. Pain of varying intensity was found in 6.7% of patients, varying degrees of testicular hypoplasia were found in 7.6%, dysuria in 13.4%, epididymitis in history 8.4%; history of epidemic mumps 6.7%; 12.6% had depression, restlessness, sleep disorders, and 21.0% had erectile dysfunction. According to clinical examination and ultrasound, chronic prostatitis is suspected in 19.2% of patients. An increase in the number of leukocytes in the blood was found in 13.2% patients. According to ultrasound, 60.5% of the patients with azoospermia had normal testicular sizes, and 39.5% had reduced testes. The volume of the testicles in the control group was 22.3 ± 2.1 cm³ on average, varying from 18.3 to 25.1 cm³. In the group with azoospermia, the volume of the testicles was on average 16.7 ± 1.7 cm³ and varied from 8.2 to 21.1 cm³, that is, the volume of the testicles in patients with azoospermia was on average 1.3 times smaller compared to normozoospermia. In the obstructive form of azoospermia, diffuse changes were detected in both testicles, probably obturational changes. Hypoplasia of the left testicle and increased echogenicity were observed in the non-obstructive form of azoospermia. The veins of the spermatic cord were moderately dilated. There was a normal amount of free fluid in the scrotum. According to elastography, the elasticity of the testicles was above normal. Obturational processes in the testicles were suspected. The hemodynamic parameters of testicular parenchymal blood flow in infertile men obtained by ultrasound dopplerography are of important diagnostic value. The average value of the linear blood flow velocity in the arteries of the parenchyma in men with normozoospermia was 0.107 ± 0.015 m/s on the right, and 0.103 ± 0.012 m/s on the left. With azoospermia, the average value of the linear velocity of blood flow on the right was 0.086 ± 0.012 m/s, and on the left – 0.084 ± 0.008 m/s. Thus, the hemodynamic indicators of the scrotum show that the most pronounced changes are found in men with azoospermia in the absence of spermatogenesis.

Keywords: infertility; azoospermia; genitalia; linear blood flow rates; ultrasonography; extragenital pathology.

Introduction

The sustainable progress of the society of any country, as well as civilization, depends on demographic parameters, which, in turn, depend on the reproductive health of the population. Infertility in marriage is an important medical and social problem. The prevalence of infertility is becoming epidemic. In recent decades, there has been a steady trend towards an increase in the number of infertile men (Zhang et al., 2016). According to the WHO, the prevalence of infertility has increased by 50% over the past two decades, and the percentage of male-related infertility ranges from 20% to 70%. In recent years, a progressive increase in cases of male infertility has been recorded in Ukraine and all over the world (Horpinchenko & Romaniuk, 2016; Horpinchenko et al., 2016; Salonia et al., 2022). It is believed that the most common causes of male infertility are disorders of spermatogenesis and sperm functions. Sperm disorders can be associated with quality or quantity of spermatozoa or sperm emission. Defective spermatozoa are characterized by damaged DNA, which, in turn, causes an increase in the frequency of miscarriages, the development of fetal abnormalities, etc. It has been found that the disruption of the DNA structure is closely related to free radical processes and oxidative stress (Aitken et al., 2014; Fafula et al., 2019). The most difficult form of male infertility to treat is azoospermia (Horpinchenko et al., 2016; Vorobest et al., 2020; Salonia et al., 2022). Azoospermia is defined as the complete absence of spermatozoa in the ejaculate. Depending on the nature and causes of impaired spermatogenesis, azoospermia is divided into obstructive (excretory, OA) and non-obstructive (secretory, NOA) (Horpinchenko et al., 2016; Vorobest et al., 2020; Salonia et al., 2022). In male infertility, azoospermia is detected in 10–15% of cases, while the share of obstructive and non-obstructive forms is approximately 40% and 60%, respectively (Vorobest et al., 2020; Salonia et al., 2022). The problem is the differential diagnosis of OA and NOA. OA occurs as a result of secondary obstruction of the male reproductive tract. It is determined on the basis of a comprehensive study of the anamnesis, physical methods, laboratory diagnostic tests, as well as ultrasound and genetic research methods, and histological examination of testicular biopsies. NOA, which develops against the background of primary or secondary damage to the testicular parenchyma, is differentiated from OA based on such features as the consistency and volume of the testicles, hormone levels, microscopic examination of testicular biopsies, genetic studies (karyotype, microdeletions of the Y-chromosome). Treatment of OA is based on surgical resto-

ration of the patency of the vas deferens, or extraction. However, at present, the most reliable method of diagnosing azoospermia is a testicular biopsy. It allows one not only to differentiate excretory and secretory forms of infertility, but also to determine the degree of impaired spermatogenesis based on the histological picture of the biopsy (Ghalayini et al., 2011; Dohle et al., 2012; Esteves, 2013; Bernie et al., 2015; Cissen et al., 2016; Hao et al., 2017). Since obtaining biopsies is a complex and traumatic process, there is an active search for biochemical, genetic, immune markers of azoospermia and expansion of the spectrum of clinical indicators.

Based on this, the aim of the work was the clinical characteristics of patients with various forms of azoospermia.

Materials and methods

All the men were made aware of patient information leaflets and gave informed consent to participate in the study. Terms of sample selection meet the requirements of the principles of Convention of Europe Council on Human Rights, Helsinki Declaration on Protection of Human Rights and Biomedicine and the laws of Ukraine. Approval for study was taken from the ethics committee of Danylo Halytsky Lviv National Medical University.

Infertile patients with azoospermia were divided into two groups: with secretory infertility characterized by a non-obstructive form of azoospermia (group 1, n = 69) and excretory-obstructive infertility characterized by a non-obstructive form of azoospermia (group 2, n = 50). The control group (group 3, n = 46) consisted of fertile men with normozoospermia. Inclusion and exclusion criteria were used when selecting the research groups.

Inclusion criteria: reproductive age of patients (22–45 years), established fact of infertility, absence of female infertility factor in marriage, normal testosterone level, absence of acute and chronic infectious-inflammatory processes in the urogenital tract, absence of hormonal correction of infertility.

Exclusion criteria: age < 22 years and > 45 years, severe general systemic diseases, diseases that require taking drugs capable of affecting spermatogenesis, in particular hormonal drugs, varicocele, presence of spermatozoa in the ejaculate, sexually transmitted infections in the anamnesis, presence in history of previously performed testicular biopsy.

The criteria for azoospermia are the absence of sperm in the ejaculate or the absence of ejaculate. The criteria for the obstructive form of azoospermia are the absence of spermatozoa in the ejaculate with preserved spermatogenesis. The criteria for non-obstructive form of azoospermia is the absence of sperm in the ejaculate due to a violation of the process of spermatogenesis. Diagnosis of infertility, like other diseases, is based on the patient's complaints, anamnesis data, objective status and the results of special research methods. Before the ultrasound examination with dopplerography and elastography, the following tests were performed on all patients: spermogram, blood hormones – FSH, LH, total testosterone, prolactin, estradiol, inhibin B.

Ultrasound with the help of colour Doppler mapping and dopplerography was used to determine the volume of the testicles and hemodynamic changes in the parenchyma. The research was carried out on a Logiq 3 device (General Electric, USA). The state of the seminal vesicles, prostate, sonography and dopplerography of the organs of the vestibule-appendages of the testicles, vessels of the vestibule were performed with the help of ultrasound examinations (Abdulwahed et al., 2013; Horpinchenko et al., 2016). So, normally, the testicle has an oval shape, a clear and even contour, homogeneous parenchyma, average echogenicity. The protein shell is visualized as a thin continuous strip of high echogenicity located along the edge of the testicle. The mediastinum has the appearance of a hyperechoic thin strip or wedge in the upper parts of the organ.

A protrusion with a diameter of 2–3 mm can be visualized at the upper pole of the testicle. The testicle is surrounded by a small amount of serous fluid, which appears as a thin hypoechoic zone 1–3 mm wide. The epididymis is located at the upper pole of the back surface of the testicle. Its structure is heterogeneous and in terms of echogenicity, it is similar to the testicular parenchyma. In the absence of pathological changes in the appendix, only its head, measuring 10–15 mm, is determined. When diagnosing forms of azoospermia, it is important to assess the size and structure of the testicles, the presence of signs of obstruction (dilation of the ducts of the testicle and epididymis), and blood reflux. The ultrasound method mostly allows one not only to establish the final diagnosis and assess the state of regional hemodynamics, but also to carry out differential diagnosis.

Mathematical analysis of the research results was carried out using software pack Statistica 10.0 (StatSoft Inc., USA). The results are presented as the mean ± standard error (x ± SE). Analysis of variance (ANOVA) was used to compare the difference in the means between studied groups. To compare the difference between the parameters groups we used the Tukey test. Differences were considered statistically significant at $P < 0.05$.

Results

The age of the patients who underwent clinical diagnostic tests and testicular biopsy varied between 22 and 45 years. The average age of patients with testicular (secretory) male infertility was 28.6 years, and with post-testicular (excretory) – 31.5 years. The average period of infertility was 4.2 years. Among 119 examined patients with azoospermia, 58.2% were diagnosed with secretory infertility. In 41.8% patients, the presence of spermatogenesis in excretory-obturation infertility was ascertained. Among 69 patients with a secretory form of infertility, 23 were found to have azoospermia in the absence of spermatozoa and spermatogenic cells, which accounted for 34.0% of all patients with secretory infertility (in particular, 2 with leukocytospermia, which indicated damage to the tubular apparatus as a result of previous orchitis). In 66.0% patients, azoospermia was observed in the absence of spermatozoa, but in the presence of precursor cells of spermatogenesis.

Eight (11.5%) patients out of 69 (group 1) were diagnosed with concomitant diseases (Table 1). We see that arterial hypertension, diseases of the gastrointestinal tract, liver, and kidneys occur. Hereditary diseases were not detected in the examined patients.

Table 1
Frequency of extragenital pathology in examined patients with non-obstructive form of azoospermia (n = 69)

Concomitant diseases	% of all patients
Chronic gastritis, stomach ulcer	2.9
Chronic pyelonephritis	1.4
Liver disease	1.4
Arterial hypertension	5.8

One (4.3%) patient suffered from viral orchitis, one (4.3%) had an operation for phlegmon of the scrotum, three (13.0%) suffered from non-viral epididymitis, four underwent bilateral orchopexy at an early age. Pain of varying intensity was detected in 6.7% of patients, varying degrees of testicular hypoplasia were detected in 7.6%, dysuria in 13.4%, epididymitis in the anamnesis in 8.4%, epidemic parotitis in the anamnesis in 6.7%, 12.6% had depression, restlessness, sleep disorders, and 21.0% had erectile dysfunction (Table 2).

According to clinical examination and ultrasound, chronic prostatitis is suspected in 19.2% of patients. An increase in the number of leukocytes in the blood was found in 15 (13.2%) patients.

Table 2
Frequency of clinical symptoms in all examined patients (n = 69)

Clinical symptoms	% of all patients
Pain of varying intensity in the area of the external genitalia	6.7
Testicular hypoplasia	7.6
Epididymitis in the anamnesis	8.4
Epidemic mumps in the anamnesis	6.7
Dysuria in history	13.4
Depressive state, restlessness, sleep disorders	12.6
Erectile dysfunction	21.0

According to ultrasound, 44 (95.7%) patients with normozoospermia had normal testicle sizes. In 72 (60.5%) patients with azoospermia, the size of the testicles was normal, and in 47 (39.5%) they were reduced.

No structural pathology was detected in azoospermia during the ultrasound examination of the organs of the portal vein. The veins of the right and left spermatic cord were 2 mm in diameter, without signs of blood flow disorders.

Each patient donated sperm twice with an interval of two weeks. All patients of experimental group 2, on the basis of the examination and conclusion of spermograms, were confirmed to have a complete absence of spermatozoa in the ejaculate, which indicates the presence of a non-obstructive form of azoospermia (Table 3).

Table 3
Spermogram results of patients with non-obstructive form of azoospermia (x ± SE, n = 69)

Indicator	Value
Ejaculate volume, mL	≤ 1.5
pH	7.2 ± 0.5
The total number of spermatozoa, million	was not detected*
Sperm concentration, million/mL	was not detected*
Total sperm motility, %	was not detected*
Spermatozoa with progressive movement, %	was not detected*
Number of pathological forms, %	was not detected*
Concentration of leukocytes, million/mL	≤ million/mL

Note: * complete absence of spermatozoa upon examination of the semen.

The volume of the testicles was determined using an orchidometer and specified according to ultrasound data. Thus, according to ultrasound, 95.7% patients with normozoospermia had normal testicle sizes. In 60.5% patients with azoospermia, the size of the testicles was normal, and in 39.5% they were reduced (Table 4). Microcalcifications were found in 2 (4.3%) patients with normozoospermia and in 39 (32.8%) patients with azoospermia. Normal testicle sizes range from 15 to 30 cm^3. The obtained data indicate that in patients with azoospermia, in the absence of spermatogenesis, there are significant changes in both the size of the testicles and their echogenicity.

Table 4
Testicular ultrasound data of patients with azoospermia

Ultrasound indicators	Groups	
	Normozoospermia (n = 46) % of all patients	Azoospermia (n = 119) % of all patients
Sizes of testicles:		
– normal	95.7	60.5
– reduced	4.3	39.5
Echogenicity:		
– normal	76.1	23.5
– increased	6.5	24.4
– lowered	6.5	21.0
– heterogeneous	10.9	31.1
Microcalcifications	4.3	30.3

According to ultrasound, the volume of the testicles in the control group averaged 22.3 ± 2.1 cm^3, varying from 18.3 to 25.1 cm^3. In the group with azoospermia, the volume of the testicles was 16.7 ± 1.7 cm^3 on average and varied from 8.2 to 21.1 cm^3, that is, it was 1.3 times smaller than the control (Fig. 1).

We present the examination of a 29-year-old patient with obstructive azoospermia (Fig. 2). It was established: the right testicle measures 40x19x26 mm, the volume is 10.5 cm3, the left testicle is 34x25x18 mm, the volume is 8.2 cm^3. Structurally somewhat heterogeneous, echogenicity is increased. Ectasia of efferent tubules is determined. Elastographically, the testicles have significantly reduced elasticity. The appendages on both sides are not enlarged. Their contour is clear, uneven. There is a normal amount of free fluid in the scrotum. Conclusion: diffuse changes in both testicles, probably obturational changes.

We give another example of the examination of a patient with a non-obstructive form of azoospermia, aged 31 (Fig. 3). The right testicle is typically located in the scrotum. The contours are clear, even, the dimensions are 47x27x27 mm, the volume is 18 cm^3. Echogenicity is increased. Dilation of the veins of the spermatic cord is diffusely determined. The left testicle is located typically, the contours are clear and even. Dimensions 37x25x28 mm, volume 13.5 cm^3. Hypoplasia of the left testicle. Echogenicity is increased. The veins of the spermatic cord are moderately dilated. There is a normal amount of free fluid in the scrotum. According to elastography, the elasticity of the testicles is above normal. Obturational processes in the testicles are suspected.

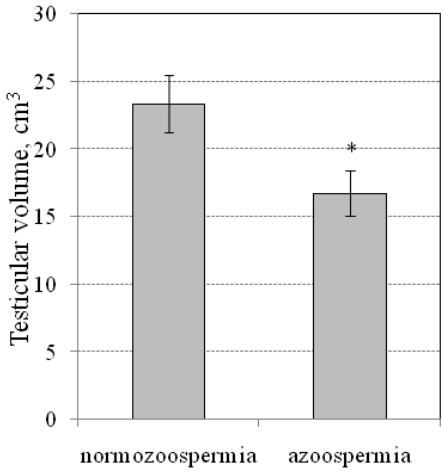

Fig. 1. Testicular volume of infertile men with azoospermia: x ± SE, n = 119; * – P < 0.01 in comparison with normozoospermia

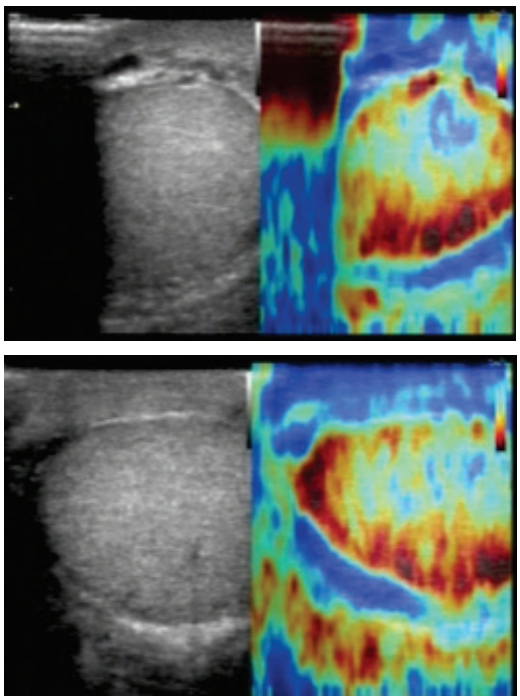

Fig. 2. Ultrasound examination with Doppler and elastography of the right and left testicles of a patient with obstructive azoospermia

The analysis of different groups of patients with non-obstructive form of azoospermia showed that among 23 men (group 1) with primary hypergonadotropic hypogonadism (increased levels of FSH and LH) 17.5% had viral orchitis in childhood, 4,3% had a reduced size of the testicles in the scrotum since childhood after surgery for phlegmon of the scrotum, 4.3% had the absence of the right testicle in the scrotum, 13.0% had non-viral orchoepididymitis. 60.9% patients deny any factors that could negatively affect fertility. In all 23 patients, the testicles were palpably hypoplastic (18–37 mm).

The hemodynamic parameters of testicular parenchymal blood flow in infertile men obtained by ultrasound dopplerography are of important diagnostic value. The average value of linear blood flow velocity in parenchymal arteries in men with normozoospermia was 0.107 ± 0.015 m/s on the right, and 0.103 ± 0.012 m/s on the left. With azoospermia, the average value of linear blood flow velocity on the right was 0.086 ± 0.012 m/s, and on the left – 0.084 ± 0.008 m/s (Fig. 4).

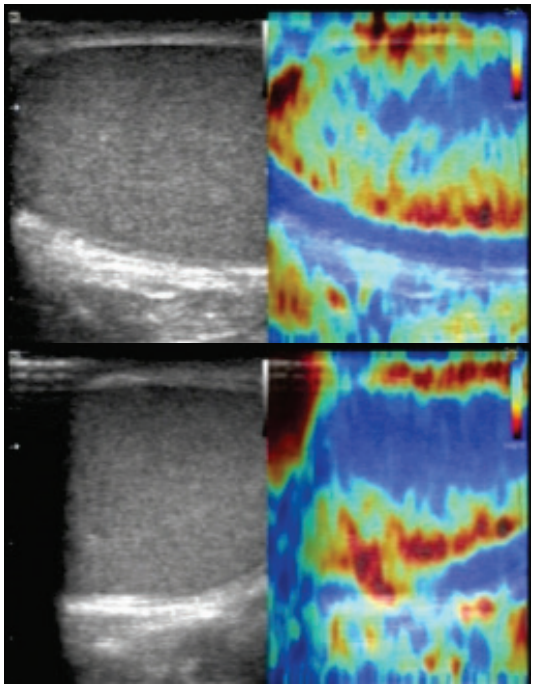

Fig. 3. Ultrasound examination with dopplerography and elastography of the right and left testicles of a patient with non-obstructive form of azoospermia

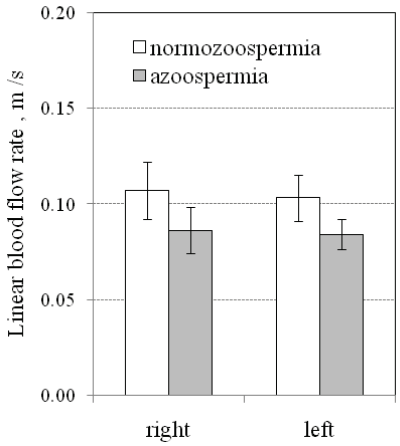

Fig. 4. Average values of the linear blood flow rates in the arteries of the testicular parenchyma in men with azoospermia: x ± SE, n = 119; differences are not significant

Thus, the hemodynamic parameters of the scrotum show that the most pronounced changes are found in men with azoospermia in the absence of spermatogenesis.

As depicted in Figure 5, positive correlations between the linear flow rates in the arteries of the testicular parenchyma and ejaculate volume was noted as moderate ($r = 0.59$; $P < 0.05$). No other significant correlation between hemodynamic parameters of the testicular artery and the routine semen measures was found.

Discussion

It is known that men with infertility, especially associated with azoospermia, belong to a difficult category of patients, due to the difficulty of diagnosing the causes and degree of the spermatogenesis disorder and the choice of treatment methods too. The fact that the average age of patients who applied to the relevant clinic for infertility is 28.6–31.5 years indicates their long journey to a specialist doctor and the probable unjustified treatment at the polyclinic level without a clearly established diagnosis. In a significant percentage of patients with NOA (11.6%), the cause of infertility can be such extragenital pathologies as arterial hypertension, diseases of the gastrointestinal tract, liver, and kidneys. Hereditary diseases were not detected in the examined patients. Among the clinical symptoms, the most common were erectile dysfunction, depressive states, and dysuria. All examined 69 patients of the secretory group had complaints about the absence of pregnancy in their wives for more than one year. The anamnesis of the patients' lives was not burdensome, it was proven that there were no injuries, epidemic parotitis, or surgical interventions on the genitals.

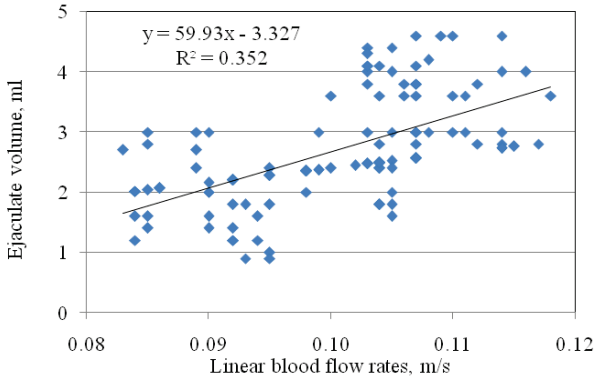

Fig. 5. Correlation relationship between the ejaculate volume and the linear blood flow rates in the arteries of the testicular parenchyma in patients with non-obstructive form of azoospermia (n = 119)

In patients with azoospermia in the absence of spermatogenesis, there are significant changes in both the size of the testicles and their echogenicity. Testicular hypoplasia is observed. Microcalcifications were found in 30.3% of azoospermic men. According to the literature, microcalcification inside the testicular parenchyma can be detected in 0.6–9.0% of men who are referred for one reason or another to ultrasound of the testicles (Richenberg et al., 2015; Pedersen et al., 2016). The relationship between testicular microcalcifications and infertility is unknown, but may be related to testicular dysgenesis, where degenerated cells exfoliate inside the seminiferous tubules and block them, and Sertoli cells are unable to phagocytose them. Subsequently, tubule calcification occurs.

The hemodynamic parameters of testicular parenchymal blood flow in infertile men obtained by ultrasound dopplerography are of important diagnostic value (Horpinchenko et al., 2016). Thus, the average value of the linear velocity of blood flow in the arteries of the parenchyma in the men with azoospermia examined by us was lower compared to the control values.

A potential cause of male infertility can be an infection of the male genitourinary tract (Gimenes et al., 2014; Salonia et al., 2022). WHO considers urethritis, prostatitis, orchitis, and epididymitis to be infections of male accessory glands (Horpinchenko et al., 2016; Salonia et al., 2022). The effect of symptomatic or asymptomatic infections on sperm quality is controversial (Gimenes et al., 2014). A systematic review of the relationship between sexually transmitted infections, such as those caused by chlamydia, mycoplasma, neisseria, trichomonads and viruses, and infertility failed to establish a strong association between sexually transmitted infections and male infertility due to the limited quality of reported data (Fode et al., 2016).

As for obstructive azoospermia, its most common cause is obstruction of the vas deferens due to epididymitis, which affects 30–67% of men with azoospermia. Congenital obstruction is usually 82% associated with at least one CF gene mutation. Other causes may be injuries or surgical intervention (Jarvi et al., 1998; Sajadi et al., 2019).

According to WHO recommendations, non-obstructive azoospermia (NOA) is defined as the absence of spermatozoa when analyzed after centrifugation, usually with a normal ejaculate volume. This conclusion should be confirmed by at least two consecutive sperm analyses (Melnyk et al., 2022). Severe impairment of spermatogenesis observed in patients with NOA is a consequence of primary testicular dysfunction or may be associated with dysfunction of the hypothalamic-pituitary-gonadal axis and oxidative stress (Horpinchenko et al., 2016; Aitken, 2020).

The diagnosis of NOA is based on two consecutive sperm tests confirming azoospermia. Patients with NOA should undergo a comprehensive examination, including genetically transmitted diseases, comorbidities

associated with health (Kasman et al., 2019). Nonobstructive azoospermia can be the first sign of a tumour of the pituitary gland or testicular germ cells (Fallick et al., 1999; Dieckmann & Pichlmeier, 2004; Ozturk et al., 2013). It has been proven that patients with NOA have an increased risk of developing cancer (Eisenberg et al., 2015).

A number of studies have shown that varicocelectomy can lead to the appearance of sperm in the ejaculate in men with azoospermia. In one such study, microsurgical varicocelectomy in men with NOA resulted in 20.8–55.0% of spermatozoa in the ejaculate (Sajadi et al., 2019).

Conclusions

It was shown that among the patients examined by us, 58.0% were diagnosed with a non-obstructive form of azoospermia, and 42.0% with an obstructive form. According to ultrasound, the volume of the testicles in patients with azoospermia was on average 1.3 times smaller than in patients with normozoospermia. Among the extragenital pathologies, the most frequent cases of the examined patients with non-obstructive form of azoospermia were arterial hypertension and chronic gastritis. The most common clinical symptoms in the examined patients were erectile dysfunction, dysuria, depressive states, and epididymitis. The volume of the testicles in patients with azoospermia was 1.3 times smaller compared to controls and they were significantly hypoplastic. Hemodynamic indicators of testicular parenchymal blood flow in infertile men with azoospermia were lower compared to control values.

Our results sustain that the evaluation of testicular hemodynamics of infertile men can be helpful in fertility assessment and for the evaluation of protective treatment.

The authors declare no conflict of interest.

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