RESEARCH ARTICLE

Altered Sperm Analysis, Where Are We Now? The Reality of the Reproductive Medicine Unit of a Tertiary University Center

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Abstract

Background: In the last decades, a substantial decline in sperm counts has been described. We aim to evaluate clinical outcomes of treatment strategies in patients referred to andrology due to abnormal sperm parameters.

Materials and methods: Observational descriptive study performed at one country's largest public reproductive unit during 5 years (2015–2019). Patients were selected according to the World Health Organization (WHO) criteria for sperm analysis.

Results: A total of 670 patients were included, with a median age of 35 (18–54) years old. During the infertility investigation, 15 patients were diagnosed with Klinefelter syndrome and two with a disorder of sexual development (46, XX). Three malignant testicular tumors were identified. Among treatments applied—varicocele correction was the most frequent (31.49%, $n = 210$), followed by antioxidant therapy (11.04%, $n = 74$). Posttreatment sperm analysis was obtained in 255 cases, demonstrating an improvement in 58.04% ($n = 148$), including normalization in 6.67% ($n = 17$) of men. An assisted reproductive technique (ART) was performed in 45.97% ($n = 308$), with a clinical pregnancy rate per initiated cycle of 26.92% ($n = 91$). Among azoospermic patients (19.55%, $n = 131$), there was a higher exposure to heat ($p = 0.02$) and a higher prevalence of previous testicular surgery ($p = 0.002$), and orchitis ($p = 0.022$). Varicocele was more frequent in non-azoospermic patients ($p = 0.007$), while Y chromosome microdeletions and chromosomal alterations were mostly found in azoospermic patients ($p = 0.004$ and $p < 0.001$, respectively).

Conclusion: Even in patients with a poor prognosis *a priori,* improvements in sperm parameters or sperm retrieval might be achieved after treatment, namely low-grade varicocele correction.

Clinical significance: An adequate investigation of male infertility can improve reproductive outcomes and contribute to an early diagnosis/secondary prevention of tumors.

Keywords: Azoospermia, Infertility, Intracytoplasmic, Male, Oligospermia, Sperm injections, Sperm retrieval, Varicocele.

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Introduction

The WHO estimates that in 50% of couples with infertility issues, the male factor isolated is found in 30% of cases and in combination with female factors in 20%.[1–5]

In the last decades, several studies have described a substantial decline in sperm counts, namely one systematic review that claimed a decrease of 50–60% (1973–2011).[3] In fact, over 90% of male infertility is due to poor sperm quality, low sperm counts, or these two concomitantly.[2] Various conditions can account for male infertility, namely anatomical defects, genetic abnormalities, systemic diseases, infections, gonadotoxins, and trauma, among others.[5] Among genetic causes, chromosomal abnormalities can deteriorate testicular function, and Y chromosome microdeletions are responsible for isolated spermatogenic defects.[3] Some childhood events (testicular trauma or torsion) or congenital defects like cryptorchidism may also contribute to the fertility issue.[3] Varicocele is one of the main causes, being present in 40% of infertile men.[1,3,6]

Nevertheless, the literature reports a significant number of cases in which no cause is identified, ranging from 30 to 40%, and thus classified as idiopathic.[1,5] Oxidative stress affects 37 million infertile men.[3] Exposure to environmental chemicals and toxic consumption

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habits like smoking, alcohol, recreational drugs, and excessive fat intake, causing obesity, are also considered risk factors for infertility.[3]

Oligospermia is the term used for a low concentration of sperm, and azoospermia means there is no sperm in the ejaculate.[2] Abnormalities regarding morphology and low motility of spermatozoa can also be present,[2] whether or not there is oligospermia. Intracytoplasmic sperm injection (ICSI) has allowed pregnancies even with very poor semen quality, like in cases of azoospermia, through surgical testicular/epididymal sperm retrieval methods.[2,3]

Moreover, there is increasing evidence in the literature suggesting that male infertility might be linked to other hidden medical health conditions, such as cardiovascular, oncological, autoimmune, and chronic systemic diseases, leading to a decrease in life expectancy.[4,5] It is thought that the combination of genetics, environmental, and lifestyle factors can contribute to these associations.[4] Considering this, a complete evaluation of the infertile man also provides a chance to improve health outcomes.[5,7]

Considering the current knowledge on male infertility, we aim to evaluate medical history and the clinical outcomes of treatment strategies applied to patients referred to andrology due to altered sperm parameters. This study contributes to the investigation of the global trends in the male infertility issue.

Materials and Methods

We performed a descriptive observational study at the reproductive medicine unit of a university hospital between January 2015 and December 2019.

Patients were selected according to the WHO Laboratory Manual for the Examination and Processing of Human Semen and Sperm-Cervical Mucus Interaction 5th Edition (2010),[8] when one or more criteria were present, as follows—sperm concentration $<15 \times 10^6$/mL accounting for oligospermia and sperm concentration $<5 \times 10^6$/mL accounting for severe oligospermia; asthenospermia defined as progressive sperm motility <32%; teratospermia defined as <4% of normal sperm morphology. The semen was collected after 3–5 days of sexual abstinence.

A descriptive analysis of the demographic and clinical characteristics of the patients was performed, including lifestyle factors, diagnostic evaluation, interventions, and outcomes. Descriptive statistics were analyzed as mean and standard deviation for the variables with normal distribution and median and interquartile range for the variables without normal distribution. Variables were also described in absolute numbers (*n*). The nominal variables were compared using Pearson's Chi-squared test or Fischer's exact test according to Cochran's rules. The normal distribution of the ordinal variables was evaluated using the Kolmogorov–Smirnov test (considering a population sample of >30 individuals in both groups).

The comparison of these variables was tested using student's *t*-tests (parametric test, applied after verifying the homogeneity of variances by Levene test) or Mann–Whitney *U* (nonparametric test). Statistical analysis was performed using the Statistical Package for the Social Sciences Statistics version 24.0®. A type I error of 0.05 was considered.

This paper was written considering the ethical and legal principles and in accordance with the recommendations of the Declaration of Helsinki of the World Medical Association. The anonymity of all the participants of this work was guaranteed, and as this study constitutes a retrospective analysis of data derived from routine clinical examination, ethical committee approval was not required.

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Results

A total of 670 patients with altered sperm parameters were included in the analysis. The demographic features, medical history of the patients, and the exogenous factors exposure are described in detail in Table 1, respectively.

Age

The median age of patients was 35 years, ranging from 18 to 54 years old.

Sperm Evaluation

Regarding initial sperm alterations—teratospermia was diagnosed in 5.22% (*n* = 35), asthenozoospermia in 6.27% (*n* = 42), asthenoteratospermia in 14.93% (*n* = 100), oligoasthenospermia in 3.88% (*n* = 26), oligoteratospermia in 9.55% (*n* = 64), oligospermia in 1.79% (*n* = 12), severe oligospermia in 5.22% (*n* = 35), oligoteratoasthenospermia (OAT) in 33.28% (*n* = 223), and azoospermia in 19.55% (*n* = 131) (Fig. 1).

Smoking

A great proportion of patients were smokers (40.75%, *n* = 273). There was no association between sperm parameters and smoking (51.13%, *n* = 203 vs 57.14%, *n* = 156 for concentration; 62.88%, *n* = 249 versus 63.00%, *n* = 172 for morphology; and 50.08%, *n* = 230 versus 58.61%, *n* = 160 for mobility, $p > 0.05$). There was also no association between smoking and azoospermia (19.44%, *n* = 77 vs 19.78%, *n* = 54, $p > 0.05$).

Y Chromosome Microdeletions

The investigation of Y chromosome microdeletions was performed in 282 men (42.09%), being detected in 15 cases. The majority was azoospermia factor (AZF) subregion C in eight cases, AZFa in three cases, AZFb in two cases, and the remaining two cases had the three types concomitantly.

Among the patients with Y chromosome microdeletions, from the eight AZFc patients, three had OAT and five azoospermia; from the AZFa, all three had azoospermia; from the AZFb, one had asthenoteratospermia and one azoospermia; the two patients with deletions in the three regions had severe oligospermia and azoospermia, respectively.

Chromosomal Abnormalities

The analysis of chromosomal changes was performed in 289 men (43.13%), and an alteration was detected in 30 cases. Klinefelter syndrome was the main diagnosis, with 53.33% (*n* = 16), followed by 46, XX (a disorder of sex development) in 6.67% (*n* = 2); there was one case with 47, XYY, one with 46, X del(Y)q11.23-q12, and another with a balanced carrier translocation. The remaining singular cases

Table 1: Demographics, passed medical history, and exogenous factors exposition of the patients included

Variable	*Population cohort (n = 670)*
Biometry	
Weight excess[a)]	37.31% (*n* = 250)
Obesity[b)]	14.78% (*n* = 99)
Endocrine disturbs	
Congenital adrenal hyperplasia (CAH)	0.15% (*n* = 1)
Diabetes mellitus	1.19% (*n* = 8)
Other conditions	
Infiltrative disease	0.90% (*n* = 6)
Spinal cord injuries	0.15% (*n* = 1)
Human immunodeficiency virus ± hepatitis C/B	0.30% (*n* = 2)
Multiple sclerosis	0.30% (*n* = 2)
Ankylosing spondylitis	0.30% (*n* = 2)
Oncological disease	1.04% (*n* = 7)
Testicular or genitourinary tract disorders	
Orchitis	0.75% (*n* = 5)
Unilateral/bilateral cryptorchidism	4.63% (*n* = 31)
Testicular cord torsion	0.75% (*n* = 5)
Testicular trauma	0.90% (*n* = 6)
Testicular tumor	0.60% (*n* = 4)
Orchidopexy	4.33% (*n* = 29)
Unilateral orchidectomy	2.09% (*n* = 14)
Previous varicocele correction	1.49% (*n* = 10)
Other testicular surgery	1.94% (*n* = 13)
Hypospadias	0.15% (*n* = 1)
Inguinal hernia	4.48% (*n* = 30)
Zinner Syndrome[c)]	0.15% (*n* = 1)
Chronic kidney disease	1.04% (*n* = 7)
Exogenous factors exposition	
Heat	4.03% (*n* = 27)
Toxic chemicals	0.60% (*n* = 4)
Chemotherapy	1.04% (*n* = 7)
Radiotherapy	0.45% (*n* = 3)
Smoking habits	40.75% (*n* = 273)
Moderate to frequent alcohol consumption	6.72% (*n* = 45)
Narcotics consumption	1.19% (*n* = 8)
Other drugs[d)]	3.88% (*n* = 26)

[a, b)] Considering body mass index above 25 or 30 kg/m^2, respectively; [c)] Rare disease, composed of renal agenesis plus seminal vesicle cysts and ejaculatory ducts obstruction; [d)] Including sulfasalazine, cyclosporine, corticosteroids, tacrolimus, methotrexate, azathioprine, sirolimus, mycophenolate mofetil, selective serotonin reuptake inhibitors, venlafaxine, carbamazepine, topiramate

were classified as "others." Regarding the 16 patients with Klinefelter syndrome, in 68.75% (*n* = 11), an hypergonadotropic hypogonadism was identified; two patients had OAT, and 14 were azoospermic. Only one patient had a previous genetic diagnosis before being referred to ART. The other 15 patients had their diagnosis during the infertility workup.

Hormonal Evaluation

The parameters were evaluated in 59.70% (*n* = 400) of the patients—3.50% (*n* = 14) had hypogonadotropic hypogonadism, 15.00% (*n* = 60) had hypergonadotropic hypogonadism, 2.00% (*n* = 8) hyperprolactinemia, and 0.75% (*n* = 3) hypothyroidism.

Imaging Evaluation

In 58.21% (*n* = 390) of the patients, a scrotal ultrasound was performed, whose results are described in Table 2. The classification system of varicocele used was Sarteschi's classification. Transrectal ultrasound (TRUS) was performed in 0.90% (*n* = 6) of the patients, with the following reports—ejaculatory duct obstruction (EDO) conditioning seminal vesicles ectasia in 28.57% (*n* = 2), hypoplastic vas deferens with no obstruction signs in 14.29% (*n* = 1), prostatitis sequelae in 28.57% (*n* = 2), and ectasia vas deferens in 14.29% (*n* = 1).

Sperm Retrieval Procedures

Conventional testicular sperm extraction (TESE) was performed in 6.57% (*n* = 44) of the patients, percutaneous epididymal sperm aspiration (PESA)/TESA in 1.49% (*n* = 10), and both procedures in 4.63% (*n* = 31). The laboratory reports revealed no spermatozoon in 61.18% (*n* = 52), immeasurable/rare spermatozoa in 21.18% (*n* = 18), and measurable to a significant number in 17.65% (*n* = 15).

The mobility report revealed 14 cases with only immobile spermatozoa, 10 with *in situ*, and nine with mobile.

Regarding pathology analysis—no alterations suggestive of obstructive cause were found in 1.33% (*n* =1), a Leydig tumor in 2.67% (*n* = 2), Sertoli-cell only in 30.67% (*n* = 23), atrophy in 1.33% (*n* = 1), a seminoma in 1.33% (*n* = 1), slight hypospermatogenesis in 14.67% (*n* = 11), moderate/significant spermatogenesis in 38.67% (*n* = 29), changes consistent with Klinefelter syndrome in 1.33% (*n* = 1) and in the other cases there was not enough material for diagnostic purposes.

Testicular Tumors

There were 5 cases of testicular tumors—one Leydig tumor, which had been previously diagnosed and treated before evaluation in our unit, two Leydig tumors and one seminoma diagnosed during infertility investigation, and one case of seminoma diagnosed when the patient was still under reproductive follow-up and after admission in the emergency department due to acute testicular complaints.

Causal Factors and Female Factor

We have classified the causal factors of male infertility into five main groups, acknowledging that patients may have more than one contributing factor—varicocele in 41.79% (*n* =280), secretory azoospermia in 19.10% (*n* = 128), environmental/behavioral factors in 15.22% (*n* = 102), others in 12.25% (*n* = 82) and idiopathic in 11.64% (*n* = 78). A female factor was contributing to the couples' infertility in 51.04% (*n* = 342) of cases—18.51% (*n* = 124) had an endometriosis diagnosis, 11.94% (*n* = 80) cases had a tubal factor identified, 7.46% (*n* = 50) presented a diminished ovarian reserve, in 5.82% (*n* = 39) anovulation was diagnosed and in 2.09% (*n* = 14) there was a uterine cause.

Treatment Strategies and Posttreatment Semen Analysis

Varicocele correction was the most frequent treatment in 31.49% (*n* = 210) of cases, antioxidant therapy in 11.04% (*n* = 74), hormone treatment (anastrozole/clomiphene citrate) in 3.88% (*n* = 26), antibiotics in 2.09% (*n* = 14), venotropics in 0.15% (*n* = 1). In 56.12% (*n* = 376), only lifestyle changes were recommended.

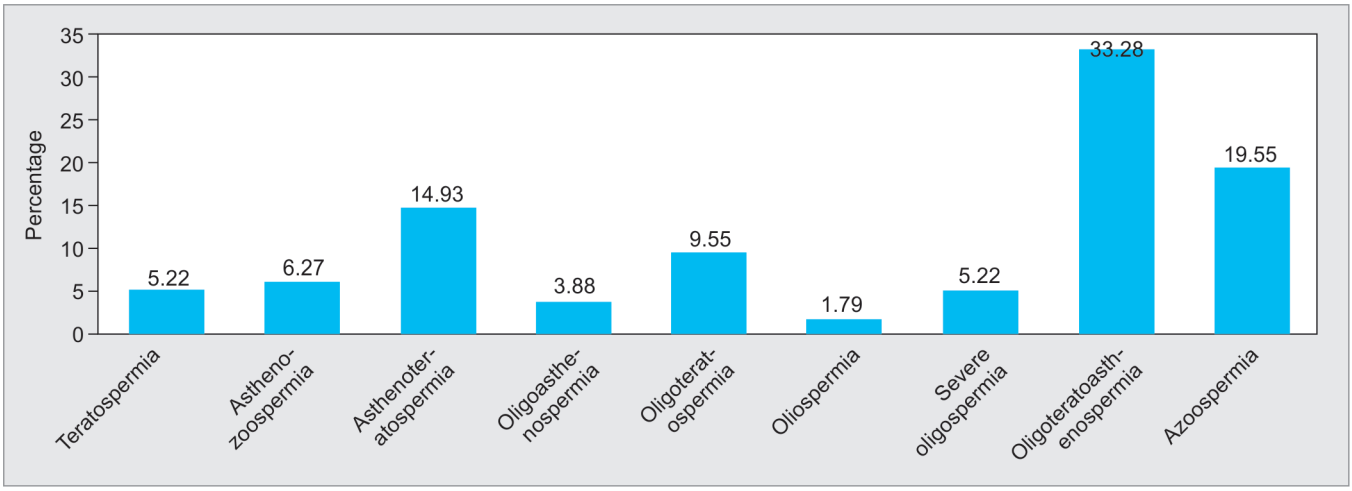


Fig. 1: Initial sperm evaluation ($n = 670$)

Table 2: Echo Doppler results

Variable	*Population cohort ($n = 390$)*
Grade 1 varicocele[a)]	13.59% ($n = 53$)
Grade 2 varicocele[a)]	26.22% ($n = 102$)
Grade 3 varicocele[a)]	30.08% ($n = 117$)
Grade 4 varicocele[a)]	8.74% ($n = 34$)
Unilateral/bilateral hydrocele	5.66% ($n = 22$)
Signs of chronic inflammation	5.40% ($n = 21$)
Reduced size testicle(s)	3.60% ($n = 14$)
Absence of one testicle	0.51% ($n = 2$)
Epididymal cysts/spermatocele	4.11% ($n = 16$)
Testicular fibrosis	0.51% ($n = 2$)
Benign nodular lesion/testicular tumor	1.29% ($n = 5$)
Cryptorchidism	1.03% ($n = 4$)
Testicular heterogeneity	2.06% ($n = 8$)
Epididymal fibroma	0.26% ($n = 1$)
Testicular microlithiasis	1.29% ($n = 5$)

[a)]The varicocele classification system used was Sarteschi's classification

During these 5 years, there was a drop out of some patients due to separation from their partner or opting for a private center for infertility treatment. Considering only the patients submitted to some treatment/intervention (excluding lifestyle changes only), the posttreatment sperm analysis results of 255 cases show an improvement of 58.04% ($n = 148$). Figure 2 specifies the posttreatment sperm evaluation of patients submitted to varicocele correction and antioxidants, respectively.

In the 17 patients whose sperm parameters normalized, there were five cases of OAT (four varicocele correction and one antioxidant), four cases of asthenoteratospermia (four varicocele correction), three cases of teratospermia (two varicocele correction and one antioxidant), two cases of asthenozoospermia (two varicocele correction) and oligospermia (one varicocele correction and one antioxidant) and one case of oligoasthenospermia (antioxidants).

Assisted Reproductive Technique (ART)

Overall, and until the end of 2019, ART has performed in 45.97% ($n = 308$) patients—one single cycle in 90.58% ($n = 279$), two in 8.77% ($n = 27$), three in 0.32% ($n = 1$) and four in 0.32% ($n = 1$). At the first attempt, ICSI ± intracytoplasmic morphologically selection sperm injection (IMSI) was used in 81.49% ($n = 251$), *in vitro* fertilization (IVF) in 13.64% ($n = 42$) and intrauterine insemination (IUI) in 4.87% ($n = 15$). At the second attempt, ICSI ± IMSI was performed in 81.48% ($n = 22$) and IVF in 18.52% ($n = 5$). Only two cases had a third attempt and one a fourth one.

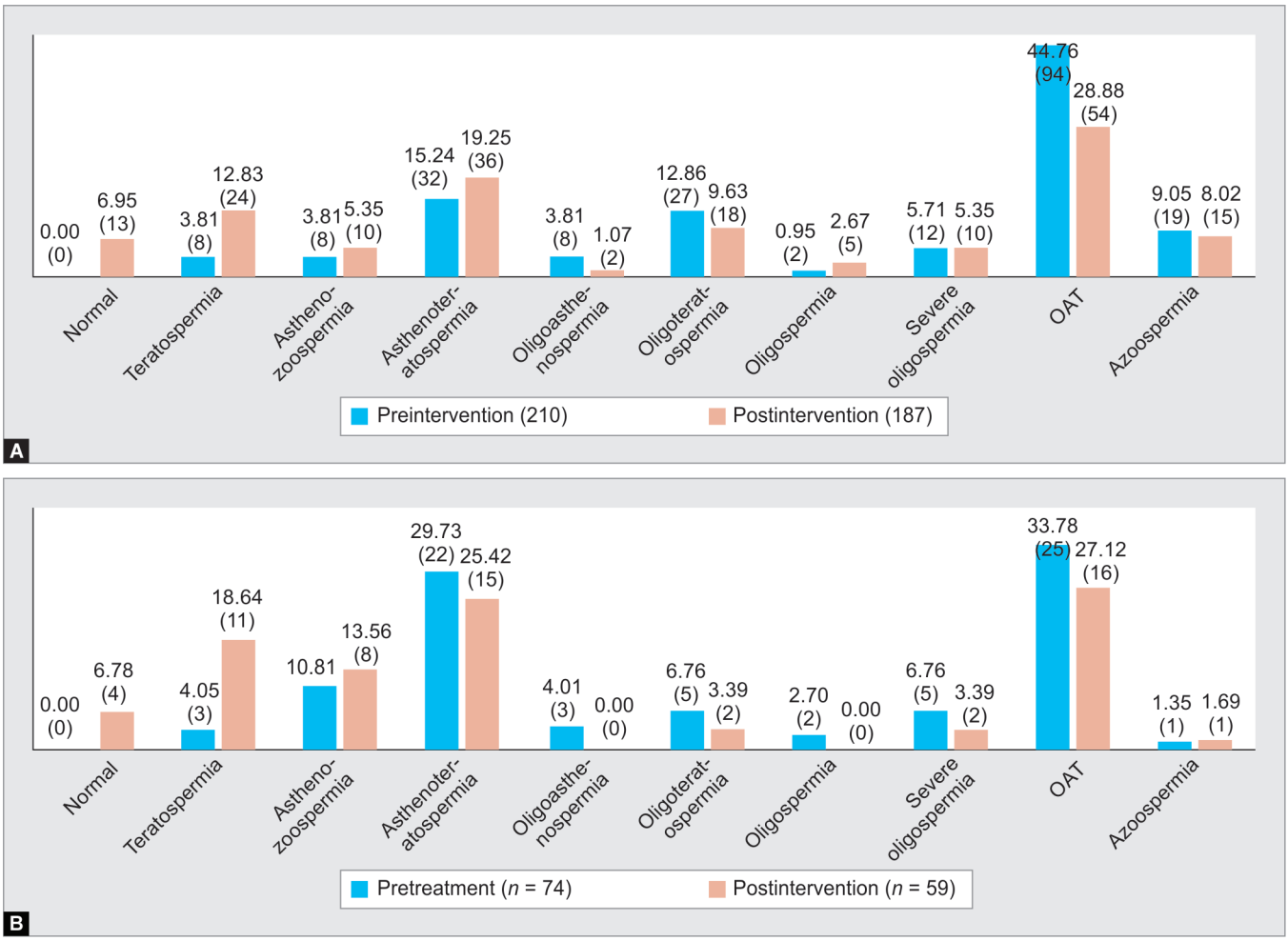
Reproductive Outcomes

The clinical pregnancy rate per initiated cycle (total number of cycles 338) was 26.92% ($n = 91$), and the rate of spontaneous abortion/ectopic pregnancy/medical interruption of pregnancy was 13.19% ($n = 12$). The live birth rate was 25.65% ($n = 79$) when considering the 308 patients submitted to ART. At the same time, 10.00% ($n = 67$) of the patients had a spontaneous pregnancy. Sperm donation was offered to 12.53% ($n = 84$). Among the 67 spontaneous pregnancies, 8.96% ($n = 6$) were patients with sperm parameters normalization after treatment/intervention. In this group of 17 patients with sperm normalization, the other two had a successful pregnancy obtained by ART, thus achieving a rate of 47.06% ($n = 8$) of newborns in this subgroup.

Azoospermia

Most cases of azoospermic patients ($n = 131$) were secretory azoospermia (98.47%, $n = 129$) and only 2.29% ($n = 3$) obstructive azoospermia (0A). The causes of azoospermia are shown in Table 3. When comparing the azoospermic patients with the non-azoospermic patients, in the former, there was a higher exposure to heat ($p = 0.02$) and a higher prevalence of previous testicular surgery ($p = 0.002$) and orchitis ($p = 0.022$). The presence of varicocele was higher in the non-azoospermic patients ($p = 0.007$), and Y chromosome microdeletions and chromosomal alterations were more frequent in the azoospermic patients ($p = 0.004$ and $p < 0.001$, respectively). Hypergonadotropic hypogonadism was also more frequent in azoospermic patients ($p < 0.001$).

Testicular sperm extraction (TESE) and/or PESA/TESA were performed in 76 azoospermic patients. Regarding treatment/interventions, these were performed in 18.32% ($n = 24$) of these patients, with 19 varicocele corrections, five hormonal therapy, and one with antioxidant therapy. Of these, 25.93% ($n = 7$) had sperm parameters improvement—four OAT, two severe oligospermia,



Figs 2A and B: (A) Pre (210) and postintervention (187) sperm analysis of patients submitted to varicocele correction and (B) Pre (74) and posttreatment (59) sperm analysis of patients who underwent antioxidant therapy, % (*n*) (OAT, oligoteratoasthenospermia)

and one asthenozoospermia. These seven patients had all been submitted to varicocele correction (three grade II and four grade III), and one had also been on hormonal therapy. Thus, ART was applied in 17.56% ($n = 23$) leading up to one spontaneous abortion [frozen embryo transfer (FET) after ICSI + TESE] and four successful evolutive pregnancies (one FET after ICSI + TESE, two ICSI + PESA/TESA, and one ICSI + ejaculate. No spontaneous pregnancies were reported in these patients. Sperm donation was offered in 45.80% ($n = 60$) of azoospermic patients.

Discussion

Age

There is no clear definition of advanced paternal age in literature.[9] A median of 35 and a maximum of 54 years old in our sample reflect the trend of increasing average paternal age reported across European countries.[9] Indeed, it is well established that aging affects sperm quality and, thus, fertility rates and ART outcomes.[9]

Sperm Evaluation

In 2021, a cohort study with 1824 infertile men reported a rate of isolated teratospermia of 11.9%, isolated asthenozoospermia of 13.9%, and isolated oligospermia of 4.1%.[10] In comparison, our rates of isolated terato and asthenozoospermia were less than half, but our rate of isolated oligospermia (including severe cases) was higher (7.01 vs 4.1%). Moreover, the literature describes a prevalence of azoospermia among infertile men of 10–15%;[11] thus, here, we report a higher rate than expected (19.55%). Nevertheless, in our study, we have only included infertile men with altered sperm parameters or absence of spermatozoa, while existing studies may have included infertile men with normal sperm evaluation.

Smoking

Tobacco's effect on sperm parameters has been demonstrated by several studies in the last decades.[12] In 2012, The American Society of Reproductive Medicine published the following state "semen parameters and results of sperm function tests are 22% poorer in smokers than in nonsmokers, and the effects are dose-dependent."[12,13] Although many studies consensually report an effect on sperm concentration and some in morphology, a recent meta-analysis did not find changes in sperm motility.[14] In this meta-analysis, the rate of smokers among their sample of infertile men was 48.57%.,[14] which is higher than ours. We did not find significant differences either in motility, concentration, or morphology between smokers and nonsmokers, but once again, our population has a 100% prevalence of sperm changes and also the different number

Table 3: Identified possible causes for the patients with azoospermia

Variable	*Population cohort (n = 131)*
Smoking habits	41.22% (*n* = 54)
Alcohol consumption	7.63% (*n* = 10)
Weight excess/obesity	48.09% (*n* = 63)
Heat exposure	7.63% (*n* = 10)
Hypogonadotropic hypogonadism	3.05% (*n* = 4)
Hypergonadotropic hypogonadism	28.24% (*n* = 37)
Oncologic condition	1.53% (*n* = 2)
Drugs/radiation	3.05% (*n* = 3)
Varicocele	41.22% (*n* = 54)
Y chromosome microdeletion	7.63% (*n* = 10)
AZFa	2.29% (*n* = 3)
AZFb	0.76% (*n* = 1)
AZFc	3.82% (*n* = 5)
AZFa + AZFb + AZFc	0.76% (*n* = 1)
Chromosomal alteration	16.79% (*n* = 22)
Klinefelter syndrome	10.69% (*n* = 14)
46, X, del (Y) (q11.23-q12)	0.76% (*n* = 1)
Mosaicism XY/XXY	0.76% (*n* = 1)
Balanced reciprocal translocation not specified	1.53% (*n* = 2)
46, XX (SRY+)	0.76% (*n* = 1)
47, XY, + mar	0.76% (*n* = 1)
Mosaicism 45, X^2/46, XYY[48]	1.53% (*n* = 2)
Testicular surgery	16.79% (*n* = 22)
Orchitis	2.29% (*n* = 3)
Testicular trauma	1.53% (*n* = 2)
Testicular torsion	1.53% (*n* = 2)
Cryptorchidism	6.11% (*n* = 8)
Schwannoma plus sacrococcygeal tumor[a)]	0.76% (*n* = 1)
EDO conditioning seminal vesicles ectasia[a)]	1.53% (*n* = 2)
Idiopathic	11.45% (*n* = 15)

[a)]Cases of OA

of cigarettes smoked per day, which is not specified, may have contributed to these findings. The impact of tobacco on male fertility still needs to be clarified.[12]

Overweight

Our population had an overweight prevalence of 37.31% (*n* = 250) and 14.78% (*n* = 99) obesity. The current trend for a decline in semen parameters parallels the increasing prevalence of obesity worldwide. Several studies support the association between obesity and altered semen parameters, increased sperm deoxyribonucleic acid (DNA) damage, and decreased pregnancy rates. Studies have shown that the endocrine and environmental factors accounting for reduced fertility potentials in overweight men seem reversible in the way that dietary measures are part of the first line of treatment in a conservative approach.[15]

Y Chromosome Microdeletions

Y chromosome microdeletions are responsible for severe degrees of dyszoospermia, ranging from teratozoospermia to azoospermia, according to the Y haplotype.[16] AZFc region is affected in about 60% of cases,[16] which is consistent with our results. All except one of our patients with Y chromosome microdeletions had oligospermia or azoospermia, as expected. Although AZFb region deletions are generally considered to have a nil chance of sperm retrieval, there are some cases reported in the literature of aberrant AZFb deletion patterns compatible with sperm production,[17,18] justifying our single case of AZFb with asthenoteratospermia. The AZFa deletions are frequently the most severe form, which is consistent with our three cases of azoospermia in these patients. When a combination of deletions occurs, various spermatogenetic phenotypes can be found.[19] Since the combination of AZFb + c deletions has a poor prognosis and AZFa deletions are generally severe, it was expectable that our two cases of AZFa + b + c would present oligospermia and azoospermia.

Chromosomal Abnormalities

The most common chromosomal abnormalities among infertile men are numerical, such as 47, XXY (Klinefelter syndrome),[20] which was also the case in our sample. Nevertheless, chromosomal deletions, translocations, inversions, and insertions have also been described.[20] Reported karyotypic abnormalities among infertile men is up to 15%,[21] which is in accordance with our findings. In 2017 a case series and literature review of 46, XX patients diagnosed during fertility evaluation reported a total of 55 cases in this setting; thus, with this study, we are adding two more patients to the described ones.[22] This is a very rare condition, reported in 1:20,000 males, with different phenotype-genotype correlations.[22,23] In our two cases, the patients had testicular atrophy, which is always present in this genetic condition,[22] and a hypergonadotropic hypogonadism. One was 32 and the other 39 years old, both with normal male phenotype and weight excess. They were directly referred for sperm donation. Not in accordance with the literature that describes the 47, XXY as the second cause of chromosomal abnormalities in infertile male patients,[24] curiously, the 47, XXY karyotype was not our second most frequent finding. According to the literature, the diagnosis occurs later in life due to an undistinguishable phenotype from 46, XY male,[24] as in our case of a 39-year-old man with a normal phenotype and weight excess, smoking habits, and a bilateral hydrocele detected on scrotal echo Doppler. An OAT was the initial diagnosis, and a shared decision for sperm donation was chosen by the couple due to the low probability of pregnancy after ART and concerns about offspring transmission risks.

The absence of a previous diagnosis of all our patients but one Klinefelter patient is consistent with the literature, which reports a low awareness of this condition and a misconception that all these patients have the classical phenotype of gynecomastia, hypogonadism, a tall stature, and limited facial and body hair,[19,20] whilst currently it is well established that it can present with a highly variable phenotype. According to literature, findings of serum testosterone inferior to the normal limit can range from 65 to 85% of adult patients with Klinefelter syndrome.[25] This explains why we had 11 Klinefelter patients with hypergonadotropic hypogonadism and the remaining five with a slight hypergonadotropic eugonadism or a normal hormonal production.

Hormonal Evaluation

Studies have demonstrated that endocrinopathies are present in 9.6% of infertile men,[26] a value slightly lower than our overall rate of 12.67%. Among the three non-Klinefelter cases of hypogonadotropic hypogonadism, there was a case of congenital

adrenal hyperplasia and a mosaic—45, X/46, XYY. The cases of hyperprolactinemia and hypothyroidism had prolactin and thyroxin values slightly above the normal range, thus not enough to be considered the main cause of the fertility issues.

Imaging Evaluation

Scrotal ultrasound is the most frequent imagiological exam performed in infertile men due to its advantages of safety, noninvasive, and wide availability. As reported, in our study, we have considered varicocele as the causal factor of infertility in 41.79% since some patients with ultrasound-diagnosed low-grade varicocele had other relevant condition(s) accounting for infertility. This is slightly higher than the varicocele prevalence reported in literature for infertile men—up to 40%.[1,3]

Transrectal ultrasound (TRUS) has a significant role in evaluating cases of suspected OA through detecting situations of congenital absence of vas deferens, obstructive ejaculatory ducts conditions, median prostate cysts, seminal vesical ectasia, or emptying alterations, prostate/seminal vesicle inflammation or stasis.[27] Six patients were submitted to TRUS, and two were diagnosed with EDO. EDO can be congenital or acquired, and according to the literature, it is responsible for 1–5% of male infertility,[11] clearly above our findings. The condition has different degrees of manifestation, from oligoasthenospermia to OA,[11] and our two patients had the latter one. Dilated seminal vesicles are expected, and TRUS can confirm its diagnosis.[11] Our other patient with OA, who had as the only relevant clinical history a schwannoma plus sacrococcygeal tumor, was classified as OA due to a TESE suggestive of obstructive cause.

Sperm Retrieval Procedures

The surgical retrieval of sperm is crucial in the treatment of infertility in azoospermic men. The available procedures included conventional TESE and/or TESA/PESA, according to the etiology of the azoospermia. Hence, in this study, 85 men were submitted to TESE and/or TESA/PESA, and the majority of them, 89.41% ($n = 76$), were azoospermic. TESA/PESA was initially preferred in cases of OA. In some cases, when the first decision of TESA/PESA fails, andrologists also perform TESE, which generally shows a lower quantity and quality of sperm.[28] Despite this, TESE is the treatment choice in cases of non-OA.[29]

Testicular Tumors

We report three cases of malignant testicular tumors discovered during the infertility investigation (two Leydig tumors and one seminoma). Not often, scrotal ultrasound may prove to be a useful tool in detecting occult and early-stage testicular masses that would go unnoticed until discovered by palpation or producing symptoms. It has been demonstrated that infertile men have an increased risk of testicular cancer, and poor semen parameters can be considered a biomarker of this tumor.[30] The prevalence of infertile men with testicular cancer is described as 0.5%,[31] which is comparable to our 0.6% if we exclude the case diagnosed during the follow-up. In 2004, a study including 560 infertile men reported 1.4% of focal testicular lesions, a slightly higher rate than ours (1.29%, according to Table 2).[32] In comparison, a study with 1250 infertile men in 2008 reported a non-palpable testicular cancer in 0.16%;[33] thus, in our study, we emphasize the importance of scrotal ultrasound since of 670 patients; three cases were diagnosed during the infertility diagnostic workup.

Causal Factors

Similar to varicocele prevalence, our azoospermia rate of 19.10% is higher than the 10–15% described in the literature among infertile men.[34] Considering the group of patients classified as idiopathic, we have excluded every patient who had any environmental/behavioral factor possibly contributing to infertility, and this might explain our rate being much lower than the expected 30–50% described in previous studies.[3] For example, being overweight and tobacco consumption were considered contributive factors, regardless of the number of cigarettes per day.

Treatment Strategies and Posttreatment Semen Analysis

Varicocele Correction

Varicocele surgery corrected varicoceles with a grade lower than three or four. In fact, a Cochrane review has claimed that treating varicocele in unexplained subfertility may have a role in improving the chance of offspring, although this is based on low-quality data.[35] Another meta-analysis, including oligospermic and azoospermic patients, concluded that varicocele correction previous to ART improves semen parameters, pregnancy, and live birth rates. These findings are in accordance with our treatment options. Four patients with OAT submitted to varicocele correction had a normal posttreatment semen analysis.

Antioxidants

Oxidative stress results from a gain in the levels of reactive oxygen species (ROS) and loss of antioxidants and is currently accepted to be one of the main male infertility causes.[36] Oral antioxidants were the second most frequent therapy chosen for our patients, as this supplementation may decrease ROS levels in semen with potential benefits in sperm parameters. In four patients, sperm parameters were normalized after antioxidant therapy.

Hormonal Therapy

The reversible aromatase inhibitor anastrozole increases intratesticular testosterone, which is believed to potentially improve spermatogenesis, especially in obese or hypoandrogenic oligospermic patients, and thus used as empirical therapy.[37] Clomiphene citrate has also been used off-label in this setting, providing the reason for its use in our study.

Antibiotherapy

Antibiotics were used in some cases with inflammatory signs, especially when leukocytospermia was present.

There was an improvement in the rate of OAT and azoospermia in our population of men adopting these previous strategies. Our findings suggest that it is worth applying some treatment strategy even in patients with no spermatozoa at all or with a poor perspective of sperm retrieval.

Lifestyle Changes

In patients for whom no specific treatment strategy was applied, lifestyle changes were recommended. Weight loss, smoking and alcohol cessation, an adequate diet, and physical activity have been shown to significantly reduce levels of ROS and improve natural body antioxidants.[36]

Assisted Reproductive Technique (ART)

Intrauterine insemination (IUI) is the least invasive and most expensive assisted reproduction method. It was the chosen

technique in some patients with less altered semen parameters, in whom the posttreatment semen analysis showed a relevant improvement, and/or in those couples whose female patient was ≥40 years old. The latter criterion was defined by Portuguese ART law for public health services. Varicocele correction may allow a shift of ART procedures from ICSI/IVF to IUI. For IVF procedures, a minimum of 50,000–500,000 motile spermatozoa are required,[38] thus preventing its use in many cases of male infertility, and that is why ICSI has changed the paradigm of male infertility over the last decades. Nevertheless, there is no reason to use ICSI in all cases of male infertility. As proof of this, a retrospective cohort study with nonsevere male factor infertility reported similar live birth rates between IVF and ICSI, neither the pregnancy nor perinatal outcomes were better when ICSI was used in the first cycle vs IVF.[34] Although larger prospective studies are needed in this field, this study supports our choice of IVF when less severe semen parameter changes were present. ICSI takes longer to perform, involves more costs, and there are still unsolved concerns about possible gamete damage or health conditions caused by children born by this method. IMSI was also performed in many cases in order to select the best morphology of the sperm to inject. A Cochrane review concluded that there is no current evidence to support or refute the use of IMSI, and the existing studies claiming a possible increase in clinical pregnancy with this technology are of low quality.[39]

Reproductive Outcomes

Considering all ART used in our study, our clinical pregnancy rate per initiated cycle is similar to the initial pregnancy rate per ICSI cycle of 20–30% reported in a retrospective cohort study, including infertile couples with male factors.[40] Since our sample also had a high prevalence of female factors, and this constitutes an important bias, we must compare these data with caution. Additionally, we had a considerable rate of spontaneous pregnancies, although no verification of biological paternity was performed.

Azoospermia

In azoospermic patients, there was a higher prevalence of exposure to heat, previous testicular surgery and orchitis, Y chromosome microdeletions, chromosomal alterations, and hypergonadotropic hypogonadism, corresponding to some of the most common causes of azoospermia.

Although there are many causes of azoospermia, the etiologies of this disorder fall into three general categories—pretesticular, testicular, and posttesticular. Every etiology is associated with a different prognosis; the pretesticular and posttesticular abnormalities are commonly treatable; conversely, testicular disorders are generally irreversible with the exception of varicocele, and the success rates for interventions associated with intrinsic testicular abnormalities are significantly lower.[11]

Although sperm can be found in the ejaculation of azoospermic men following varicocele repair in 21–55% of cases, spontaneous pregnancies are extremely rare.[11] In our study, 18.32% (n = 24) received therapeutic interventions, which 19 underwent varicocele repair, and there was an improvement of sperm parameters in seven patients (25.93%), with four successful evolutive pregnancies following ART. There were no spontaneous pregnancies, which is in line with previous studies.

This study involved a large number of patients that were followed at a reproductive unit of a public university hospital, the largest reproductive center in our country.

Conclusion

Despite being a descriptive study, several conclusions can be drawn from our study—varicocele was the leading cause of spermatic changes, as expected; overweight, obesity, and smoking habits had a high prevalence in our population, even in patients with a poor prognosis *a priori*, improvements in sperm parameters or sperm retrieval might be achieved after an attempt of treatment, including with low-grade varicocele correction; a proper investigation of male infertility not only improves reproductive outcomes but also contributes to an early diagnosis/secondary prevention of tumors.

Ethical Considerations

This paper was written considering the ethical and legal principles and in accordance with the recommendations of the Declaration of Helsinki of the World Medical Association. The anonymity of all the participants of this work was guaranteed, and as this study constitutes a retrospective analysis of data derived from routine clinical examination, ethical committee approval was not required.

Author Contributions

Conceptualization—FS and BF. Formal analysis—FS, BF, AFF, and AC. Methodology—FS, BF, AFF, and AC. Visualization—MHC. Writing–original draft—FS and BF—writing–review and editing—AFF, AC, LS, BP, APS, PC, and TAS.

Project administration—TAS.

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