



UNIVERSIDADE
LUSÓFONA

Centro Universitário de Lisboa
Escola de Psicologia e Ciências da Vida
Mestrado em Embriologia e Reprodução Humana

Overcoming Absolute Asthenozoospermia: A Case Study on Testicular Sperm Extraction in Male Infertility Treatment

Dissertação defendida em provas públicas para obtenção do grau de Mestre em Embriologia e Reprodução Humana, orientada pela Doutora Sónia Jorge e Professora Doutora Patrícia Rodrigues

Carolina Matos Conde de Álvares Pereira, n°22201943

2025

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Infertility Treatment

VERSÃO FINAL

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Resumo

A infertilidade é um problema a nível global, e o fator masculino tem nela um papel importante. A motilidade, ou a falta dela, é um dos seus aspetos essenciais.

A astenozoospermia absoluta, definida como 100% de espermatozoides imóveis, apresenta um desafio significativo em procriação assistida. Este caso de estudo avalia a possibilidade do uso de extração de esperma testicular [TESE], juntamente com ICSI, para a ultrapassar.

Um homem de 45 anos com astenozoospermia absoluta realizou múltiplos ciclos de ICSI sem sucesso, o último devido a uma falha completa de fertilização, depois dos oócitos terem sido micro-injetados com espermatozoides imóveis, vindos do ejaculado.

No ciclo atual, uma TESE foi realizada com o propósito de recolher esperma testicular, revelando espermatozoides como motilidade não progressiva. ICSI com estes espermatozoides resultou numa taxa de fertilização de 66,67%, um aumento significativo [$p=0,009$] face ao ciclo anterior.

O casal foi assim capaz de obter embriões, um dos quais foi transferido. Apesar de não ter resultado em gravidez, os resultados sugerem que esperma testicular pode ser uma opção viável de ultrapassar falhas de fertilização no caso de astenozoospermia absoluta. Será necessária mais investigação para determinar a utilidade clínica de utilizar TESE nestes casos de motilidade severamente comprometida.

Palavras-chave: astenozoospermia absoluta, TESE, ICSI, infertilidade masculina, falha de fertilização

Abstract

Infertility is a global health issue, and the male factor plays a significant role. Motility, or lack thereof, is one of its key aspects.

Absolute asthenozoospermia, defined by 100% immotile spermatozoa, presents a significant challenge in assisted reproduction. This case study evaluates the possibility of using testicular sperm extraction [TESE], in conjunction with ICSI, to overcome it.

A 45-year-old male with absolute asthenozoospermia underwent multiple failed ICSI cycles, the latest due to complete fertilization failure, after the oocytes underwent ICSI with immotile ejaculated sperm.

In the current cycle, TESE was performed to retrieve testicular sperm, revealing spermatozoa with *in situ* motility. ICSI with these sperm resulted in a fertilization rate of 66.7%, a significant improvement [$p=0,009$] over the previous cycle.

The couple was therefore able to obtain embryos, one of which was transferred. Although pregnancy was not achieved, the results suggest that testicular sperm may provide a viable option for overcoming fertilization failure in cases of absolute asthenozoospermia. Further research is needed to determine the broader clinical utility of TESE in cases of severely compromised motility.

Keywords: absolute asthenozoospermia, TESE, ICSI, male infertility, fertilization failure

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List of abbreviations, acronyms and symbols

- **APA** – American Psychological Association
- **ART** – assisted reproductive technology
- **ATP** – adenosine triphosphate
- **COC** – cumulus-oocyte complex
- **ICSI** – intracytoplasmic sperm injection
- **IUI** – intrauterine insemination
- **IVF** – *in vitro* fertilization
- **MI** and **MII** – metaphase I and II, in the context of oocytes
- **MAR** – medically assisted reproduction
- **PB** – polar body
- **PCD** – primary cilia dyskinesia
- **PN** – pronucleus/pronuclei
- **PVS** – perivitelline space
- **ROS** – reactive oxygen species
- **TESE** - testicular sperm extraction
- **WHO** – World Health Organization
- **ZP** – *zona pellucida*

1. Introduction

1.1. Brief Overview of the Male Gamete

A spermatozoon is a highly specialized haploid cell, designed to fertilize an oocyte. The female gamete, the oocyte, is a large, immotile cell, and provides the necessary materials for early life development, in addition to the maternal genes. The spermatozoon, in the contrary, contains the male genes, and through its motility carries them through the female reproductive

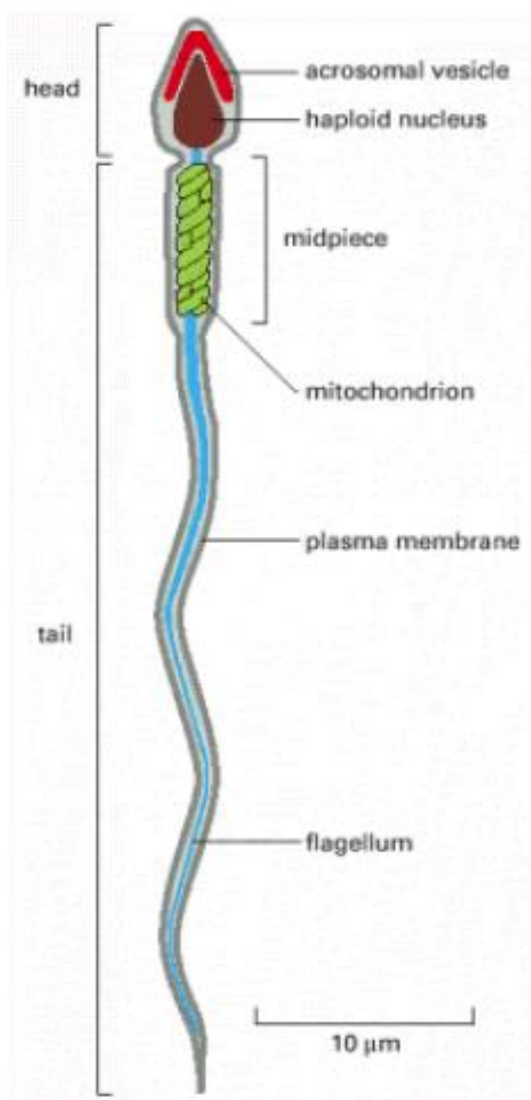


Figure 1: Human spermatozoon

(Alberts et al, 2002)

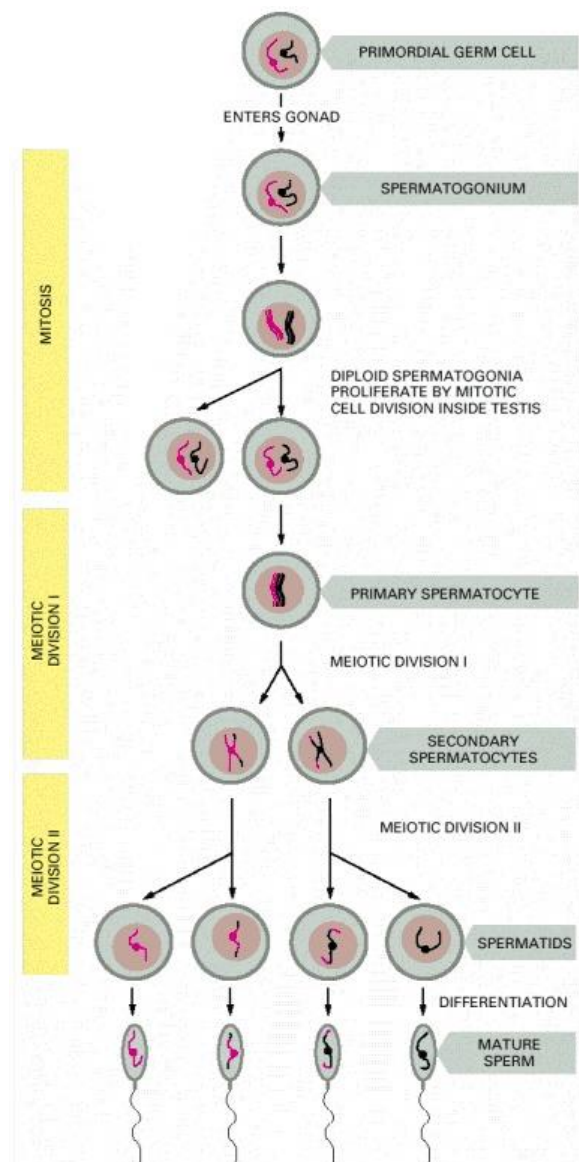


Figure 2: Spermatogenesis

(Alberts et al, 2002)

tract, enabling the encounter of the gametes necessary for fertilization (Alberts et al., 2002), and the subsequent development of life.

They are small cells, lacking all cytoplasmic components that are not needed for the spermatozoon's specific function. They consist of a head and a tail, in which the head contains the tightly packed haploid nucleus, and the tail confers it its motility [Fig. 1]. The “midpiece” in the tail, contains the mitochondria necessary to power it through the production of ATP [Fig. 1].

The creation of these cells is referred to as spermatogenesis [Fig. 2].

In humans, spermatogenesis begins during puberty. Primordial germ cells, that migrated to the testes during embryogenesis, will develop into spermatogonia. As a man reaches sexual maturity, these spermatogonia will proliferate through continuous mitosis. The daughter cells will either have the capacity to continue doing the same indefinitely, as stem-cell spermatogonia, or can instead, after a limited number of mitoses, mature into primary spermatocytes. These will go through meiosis I, becoming haploid cells through the separation of homologous chromosomes— secondary spermatocytes. The meiotic division II will then generate spermatids, haploid cells with one chromatid per chromosome (Alberts et al., 2002).

These spermatids will undergo morphological differentiation, particularly the loss of the majority of the cytoplasm, and become spermatozoa. These cells will leave the testes into the epididymis, where they are stored and continue to mature (Alberts et al., 2002).

In humans, the process of forming a spermatid from a spermatocyte takes around 24 days. The transformation from a spermatid into spermatozoon will take another five weeks (Alberts et al., 2002).

1.2. Male infertility

Clinical infertility is defined as the inability of a couple to achieve a pregnancy after a year of regular, unprotected sexual intercourse (WHO TEAM, 2023). Reproductive success is defined at the couple level, and so the cause of infertility can be attributed to either or both members of the couple (Eisenberg et al., 2023), as well as be undetermined. WHO reports that an estimated 17,5% of couples globally will experience infertility in their lifetime (WHO TEAM, 2023). There is insufficient data at a global level to determine with certainty the

geographic distribution of infertility, but the prevalence seems to be higher in central and eastern Europe, Australia, and North America (Eisenberg et al., 2023).

Worldwide, sperm quality, particularly sperm count, continues to decline, and so male infertility can and should be considered a public health, rather than just men's health, issue. It is increasingly more likely that a physician will encounter and be responsible for the treatment and health of men with infertility or abnormal sperm parameters (T. Chen et al., 2022).

Male infertility accounts for around 20-30% of all infertility cases and contributes to another 20-30%. It is estimated that infertility affects more than 30 million men globally (Agarwal et al., 2015).

There can be multiple causes for infertility in regard to the male factor. The majority is due to primary testicular defects; this includes abnormal sperm parameters without an identifiable cause. Other causes include endocrine disorders, namely hypogonadism, and sperm transport disorders. It can also be idiopathic, in cases where the male patient shows normal sperm parameters but still remains infertile (Esteves et al., 2011a; Leslie et al., 2024).

Male infertility varies by sociodemographic factors, including but not limited to geographic location, ethnicity and age. Secondary infertility is also significantly more common than primary infertility (Eisenberg et al., 2023).

There has been an increase on the use of male infertility as a biomarker for general health. Infertility and subfertility are associated with lower health and higher incidence of comorbidities, as well as an increased risk of all-cause mortality (T. Chen et al., 2022; Eisenberg et al., 2023). It can also lead to negative psychological and socioeconomic outcomes (T. Chen et al., 2022). Due to this high association with other facets of health, a man undergoing fertility treatment or being evaluated for abnormal sperm analysis could benefit from a more holistic approach, not limited to fertility counselling, but general health maintenance (T. Chen et al., 2022).

As fertility rates decrease globally, they are associated with more than just the retardation of parenthood. Despite this, research on male infertility remains insufficient. Low fertility rates will of course lead to population decline as well as an age structure with a more expansive elderly population—a situation that could be unsustainable (Eisenberg et al., 2023) as it brings forward several social and economic concerns.

Despite the recognition that infertility is a disease of the couple, ART remains largely centered on the female partner. This imbalance not only reinforces the outdated perception of reproduction as primarily the responsibility of women, but also places almost all physical strain associated with ART on them, even in cases where the underlying infertility is linked to the male factor (Eisenberg et al., 2023). Expanding the focus of ART to include more targeted treatments and prevention for male infertility could improve fertility outcomes, reduce costs, and minimize the need for more invasive procedures and treatments (Eisenberg et al., 2023).

Moreover, integrating male fertility into routine medical care could provide an opportunity for early detection and/or prevention of broader health issues, as infertility may serve as a marker for other medical conditions (T. Chen et al., 2022), as previously mentioned.

As such, increasing awareness, research, and specialized medical training in male reproductive health could make infertility treatment more equitable, ultimately benefiting not only men but also their partners, possible future offspring, and society as a whole (Eisenberg et al., 2023).

1.2.1. Causes of Male Infertility and Models of Classification

As mentioned previously, male infertility can have a myriad of causes. There are multiple ways to classify them.

One form of classification divides causes according to which section of the reproductive process they affect. These are pre-testicular, testicular, and post-testicular factors (Leslie et al., 2024).

Pre-testicular factors include endocrine disorders, such as hypogonadotropic hypogonadism, systemic issues such as erectile dysfunction, and genetics factors, including but not limited to chromosomal abnormalities. Coital disorders, such as retrograde ejaculation and anejaculation, can also be entered into this category (Leslie et al., 2024).

As the name indicates, testicular factors concern all issues regarding the testes, as well as the epididymis. The testicular causes include testicular tumors, orchiectomy, primitive testicular dysfunction, cryptorchidism, and atrophic testes. Varicocele is also included here due to its impairment of thermoregulation in the testes. Regarding epididymal causes, these include epididymal dysfunction, cysts, spermatoceles and epididymitis (Leslie et al., 2024).

Lastly, post-testicular factors are those that refer to issues that affect the transit of the sperm, outside the testes, and that impede fertilization due to the lack of encounter of the male and female gamete. These include lesions of the seminal tract, inflammatory diseases, congenital absence of the *vas deferens*, as well as vasectomies. Erectile dysfunction and premature ejaculation are re-included in this category. This category also includes barrier contraceptives, such as the use of condoms or diaphragm (Leslie et al., 2024). These last ones will of course not be considered a cause of infertility, in accordance with the WHO definition of it, that notes only the lack of pregnancy after 12 months of unprotected intercourse (WHO TEAM, 2023).

Alternatively, these causes can also be divided according to their specific etiology: acquired or congenital urogenital abnormalities, endocrinological, immunological or idiopathic causes, genetic or environmental factors, malignancies, drugs, sexual dysfunction, urogenital tract infections, and others (Leslie et al., 2024).

The last classification system mentioned here would be to categorize the causes for male infertility in accordance with their possibility of treatment, whether that is medical or through ART.

Most causes fall into uncorrectable male infertility or subfertility. These would be issues in sperm parameters that negatively affect or impede the possibility of either fecundation or subsequent pregnancy. They make up for seventy percent [70%] of cases and usually need the use of ART to overcome their deficiencies (Leslie et al., 2024).

Eighteen percent [18%] of cases are treatable, through surgical interventions, medication and/or lifestyle changes. These include cases of obstructive azoospermia, ejaculatory duct and prostatic midline cysts, gonadotropin deficiency, sexual function disorders, sperm autoimmunity, varicoceles, and reversible effects of toxins (Leslie et al., 2024).

The remaining percentage refers to untreatable male sterility, due to conditions such as primary seminiferous tubular failure, Sertoli cell-only syndrome, and bilateral orchiectomy (Leslie et al., 2024).

1.2.2. Semen Analysis and Sperm Quality Parameters

A laboratory semen analysis is necessary before any assisted reproduction technology [ART]. Here, multiple parameters, with variable importance, are evaluated, and can determine which course of action to take in the subsequent fertility treatments.

It is to be noted that the current edition of the “WHO laboratory manual for the examination and processing of human semen” doesn’t state reference values for any of the following sperm parameters. Previously, they have published these values, counting the fifth percentile of fertile men. These values were often mistakenly taken as a hard limit between fertile and infertile men, and so the current edition chose to omit them in order to broaden the interpretation of results of semen analysis (World Health Organization, 2021). For the sake of convenience, the 5th percentile is noted here as a one-sided, lower reference value in the following parameters, though, once again, it cannot be interpreted as immediately indicating infertility for anything below these values, and any values should be taken in conjunction with each patient’s clinical data and medical history.

Before any microscopic analysis, a macroscopic assessment is made. In the immediate sample handling, before liquefaction occurs, the volume of the collected sample is noted through weight, assuming a density of 1g/ml (World Health Organization, 2021). The lower reference value for volume is 1,5 milliliters (T. G. Cooper et al., 2010). Low semen volume can indicate obstruction of the ejaculatory duct, congenital bilateral absence of the vas deferens, androgen deficiency, and issues in the collection of the ejaculate. Contrarily, abnormally high semen volume can be associated with inflammation in accessory organs (World Health Organization, 2021).

After 30 to 60 minutes, the macroscopic appearance of the semen must be assessed, namely liquefaction and viscosity (World Health Organization, 2021). If pH is to be measured, it should be done at this stage, as waiting too long can mistakenly show higher pH values, due to the gradual loss of CO₂, and consequent alkalinization of the sample. A semen pH below 7.0, in a sample without spermatozoa, could indicate congenital bilateral absence of the vas deferens (World Health Organization, 2021).

It is also during this time that the microscopic analysis commences.

The microscopic appearance of the semen is noted, and multiple microscopic parameters evaluated. The standard parameters measured are concentration, morphology, and motility. Motility, both progressive and total, should be the first parameter assessed, due to its tendency to decrease through outside exposure. More on motility later.

Testing for sperm vitality should also happen as soon as possible after liquefaction, and WHO recommends this complementary exam if motility is below 40% (World Health Organization, 2021). The same is true for tests regarding anti-sperm antibodies.

Concentration and morphology are usually accessed with a fixed, diluted sample. Concentration refers to the number of spermatozoa per volume of semen. The measure of concentration, in addition to the previously measured volume, also allows the determination of total sperm count. This tends to have more importance than the sperm concentration itself, in ART (World Health Organization, 2021). It is often this total sperm count that determines which treatment to follow, as intrauterine insemination [IUI] would require the highest, and intracytoplasmic sperm injection [ICSI] the lowest.

The previous lower reference value given by WHO was 15 million spermatozoa per milliliter, and 39 million spermatozoa as the total sperm count of the ejaculate (T. G. Cooper et al., 2010). A low sperm count is referred to as oligospermia. Azoospermia refers to the inability to find any sperm in the ejaculate (World Health Organization, 2021).

In regard to morphology, the proportion of normal spermatozoa must be registered, but the laboratory should also note the proportion of deficits of each segment— those being head, midpiece, and principal piece, the last two being part of the flagellum.

High rates of abnormal morphology can be associated with defective spermatogenesis and epididymal pathologies, as well as issues in the DNA, such as high fragmentation, aneuploidies, structural chromosomal aberrations and immature chromatin. Abnormal morphology seems to be linked to lower fertilizing potential (World Health Organization, 2021). The reference value for normal forms is 4% (T. G. Cooper et al., 2010; Eisenberg et al., 2023). Decreased normal morphology is referred to as teratozoospermia.

One of the factors most linked to fertilizing potential, out of the ones assessed during microscopic semen analysis, seems to be motility (Dcunha et al., 2022; Donnelly et al., 1998; Shulman et al., 1998).

1.3. Sperm Motility

Sperm motility is an essential parameter of male fertility, both in natural conception and in medically assisted reproduction [MAR].

Sexual reproduction requires the unification of male and female gametes (Gaffney et al., 2011). *In vivo*, as well as in IUI and to some extent in conventional *in vitro* fertilization [IVF], this process requires the active swimming of the sperm cell to the oocyte. Only a minority of the initial spermatozoa will reach the fallopian tubes, and even less the specific site of fertilization (Gaffney et al., 2011). Progressive motility is therefore essential for fertilization.

After the conclusion of the spermatogenesis, sperm cells are already morphologically differentiated, but immature (Gervasi & Visconti, 2017; James et al., 2020). Epididymal maturation is responsible for their acquisition of progressive motility, as they transit through each unique luminal environment of each region of the epididymis (James et al., 2020). It is here, in conjunction with the capacitation that happens later in the female reproductive tract, that sperm cells gain fertilizing capacity (Gervasi & Visconti, 2017). The epididymis also stores, protects and concentrates sperm (James et al., 2020).

Sperm motility largely depends on the structure and function of the flagellum (Dcunha et al., 2022). The flagellum is a motile appendage (Gaffney et al., 2011) and the “tail” of the spermatozoon. It consists of the mid, principal, and end piece (Dcunha et al., 2022).

Along the centerline of the flagellum runs the axoneme, a phenotypically conserved structure consisting of a central pair of microtubules, surrounded concentrically by nine pairs of microtubule doublets [known as the 9 + 2 structure] (Gaffney et al., 2011). The central pair is connected to each doublet through radial links, and the doublets are adjacently connected through nexin bridges (Gaffney et al., 2011). Inner and outer dynein project from the microtubule doublets, and it's the dynein arms that will consume adenosine triphosphate [ATP] and aid the sliding of the microtubules (Dcunha et al., 2022), creating the shear and bending that confer motility to the spermatozoon (Gaffney et al., 2011).

The flagellum meets the head of the spermatozoon at the connecting piece. This piece shows elasticity and has been theorized to be responsible for alternating the bends that propagate down the flagellum (Gaffney et al., 2011). The mid piece contains a sheath of mitochondria, that produces ATP, and is therefore responsible for meeting the energy needs of the flagellum (Gaffney et al., 2011).

Regarding the connection of sperm motility with fertility, the presence of motile sperm [in addition to other factors, such as morphology and concentration] is useful in predicting fertility potential among infertile patients (Dcunha et al., 2022).

The motility of the sperm is also necessary to determine the course of treatment, as it is a factor who heavily influences the outcomes of IIU, IVF and ICSI cycles (Donnelly et al., 1998; Shulman et al., 1998). For following a IIU protocol, a minimum of 5 million motile sperm should be able to be extracted from the ejaculate. In conventional IVF, that number is between 2 and 5 million. Below that, ICSI is recommended (Dcunha et al., 2022).

In ICSI, although the spermatozoon does not have to independently move towards the oocyte, its mobility is useful for the embryologist to locate viable sperm, and to do so quickly. Microinjection with non-motile sperm tends to yield poor results, due to the non-determination of its viability (Kahraman et al., 1996), therefore, in ICSI, it is better to use motile spermatozoa, which are always living. Indirectly, in a sample with low motility, it can be challenging to find those motile spermatozoa, which can delay the ICSI procedure (Dcunha et al., 2022), and therefore affect negatively the outcomes, as the time the oocyte spends outside the incubator, in suboptimal conditions, should be minimized (ESHRE, 2016).

According to the sixth edition of the “WHO laboratory manual for the examination and processing of human semen” (World Health Organization, 2021), sperm mobility can be classified into four distinct categories, based on the movement and velocity of the spermatozoa. The categories are:

- rapidly progressive, in which the spermatozoon moves at least half of the tail length in one second, in either linear or large circular movements;
- slowly progressive, in which the spermatozoon moves between the length of its head and half of the length of the tail in one second, in either linear or large circular movements;
- non-progressive, in which the spermatozoon presents tail movement, but is otherwise incapable of progression, also known as *in situ* motility;
- immotile, in which there are no active tail movements.

In the previous edition of the manual (World Health Organization, 2010), there was no distinction between rapidly progressive and slowly progressive. Instead, they were combined into a single category, “progressive”, that doesn’t account for the speed of the spermatozoon, only its active movement. This decision was reversed, as the velocity of the spermatozoon seems to be of biological significance (World Health Organization, 2021).

1.3.1. Asthenozoospermia

Asthenozoospermia can be classified as decreased sperm motility, and is one of the most relevant factors contributing to male infertility (Dcunha et al., 2022). It is present in 40% of infertile men (Dirami et al., 2013).

Following previous WHO guidelines, asthenozoospermia could be established by spermatozoa presenting a total motility inferior to 40%, as well as a progressive motility below 32%.

Sperm motility and sperm vitality are two different parameters, as the latter refers to all living sperm, motile or not— but motility can give insights into vitality. As mentioned, WHO recommends testing the vitality if total motility is below 40% (World Health Organization, 2021).

Micro-injecting oocytes with immotile spermatozoa yields very poor fertilization results, and out of all the initial semen analysis parameters [concentration, morphology, motility], the presence of at least one living spermatozoon, easily found through its motility, per oocyte is the single greatest criteria for a successful ICSI (Nagy et al., 1995).

1.3.1.1. Risk factors and causes for asthenozoospermia

There are numerous risk factors that are known to affect sperm motility (Dcunha et al., 2022). These factors range from genetic causes to lifestyle and environmental ones.

Regarding genetic causes, multiple genetic defects have been identified as having correlation to impaired sperm motility. Amongst these, the most common seem to be primary ciliary dyskinesia [PCD] (Dcunha et al., 2022). When PCD is associated with *situs inversus*, it is referred to as Kartagener syndrome, an autosomal recessive disorder (Guichard et al., 2001).

PCD is a relatively rare multiorgan disease, caused by mutations in genes associated with the structure and function of motile cilia. This affects multiple facets of the body, most notably the respiratory tract, but is also linked to numerous issues in the reproductive systems of both sexes. In men, the disfunction of cilia in the efferent ducts can affect sperm concentration. The sperm flagella, namely the axoneme, shares a similar structure with these cilia, particularly the dynein arms, and so PCD can be linked to defects in the ultrastructure of the spermatozoon, and as such correlated with asthenozoospermia (Dcunha et al., 2022; Schreck et al., 2024).

As mentioned previously, sperm motility is dependent on the energy provided by the mitochondria (Dcunha et al., 2022), as the organelle generates ATP, which is delivered to the axoneme and utilized for the propulsion of the flagella (Kao et al., 2004). Because of this, motility is contingent on the functional integrity of both the mitochondria and the mitochondria sheath (Dcunha et al., 2022; Paoli et al., 2011). Deletions in the sperm mitochondrial DNA have been associated with asthenozoospermia (Kao et al., 2004), and could be used as an indicator for sperm quality (Kao et al., 2004).

The presence of anti-sperm antibodies can also affect progressive motility (Shibahara et al., 2005), through the entanglement of the spermatozoa (Dcunha et al., 2022).

Prolonged sexual abstinence can affect motility due to the prolonged stay in the epididymis, which exposes the sperm cells to oxidative stress (Dcunha et al., 2022). Oxidative stress affects multiple facets of male fertility, including sperm mobility (Hussain et al., 2023). The WHO recommendation for sexual abstinence prior to semen analysis and fertility treatment is a period between 2 and 7 days (World Health Organization, 2021), although men with asthenozoospermia can benefit from a shorter period, as well as frequent ejaculation for an amount of time, in order to diminish the reproductive tract reserves of sperm (Wilton et al., 1988).

Lifestyle, demographic and occupational factors have also been associated with diminished sperm mobility (Dcunha et al., 2022); such include alcohol and cigarette consumption (Silva et al., 2017), intense exercise (Józków & Rossato, 2016), high body mass index (Belloc et al., 2014) prolonged use of electronics (H. G. Chen et al., 2022), sleep deprivation and psychological stress. Regarding occupation, positions that require exposure to heat, radiation or certain chemicals can jeopardize sperm motility (Vaziri et al., 2011). Certain medications have also been shown to adversely affect sperm motility (Dcunha et al., 2022).

As immotility can indicate non-vitality, necrozoospermia, although rare, can be a cause of asthenozoospermia (Ortega et al., 2011). Necrozoospermia can be defined as a pathological decrease in sperm vitality (Agarwal et al., 2021). Among other sites, it often happens in the epididymis, due to both environment and a possible inherent structural instability in the spermatozoa (Ortega et al., 2011). It can suffer DNA damage due to reactive oxygen species [ROS] and lose cytoplasmic compartments by diffusion through the membrane, causing it to be apoptotic or lose viability. This can be associated with various factors, such as infections, inflammation and abnormalities in the gonads, genital tract and accessory glands, anti-sperm

antibodies, hyperthermia, prolonged anejaculation, exposure to toxins, advanced age and cancer.

1.3.1.2. Absolute and complete asthenozoospermia

Complete asthenozoospermia is defined as 100% immotile spermatozoa in the ejaculate, and it is found at a frequency of 1 in 5000 men (Castañeda et al., 2018).

It is relevant to distinguish between patients with complete asthenozoospermia: those with virtual asthenozoospermia, that show some motile spermatozoa after extensive sperm processing, and those with absolute asthenozoospermia (Ortega et al., 2011). The latter can be associated with metabolic deficiencies, ultrastructural abnormalities of the flagellum, and necrozoospermia. It can also be idiopathic (Ortega et al., 2011). As much as possible, the source of the absolute asthenozoospermia should be elucidated and corrected (Ortega et al., 2011).

In cases in which the cause cannot be elucidated or corrected, the only way to achieve fertilization is through the microinjection of oocytes (Ortega et al., 2011), and even then, fertilization and pregnancy rates are very low (Kahraman et al., 1996), as mentioned above. For achieving a viable pregnancy, finding motile, and therefore living, spermatozoa could be crucial. When motile spermatozoa are completely absent in the ejaculate, alternative methods of sperm extraction can be considered (Al-Malki et al., 2017; Kahraman et al., 1996).

1.4. Alternative Sperm Retrieval Techniques

Alternative sperm retrieval techniques are here referred as ways to obtain sperm besides in the ejaculate, particularly testicular and epididymal sperm.

The sperm in these various locations will not be identical. Morphology is one of the factors that differ between testicular, epididymal, and ejaculate sperm. Testicular sperm has the lowest percentage of normal morphology, and epididymal the highest (Steele et al., 2000). The lower normal morphology in the testes could be indicative of immaturity, rather than genuine abnormality. Generally, motility will also progressively increase, due to epididymal maturation.

The literature shows mixed results regarding the outcomes of ART with various sperm sources. Some indicate that TESE, in cases of oligozoospermia, yields worse results than using ejaculate sperm (Kendall Rauchfuss et al., 2021). In others, motility plays a factor, in that ICSI with immotile ejaculate sperm yields better results than with TESE, and the opposite for motile

sperm (Stalf et al., 2005). This last study proposes that the degree of maturity is a positive factor in fertility and so, that ejaculate sperm should be used when possible, and in the case of azoospermia, epididymal sperm is preferable to testicular (Stalf et al., 2005).

Alternative sperm retrieval techniques are not heavily associated with the treatment of asthenozoospermia, especially not in isolation. They are instead directly linked to obstructive and non-obstructive azoospermia (Esteves et al., 2011b), as evidently if there is no sperm in the ejaculate the only option would be to search for viable sperm in the stages prior to it.

Nonetheless, the extraction of testicular or epididymal sperm could be beneficial in other situations, as it can have a number of advantages over the ejaculate sperm [which nevertheless is preferable when available, as stated previously]. One of these advantages would be lower DNA fragmentation. As mentioned, due to oxidative stress, sperm DNA can be damaged during post-testicular transport and storage, both in the epididymis and beyond (Shin & Turek, 2013). In cases where DNA fragmentation is theorized to be a significant issue in ART cycles, the extraction of testicular sperm could bypass these issues.

Alternative sperm retrieval techniques can also be considered in cases of necrozoospermia, both absolute and whenever there is an insufficient number of viable sperm in the ejaculate, as they can raise this number (Ortega et al., 2011).

Some studies show that as long as viability is comparable, confirmed by either motility or vitality testing, so are the rates of pregnancy for the various sperm sources (Shin & Turek, 2013), for fresh samples.

There are currently multiple techniques that can be employed to extract sperm from either the testes or the epididymis, including (Esteves et al., 2011b):

- Percutaneous epididymal sperm aspiration [PESA]
- Microsurgical epididymal sperm aspiration [MESA]
- Testicular sperm aspiration [TESA]
- Testicular sperm extraction; single or multiple biopsies [TESE]
- Microsurgical testicular sperm extraction [Micro-TESE]

Each technique will have its own set of advantages and disadvantages, referring to cost, expertise and materials needed, speed, possible discomfort to patient, and more. The reason why an alternative sperm retrieval is being considered in the first place should also be a

factor involved in picking which protocol to follow, as the techniques will be better suited for different situations.

No one technique is optimal, and each embryology or medical team should choose the one best suited for their needs, capability, and specific case.

1.4.1. TESE



Figure 3: Illustration of a single-incision testicular sperm extraction [TESE]

(Esteves et al, 2013)

In conventional TESE [Fig. 3], a standard open surgical biopsy technique is used to extract testicular parenchyma and as such retrieve sperm from the testes. A single or multiple extractions can be made from the same incision, and it is also possible to make several incisions in different parts of the testis (Esteves et al., 2011b). The incision is closed with a running, non-absorbable suture (Esteves et al., 2013).

As other alternative sperm retrieval techniques, it is most used in cases of azoospermia, in order to obtain spermatozoa when there is none in the ejaculate.

The multi-biopsy TESE method, with either single or multiple incisions, was developed to increase the chances of obtaining sperm by increasing the number of samples collected, in cases of non-obstructive azoospermia. This is because, in these cases, sperm can be more difficult to find as its distribution can be sparse and irregular (Esteves et al., 2011b). This distribution is more predictable in cases of obstructive azoospermia, and there is generally more sperm, and as such often a single incision is made, or a single biopsy obtained (Shin & Turek, 2013).

The obtained samples are then processed by either mechanical shredding or enzymatic digestion. This step frees sperm from the surrounding tissue and facilitates sperm search. The results between techniques are comparable (Esteves et al., 2011b).

TESE is usually performed in advance to and in association with ICSI, either with a fresh sample, or cryopreserved and later thawed (Shin & Turek, 2013). The first tends to yield better results, likely due to an easier immediate assessment of vitality (Shin & Turek, 2013). As such, TESE is usually performed on the same day as the egg retrieval (*Sperm Retrieval Procedures* | *Johns Hopkins Medicine*, n.d.).

It is an invasive option of obtaining viable sperm, when compared to both the ejaculate and other sperm retrieval techniques. It commonly uses local anesthesia (*Sperm Retrieval Procedures* | *Johns Hopkins Medicine*, n.d.). It also causes some general post-operative discomfort (Esteves et al., 2011b).

Out of the techniques mentioned, it presents the highest risk of testicular damage. In cases involving multiple biopsies and multiple incisions, generally in non-obstructive azoospermia, it can pose a risk of testicular atrophy (Shin & Turek, 2013).

2. Case Description

A male patient, 45 years old, had been undergoing fertility treatment at the clinic for several months with his 44-year-old female partner. The couple presents secondary infertility, having had a pregnancy in 2021, which was medically terminated due to trisomy 18.

The couple has completed two IVF cycles and one additional transfer with cryopreserved embryos at the current clinic.

Prior to the cycles, a semen analysis was conducted, in April of 2023. The patient presented a volume of 3,7ml, sperm concentration of 8×10^6 spz/ml, 1% normal morphology, and 0% motility. In this semen analysis a vitality test performed, and it revealed 18% vitality.

The first cycle, in June 2023, yielded four embryos, two of which were transferred, and the remaining two were cryopreserved, all at the third day of embryo development. The cryopreserved embryos were transferred in September of the same year. None of these transfers resulted in pregnancy. Semen parameters of the patient at this time were: volume of 2,5ml, sperm concentration of $2,4 \times 10^6$ spz/ml, and 1% normal morphology, [oligozoospermia and teratozoospermia, respectively] and 0% motility. Motile spermatozoa were found after treatment of the sample with SpermMobil®.

In October, the couple went through another ICSI cycle. Due to the complete lack of sperm motility, even after treatment of the sample with SpermMobil®, six oocytes were microinjected with immotile sperm, and none were correctly fertilized, rendering a transfer impossible. In this cycle, the patient had a sperm concentration of 8×10^6 spz/ml and 1% normal morphology, both of which are considered low. The volume of the sample was 3ml.

The patient was referred to genetic testing for cystic fibrosis, where he was determined to not be a carrier for any of the most commonly tested mutations associated with this condition.

In November, the female patient went through another oocyte retrieval, and cryopreserved three MII oocytes.

The medical team and the couple decided to attempt another ICSI cycle, this time using testicular sperm, in an attempt to improve motility and vitality.

2.1. Current cycle

2.1.1. Sperm analysis

Even though the patient was to go through a testicular biopsy, a fresh ejaculate was still collected prior to it, at six days of sexual abstinence. Subsequent analysis of this sperm revealed a concentration of $4,4 \times 10^6$ spz/ml, 1% normal morphology, and 100% immotile spermatozoa. Based on these parameters, as well as the results of the previous cycles and the couple's wishes, a testicular sperm extraction was performed.

2.1.2. Oocytes

Nine oocytes were retrieved during follicular aspiration the day of the biopsy, seven of which were in metaphase II [MII], indicated for ICSI. The female partner also had three frozen oocytes, which were thawed. Two survived the thawing process.

In total, nine oocytes were available for TESE-ICSI.

2.1.3. TESE

Two fragments were collected from the right testis, both with multiple spermatozoa, from a single incision, as the patient was not azoospermic.

Ten spermatozoa were found per field at 400x magnification.

Regarding motility, at a 400x magnification, one spermatozoon with *in situ* motility was found per field. A swim-out protocol was followed, but the spermatozoa lacked the sufficient motility to swim to the edges of the drop.

2.1.4. TESE-ICSI

Each of the nine oocytes was micro-injected with a spermatozoon presenting *in situ* motility.

3. Objective

The objective of this case study was to evaluate the feasibility and effectiveness of TESE as a viable approach to improve fertility outcomes in individuals with absolute asthenozoospermia. This condition is characterized by the complete absence of sperm motility, rendering conventional assisted reproductive techniques relying on ejaculated sperm largely ineffective, due to the role that sperm motility plays in its selection.

Since testicular sperm may bypass some of the factors contributing to immotility in the ejaculate, this study aimed to assess whether sperm retrieved directly from the testes could be successfully used in assisted reproductive technologies, particularly ICSI. By analyzing fertilization rates, embryo development, and clinical pregnancy outcomes following the use of testicular sperm in ICSI, this research sought to provide insights into the potential role of TESE in overcoming the reproductive challenges associated with absolute asthenozoospermia.

4. Material and methods

4.1. Data and methodology

All patient data and protocols were provided by the Medically Assisted Procreation Unit of *Hospital Lusíadas Lisboa*, Lisbon.

Informed consent was obtained from the patients for the use of their data, under a regime of complete anonymity, in scientific works for public presentation and/or publication. Additionally, they also consented to the use of their embryos, not used by them within a three-year period [or six, if renewed] for scientific research projects. This last permission was not and will not be utilized, as all non-transferred embryos were discarded due to their quality.

This case study follows the seventh [7th] edition of the American Psychological Association [APA] citation style for all references and citations.

4.2. Oocyte retrieval and denudation

Fresh oocytes were obtained the day of the ICSI, through a transvaginal oocyte retrieval.

The cumulus-oocyte complexes (COCs), alongside the follicular fluid are retrieved by the medical practitioner with an aspiration needle [K-OSN-1735-B-90, Cook®], and deposited into 60x15 Petri Dish [150270, Nunc™ IVF Dishes, Thermo Scientific™]. In the laminar flow chamber, at 37 degrees Celsius, the COCs are separated from the follicular fluid and into a previously calibrated 30x15mm Petri Dish [Nunc™ IVF Dishes, Thermo Scientific™], with Flushing Medium [10840060A, ORIGIO®], in order to wash them. With the same purpose, they are subsequently transferred into another identical dish. From there, they are transferred into the first well of a previously prepared four-well dish (144444, Nunc™ IVF Multi Dish, Thermo Scientific™). Each well contains 0,5 ml of fertilization medium [10310010A, ORIGIO®], overlaid with liquid paraffin [10105060A, ORIGIO®]. They are stored in an incubator until denudation.

Denudation happens in the laminar flow chamber. The COCs are transferred from the four-well dish into a previously prepared into 60x15 Petri Dish [150270, Nunc™ IVF Dishes, Thermo Scientific™], containing a central 100 µl droplet of hyaluronidase [15115001A, ORIGIO®], surrounded by six 100 µl droplets of Sperm Preparation Medium [10691010A, ORIGIO®]. Whichever COCs are not being actively handled are kept in the incubator.

The COCs are first placed in the hyaluronidase droplet. This is followed by gentle repetitive aspiration and expulsion using a Pasteur pipette [PPS150-100PL, Hunter Scientific] to further remove the surrounding cumulus cells. The oocytes are then sequentially transferred through the multiple washing droplets to ensure the removal of residual enzyme and debris, with a 134-145µm pipette [15533, Vitrolife™]. The resulting free oocytes are transferred into a clean well of the four-well dish. It is also at this stage that their degree of maturation is accessed.

4.2.1. Oocyte vitrification and devitrification

Oocytes are vitrified following the Irvine Scientific protocol (*Simplified MII Oocytes Vitrification Protocols*, n.d.) and using their “Vit Kit – Freeze NX” [90188]. They are stored in a Cryolock® [S-CL, Biotech, Inc] in liquid nitrogen until the moment of their devitrification.

Similarly, devitrification is done using the Irvine Scientific protocol (*Simplified Embryo and Oocyte Warming Protocols*, n.d.) and using their “Vit Kit – Warm NX” [90183]. It is performed the same day the oocytes will be utilized.

4.3. Testicular biopsy/ sperm retrieval

A fresh ejaculate was analyzed to confirm the absence of motile sperm, with the addition of SpermMobil®.

Two fragments were collected from the right teste by the medical practitioner.

4.3.1. Sperm processing

The biopsied samples were put into a 60x15 Petri Dish [150270, Nunc™ IVF Dishes, Thermo Scientific™], along with 0,5ml of Sperm Preparation Medium [10691010A, ORIGIO®], previously prepared in 75x12 tubes [352003, Falcon].

Here, the testicular tissue was cut up into very small portions with the use of disposable scalpels [PDSS24, Paramount Surgimed]. This allows for the freeing of the sperm cells from the surrounding tissues and into the culture medium.

A small sample was collected with a 150 mm Pasteur pipette [PPS150-100PL, Hunter Scientific], in order to determine the presence of spermatozoa under the microscope at 400x magnification.

The sample was washed in Sperm Preparation Medium [10691010A, ORIGIO®], placed in a Nunc™ IVF ICSI Dish [150265, Thermo Scientific™], and then overlaid with liquid paraffin [10105060A, ORIGIO®], following a swim-out protocol.

4.4. ICSI

Each oocyte was placed in a 5 µl drop of Sperm Preparation Medium [10691010A, ORIGIO®], overlaid with liquid paraffin [10105060A, ORIGIO®], in the Nunc™ IVF ICSI Dish [150265, Thermo Scientific™].

The microinjection of the oocytes happened outside of the laminar flow chamber, in the inverted microscope, at 200x ampliation. Both the holding and the injection pipettes were by Cook® [K-PIP-1035 and K-MPIP-1035, respectively].

Observations and relevant procedural details were documented in real-time throughout the ICSI process, namely the rupture of the oolemma. Additionally, characteristics of oocyte quality were also noted, like *zona pellucida* [ZP], perivitelline space [PVS], polar body [PB] and cytoplasm defects, as well as the presence of vacuoles, cytoplasmic inclusions and smooth endoplasmic reticulum.

4.5. Embryo culture

The injected oocytes were transferred to previously prepared 30x15mm Petri Dishes [Nunc™ IVF Dishes, Thermo Scientific™], each into a 25 µl drop of embryo culture medium [67010010A, SAGE 1-Step™, with HSA and phenol red, ORIGIO®], overlaid with liquid paraffin.

Embryo development was checked at multiple stages. Fertilization was accessed at Day 1, defined by the presence of two PN, and two PB. All other oocytes were discarded, with the exception of ones with 1 PN and 2 PB, that were also kept to check for further development.

Embryos were accessed again on Day 3 of development. Here, the number of cells was noted, as well as any size discrepancies between them. Embryos were graded by the following system:

- 1.0 – equal blastomeres, no fragmentation
- 2.0 – differently-sized blastomeres, no fragmentation
- 2.1 – under 10% fragmentation

- 2.2 – under 20% fragmentation
- 3.1 – under 50% fragmentation
- 3.2 – over 50% fragmentation

Transfers [maximum 2 embryos] were to be made at this stage, but non-transferred and non-degenerated embryos were taken to day 5 of development, where their quality would be accessed again, following either the blastocyst grading system from the “Istanbul workshop consensus on embryo assessment” (Balaban et al., 2011) or the system previously described, in case of retarded development.

4.6. Transfer

As mentioned, the transfer occurred with day 3 embryos, and either one or two embryos are transferred, based on their quality. The embryos were transferred from their culture medium into a central well dish [16005, Vitrolife™] with 1ml of SAGE 1-Step™.

The ultrasound-guided transfer was performed with a Frydman® [1320200, PRODIMED] catheter, previously loaded by the embryologist following the medium-air-embryo-air-medium method (Christianson et al., 2014), with a 1ml syringe [4010.200V0, HENKE-JECT®].

5. Results

	1	2	3	4	5	6	7	8	9	10	11
Day 0	OK	OK	OK	no rupture	no rupture	OK	OK	MI	ZP	no rupture	no rupture
(ICSI notes)		fragmented PB	-	-	large PVS	-	-	-	-	fragmented PB	degenerated PB
Day 1 (PN check)	1PN 2PB	Dg	2PN	OPN 2PB	2PN	2PN	2PN	-	-	2PN	2PN
Fertilization notes							1 big, 1 small	-	-	far apart	
Day 3	8 cell 2.2	-	6 cell	-	5 cell	9 cell 2.1	1 cell 2.2	-	-	6 cell 3.2	8 cell 2.1
			1 big, 5 small	-	3 big, 1 small	Transferred		-	-		5 big, 3 small
Day 5	-	-	9 cell	-	5 cell	-	-	-	-	Dg	10 cell 2.1
			1 big, 8 small	-	3 big, 1 small	-	-	-	-		

Table 1: Laboratory results of the cycle and notes

Each day of development is individually analysed below.

5.1. ICSI procedure [Day 0]

	1	2	3	4	5	6	7	8	9	10	11
Day 0	OK	OK	OK	no rupture	no rupture	OK	OK	MI	ZP	no rupture	no rupture
(ICSI notes)		fragmented PB	-	-	large PVS	-	-	-	-	fragmented PB	degenerated PB

Table 1.1: ICSI procedure notes

Although the spermatozoa did not present any progressive motility, enough spermatozoa with *in situ* motility were found in the biopsied sample to micro-inject all MII oocytes, an improvement from the fresh sample from both the current cycle as well as the previous.

Out of the nine ICSIs performed, five [55,56%] demonstrated clear oolemma rupture [noted in the above table 1.1 as “OK”], a positive sign in regard to correct fertilization and reduction of oocyte damage (Carrillo et al., 1998). The remaining four [44,44%] did not, including the ones with the two previously cryo-preserved oocytes, noted in the table as “no rupture”.

Several oocyte abnormalities were noted, in accordance with the above table 1.1, such as fragmented [numbers 2 and 10; Fig. 4A] and degenerated polar bodies [11; Fig. 4C], and a large perivitelline space [5; Fig. 4B]. It is to note that both the thawed oocytes had abnormal polar bodies, fragmented in one case and degenerated in the other.

Below are the abnormalities noted.

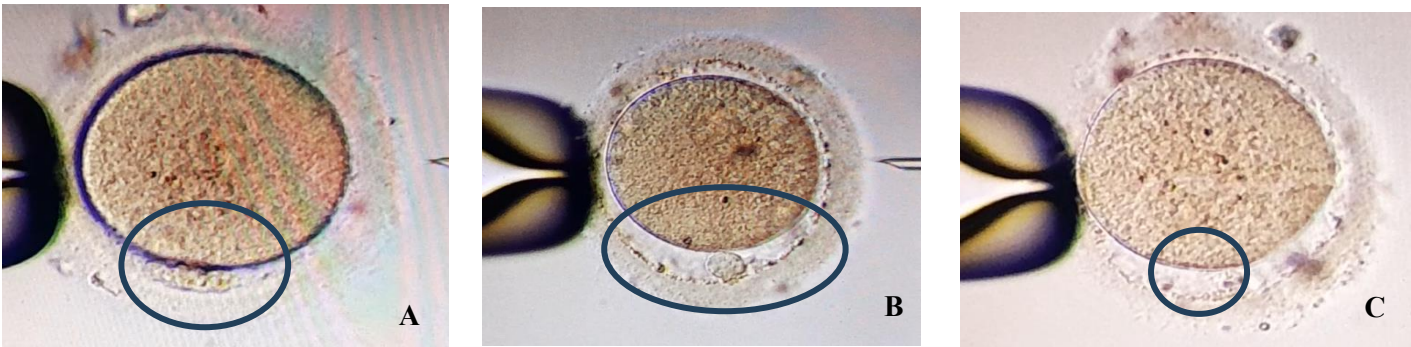


Figure 4: Abnormalities in the oocyte.

- A: Oocyte with fragmented polar body, circled. [Original]
- B: Oocyte with augmented perivitelline space, circled. [Original]
- C: Oocyte with degenerated polar body, circled. [Original]

5.2. Fertilization [Day 1]

	1	2	3	4	5	6	7	8	9	10	11
Day 1 (PN check)	1PN 2PB	Dg	2PN	OPN 2PB	2PN	2PN	2PN	-	-	2PN	2PN
Fertilization notes							1 big, 1 small	-	-	far apart	

Table 1.2: Fertilization notes

Fertilization was checked through the existence of two-pronuclear zygotes [“2PN”, Table 1.2]. Six oocytes were fertilized into 2PN [66,67%]. Regardless of the abnormalities noted during the ICSI procedure, both thawed oocytes were fertilized [100%], although in one the pro-nuclei were farther apart than what it is normal [Table 1.2]. Out of the four fresh 2PN [57,14% fertilization rate], only one showed any abnormality— a large size discrepancy between the pronuclei.

Beyond the 2PN, one oocyte was incorrectly fertilized into 1PN 2PB, meaning only pronucleus to two polar bodies, and another into 0PN 2PB, meaning the existence of two polar bodies in the absence of pronuclei. One oocyte degenerated [Table 1.2].

Six zygotes, out of the nine oocytes injected, were then followed into the next stage of development, day 3 embryos. The remaining were also kept to verify further development, but due to the incorrect fertilization they were not viable for transfer or freezing.

5.2.1. Fertilization when compared to previous cycle

Cycle	2PN [+]	2PN [-]	Total
Previous	0	8	8
Current	6	3	9

Table 2: Fertilization comparison between cycles

2PN [+]: correct fertilization, presence of two pronuclei

2PN [-]: incorrect fertilization, any other result

The fertilization rate of the current cycle, using testicular sperm with *in situ* motility, was 66,67% [6/9], while in the previous cycle, with immotile ejaculate sperm, it was 0% [0/8].

Fisher's exact test was used to determine whether the improvement of the fertilization rates could be attributed to chance. A p-value of was 0,009 obtained, and taking the common threshold of $p < 0,05$ for statistical significance, it is unlikely the improvement of fertilization and the source of sperm are unconnected.

5.3. Embryo development [Day 3; Day 5]

5.3.1. Day 3 [8 cell embryo]

	1	2	3	4	5	6	7	8	9	10	11
Day 3	8 cell 2.2	-	6 cell	-	5 cell	9 cell 2.1	1 cell 2.2	-	-	6 cell 3.2	8 cell 2.1
			1 big, 5 small	-	3 big, 1 small	Transferred		-	-		5 big, 3 small

Table 1.3: Embryonic development notes, day 3

The transfer occurred at this stage. Ideally, the embryos would have eight equal sized cells and present low fragmentation [Table 1.3].

Only one correctly fertilized embryo had eight cells, and the large size discrepancy of its blastomeres made it not the best option for transfer [number 11; Table 1.3]. There were two 6-cell embryos, one with uneven blastomeres [number 3; Table 1.3] and the other with a high degree of fragmentation [number 10, Table 1.3]. Another’s number of blastomeres was difficult to determine, but the embryo was noted as having five cells with at least three large and one small blastomeres [number 5; Table 1.3]. The last non-transferred embryo only had one cell [7, Table 1.3].

The embryo that was then transferred had nine cells and was graded 2.1 [under 10% fragmentation]. Although it did not present the ideal eight-cell count, it was visually the closest to a correct day 3 embryo, and it was both possible and likely that one of the eight blastomeres had recently undergone mitosis.

Once again, the remaining non-transferred embryos were kept to verify further development, namely their possible advancement into blastocysts on day 5.

5.3.2. Day 5 [Blastocyst]

	1	2	3	4	5	6	7	8	9	10	11
Day 5	-	-	9 cell 1 big, 8 small	-	5 cell 3 big, 1 small	-	-	-	-	Dg	10 cell 2.1

Table 1.4: Embryonic development notes, day 5

None of the embryos had advanced into blastocysts at the fifth day of development, showing either severe retardation [number 3 and 11; Table 1.4], arrested development [number 5; Table 1.4] or degeneration [number 10; Table 1.4].

Due to the poor quality of the embryos at this stage, none were cryopreserved for future cycles.

5.4. Pregnancy

Two-weeks post-transfer, the female patient's β -HCG levels were determined to be <2 mU/ml, indicating a lack of pregnancy, as the reference value for non-pregnancy is anything below 5mU/ml.

6. Discussion

6.1. Significance of Cycle Successes and Previous Failures

Although the lack of pregnancy might be discouraging, it is important to note the successes of this cycle in comparison with the previous one, and what it might mean for the possible overcoming of fertilization failure in the presence of completely immotile ejaculate sperm.

As mentioned previously, this couple had been undergoing fertility treatment for several months at the time of this current cycle, having had two failed transfers from a first ICSI cycle. The patient's sperm quality continued to diminish, and in the next cycle the fresh ejaculate showed absolute asthenozoospermia, as it did during this current cycle.

Back then, the oocytes underwent ICSI with these immotile spermatozoa, and there was a complete failure of fertilization. Sperm vitality was assessed in that cycle using SpermMobil®, but the sperm remained completely immotile even after exposure to it. This strongly suggests that non-viable sperm were injected into the oocytes during ICSI.

Both the lack of motility and the complete fertilization failure in a previous cycle with immotile sperm indicate poor vitality, a factor that would be irreversibly linked to poor fertility. More so, the SpermMobil® test should have, in principle, induced movement in live sperm (Sandi-Monroy et al., 2019), but since no movement was observed, the spermatozoa were likely non-viable.

As such, following both the wishes of the couple [who intended this to be their last cycle] and once again taking into account both their age and the downward trend of the sperm quality, the medical team chose to go ahead with the testicular sperm extraction, with the objective of possibly bettering sperm parameters, namely sperm motility [and the diminished vitality likely responsible for it].

Testicular sperm tends to show lower motility, due to maturing that happens later in the epididymis (Toshimori, 2003). In this case, however, testicular sperm showed better motility compared to the fresh ejaculate [some *in situ* motility versus no motility at all] which could indicate that the sperm might be suffering some damage during its maturity, storage or transit that affected its quality and vitality.

This is not unlikely— for instance, DNA sperm fragmentation tends to increase during maturation and transit due to oxidative stress (Shin & Turek, 2013), and this factor is also associated with lower fertility, namely higher rates of abortions, both in natural conception (Robinson et al., 2012) and following IVF cycles (Li et al., 2024; Zini et al., 2008). Although this is not completely relevant to this case— the previous abortion was medically induced due to trisomy 18, an aneuploidy that often leads to pregnancy loss, especially taking into account the higher rates of aneuploidies and miscarriages in older women’s pregnancies (Elmerdahl Frederiksen et al., 2024)— it is still worth mention.

Due to the higher sperm quality of the biopsied sperm, and the sufficient number of motile spermatozoa, all oocytes, both the freshly collected and the thawed, underwent ICSI with these motile sperm cells. The ICSI process itself was comparable to the previous cycle, but the most improvement happens in the next stage— fertilization.

As mentioned, the injection of immotile sperm in the previous cycle did not yield 2PN-zygotes from any of the eight oocytes. In this case however, out of nine injected oocytes, six developed into 2PN.

This is a significant improvement. It also indicates that at least in this case, the use of motile testicular sperm yielded much better results than immotile ejaculate sperm, and allowed for an overcoming of the complete fertilization failure that cut the previous cycle short and impeded even a transfer.

Motility is one of the most important selection parameters for the sperm used in ICSI. Although an immotile sperm cell does not mean that it is undoubtedly dead, dead spermatozoa will always be immotile, and so choosing a motile spermatozoon is always preferable, and an assurance of its viability. The higher fertility rates were therefore expected, just on the account of having used undoubtedly viable sperm, but further indicate the possibility of TESE in cases in which the sperm quality is not only severely diminished in the ejaculate [in several parameters but most notably absolute asthenozoospermia, and necrozoospermia], but it also seems that it worsens through the transit and maturation of sperm from teste to ejaculate.

6.2. Embryonic Development Assessment

In the steps beyond fertilization, embryo development was not optimal. The embryos were checked at day 3 and day 5 post ICSI, where they should be at the 8-cell stage and blastocyst stage, respectively (Balaban et al., 2011).

On the third day of development [Table 1.3], only two embryos had eight cells, and neither was adequate for transfer. The first had not fertilized correctly previously, having had only one pronucleus [Table 1.2], rendering it untransferable. The second, although it had eight cells and fairly low fragmentation [under 10%], presented blastomeres with a large size discrepancy— five large and three small cells. In early embryonic development, mitosis should generate two equal-sized daughter cells. When this division is not symmetric, the resulting blastomeres do not inherit equal halves of the cytoplasm, leading to a defective lineage in the embryo (Prados et al., 2012). Evenly divided embryos have lower rates of multinucleation and aneuploidy, compared to asymmetrically divided ones (Hardarson et al., 2001).

Moreover, in 8-cell embryos, like 2 and 4-cell, the blastomeres should be equal in size (Prados et al., 2012). The fact that this embryo had eight cells but they were not evenly sized indicated asymmetric division, and suggested that it was a worse option to transfer than the chosen embryo, which had nine cells. Although this is not the correct number for this stage, it is possible that one of the blastomeres had just undergone mitosis. Taking this into account, and the fact the embryos had around the same degree of fragmentation, the 9-cell embryo was transferred. The remaining embryos all presented retarded development [1, 5 and 6 cells], but were nevertheless kept into the fifth day of development, as was the 8-cell asymmetric embryo, when they would ideally be blastocysts.

None of the embryos developed into blastocysts, or even morula [Table 1.4]. They either did not further develop since they were evaluated at day 3, or presented extreme retardation. The uneven 8-cell embryo, previously mentioned, had ten cells at this stage. If the embryos had not reached the blastocyst stage but had shown better progress, they could have been kept into the sixth day of development. Even if they had not yet degenerated, it was extremely unlikely that the embryos were viable. As it was, considering their very poor quality and retarded development, as well as the couple's wishes for this cycle to be their last, the remaining embryos were all discarded at this stage.

6.3. Limitations of the study

Although Fisher's Exact Test pointed to an improvement of fertilization rates while using testicular versus ejaculated sperm in the case of absolute asthenozoospermia, it is important to note the small sample size, impossible to overcome in the case of a single case study.

In addition to it, it would perhaps have been beneficial to use other methods of vitality evaluation, beyond SpermMobil®. It is highly unlikely that other tests would present different results, supported by the downward trend of the patient's sperm quality, the lack of motility in the ejaculate, and the previous fertilization failures, but it would still be a helpful confirmation of the necrozoospermia in the ejaculate.

It is also significant to note the advanced maternal age when discussing this case study. Advanced maternal age is a factor very closely linked with diminished oocyte quality, increased aneuploidy and spontaneous abortion rates (Elmerdahl Frederiksen et al., 2024), and overall lower fertility (*Pregnancy at Age 35 Years or Older* | ACOG, n.d.). This indicates for increasingly lower oocyte quality in each new cycle, an untested variable. It is impossible then to disassociate this factor with the poor embryo development, both in this cycle and in the previous ones, as well as the subsequent lack of pregnancy.

Even without considering the male factor, achieving a pregnancy with one's own gamete is unlikely at the female patient's age (Fernandez et al., 2020). Multiple factors related to aging diminish the quality of the oocyte, and the possible subsequent embryo—mitochondrial dysfunctions, for instance, increase with age, and can affect necessary events such as meiotic spindle assembly, cell cycle regulation, chromosome segregation, embryo development, and implantation. Telomeres too shorten as one ages, and shorter telomeres have been associated with IVF failure, recurrent miscarriages, and faulty embryos. Lack of cohesion between sister chromatids is also linked with age, which will naturally affect chromosomal segregation (Cimadomo et al., 2018). At the female patient's age, aneuploidies are extremely common, and the odds of achieving a pregnancy and carrying it to term are diminutive (Ubaldi et al., 2017).

As such, poor embryonic development and failure to achieve a pregnancy were likely to start with, just on the account of the maternal age, making it impossible to determine just how much the sperm quality affected the outcomes.

Lastly, the cause of the absolute asthenozoospermia remains unknown, and it would be beneficial to know if the spermatozoa were normal at an ultrastructural level, in order to confirm whether the asthenozoospermia was due to the sperm's inherent quality, or if the motility degeneration and sperm death were happening during epididymal storage or upon mixing with the seminal plasma.

6.4. Future Directions and Potential of TESE in Treating Absolute Asthenozoospermia

Due to the nature of human fertility studies and the ethical concerns surrounding them, obtaining conclusive, large-scale data on the success of using testicular sperm extraction to improve fertility in cases of absolute asthenozoospermia remains challenging. This is especially true considering the invasive nature of a biopsy in comparison to the relatively non-invasive collection of sperm from ejaculate used in regular fertility treatments.

While the results of this particular case are somewhat promising, despite the lack of pregnancy, it is important to acknowledge that the small sample size, as well as the previously mentioned limitations, compromise the ability to generalize these findings across a wider population. The lack of broader statistical data makes it difficult to determine whether this approach could be considered a routine part of fertility treatments.

The small sample size might mean that the results observed here— though positive— could reflect an individual anomaly rather than a reliable, reproducible trend. A larger cohort would provide a more robust statistical foundation and offer more meaningful insights into the overall effectiveness of TESE in improving fertility outcomes for men with absolute asthenozoospermia.

It would also perchance be beneficial to test whether a shorter abstinence period could improve motility. The abstinence period for the ejaculate in this cycle was six days, which is within the WHO recommended time period (World Health Organization, 2021), but due to the necrozoospermia, the patient could benefit from a shorter period, as well as frequent ejaculation for some time prior to the IVF or ICSI cycle, in order to minimize the time the spermatozoa spends in storage and deplete the maybe defected sperm reserves (Dcunha et al., 2022; Wilton et al., 1988). If this strategy worked to better the vitality and motility rates of the ejaculate, the use of TESE or any other form of alternative sperm retrieval would be unnecessary, which would likely be a more comfortable and cost-effective option for the patient, and the couple.

Another facet future studies should take into account is the ultrastructure of the spermatozoa. It was unfortunately not determined in this case, but it could give insights into the cause of the immotility.

As such, larger studies with more diagnostic tools, including the testing of more sperm parameters in both testicular and ejaculated sperm, are needed to determine whether TESE

could become a widely accepted practice for improving fertilization rates in cases of absolute asthenozoospermia.

Until such data is available, it is impossible to make this treatment a routine approach in ART, noting the invasive nature of testicular sperm extractions. It could be, however, an option of cases such as the one studied here— where sperm quality is progressively decreasing with no clear cause, and there is a significant biologically imposed time limit to the treatment.

In sum, while the results in this case are promising, the path forward requires more research to confirm the broader applicability of TESE. The potential for improving fertilization outcomes in cases of absolute asthenozoospermia is encouraging, and with an increased use of more diagnostic techniques and larger studies, this approach may hold significant promise for the future of ART in these cases. Though not yet a routine solution, TESE offers a possibility for patients facing declining sperm quality resulting in absolute asthenozoospermia and necrozoospermia and may become an increasingly valuable option as the field hopefully progresses.

7. Conclusion

Although both the poor embryonic development and the lack of pregnancy can be discouraging, the significantly improved fertilization rates provide valuable hope for the potential use of testicular sperm extraction in cases of absolute asthenozoospermia.

The improved fertilization outcomes observed in this study suggest that TESE may offer a critical alternative for patients with severe male infertility, particularly in cases when traditional methods involving ejaculated sperm have previously failed.

While the absence of a pregnancy outcome limits the conclusions that could have been drawn, the promising fertilization rates underscore TESE's potential to help overcome fertilization failures associated with absolute asthenozoospermia and offer new opportunities for parenthood.

8. References

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