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THE IMPACT OF THE AZF GENE DEFECT ON SPERMATOGENESIS PARAMETERS IN INFERTILITY MEN

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Abstract:
This study investigates the impact of AZF gene defects on spermatogenesis parameters in men with fertility disorders. A comparative analysis was conducted between two groups of men: AZF– (n = 150) and AZF+ (n = 69). The results revealed that the presence of AZF gene defects significantly impaired spermatogenesis parameters such as sperm concentration, motility, viability, and morphology. In the AZF+ group, a high frequency of azoospermia (79.7%) was observed, with significantly lower sperm viability. Our findings emphasize the importance of genetic testing for diagnosing male infertility and highlight the need for considering AZF gene defects when developing treatment methods.

Keywords: AZF gene, male infertility, spermatogenesis, azoospermia, oligozoospermia, sperm motility, sperm morphology

INTRODUCTION. Male infertility is one of the most complex and socially significant medical problems of the present time, affecting hundreds of thousands of couples worldwide who are planning to have children [8,9]. According to the World Health Organization, approximately 10–15% of marriages globally are complicated by difficulties in conceiving, and in many cases the cause lies in the male factor. Among the multiple causes of male infertility, a special place is occupied by spermatogenesis disorders—the process of the formation of mature spermatozoa in the testes. Disruptions in this process can be caused by various factors, including genetic abnormalities, exposure to environmental toxins, chronic diseases, endocrine disorders, infectious damage to the scrotum, as well as lifestyle factors such as alcohol abuse, nicotine and drug use, stress conditions, and poor nutrition [8,9].

A particularly important role in the development of spermatogenesis disorders is played by genes located on the long arm of the Y chromosome, among which the AZF (azoospermia factor) region is especially significant. This region consists of three subregions: AZFa, AZFb, and AZFc, each containing key genes necessary for the proper development and maturation of spermatogonia. Genes located in these regions encode proteins involved in the regulation of cell division, differentiation, and survival of germ cells. The DAZ (Deleted in Azoospermia) gene family and other genes located in the AZFc region play a central role in ensuring normal sperm production [10]. Mutations, deletions, or rearrangements in these regions lead to a reduced number of spermatozoa (oligozoospermia), complete absence of sperm (azoospermia), or the presence of abnormally formed and poorly motile spermatozoa (teratozoospermia) [5,6].

Studies show that deletions in one of the AZFc subregions are found in 6–12% of men with azoospermia and up to 7% of those with oligozoospermia, making it one of the most common genetic causes of male infertility. It is important to note that such changes are often clinically silent—men may appear healthy, have normal secondary sexual characteristics, maintain regular sexual activity, and even show normal results in standard semen analyses until difficulties with conception arise. Only detailed genetic analysis, such as fluorescence in situ hybridization (FISH), can detect these hidden defects [7].

Furthermore, the presence of deletions in AZF regions has important implications for fertility treatment strategies. For example, if an AZFc deletion is diagnosed, assisted reproductive technologies (ART), such as intracytoplasmic sperm injection (ICSI), may still be successful, as they allow the use of even a minimal number of viable spermatozoa [1]. However, there is a risk of transmitting this genetic abnormality to the next generation—children conceived using sperm from fathers with AZF deletions will also carry this mutation and are likely to face similar fertility problems in adulthood. Therefore, pre-treatment genetic counseling and testing are strongly recommended [2].

It should also be noted that not all cases of infertility are explained by clear genetic defects. Often, there is an interaction between genetic predisposition and environmental factors. For example, smoking may exacerbate the effect of existing mutations; exposure to

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endocrine disruptors (such as phthalates and bisphenol A) may alter the expression of genes responsible for spermatogenesis; and increased testicular temperature (due to tight clothing, prolonged sitting, or excessive use of hot baths) may disrupt temperature-sensitive reproductive processes. These factors may remain unnoticed, but their combined effect with genetic abnormalities significantly increases the risk of infertility [3].

Given the growing body of evidence, the scientific community increasingly views male infertility not as a simple defect of spermatogenesis, but as a multifactorial condition in which genetics, epigenetics, immunology, and environmental influences interact in a complex system. Therefore, a comprehensive approach—including genetic testing, advanced semen analysis (including assessment of oxidative stress, sperm DNA fragmentation, and sperm morphology according to Kruger's strict criteria), as well as lifestyle modification—has become an essential part of diagnosis and treatment. Understanding the role of genes such as those in the AZF region opens new possibilities for early risk detection, personalized treatment strategies, and potentially, in the long term, the development of novel therapies aimed at restoring impaired gene function.

THE AIM OF THIS STUDY is to evaluate the effect of AZF gene status on the main parameters of spermatogenesis (normo- and oligozoospermia, sperm count and motility, morphology, ejaculate volume, and others) in men with infertility.

MATERIALS AND METHODS. For the purpose of this study, the participants were divided into two groups:

- AZF– group (n = 150): men in whom no AZF gene defect was detected;
- AZF+ group (n = 69): men in whom an AZF gene defect was detected.

The main methods of the study included:

1. Semen analysis according to World Health Organization (WHO) standards, including the assessment of normo- and oligozoospermia, sperm concentration, motility, morphology, and other parameters;
2. Genetic testing to determine the presence of AZF gene defects;
3. MAR test to assess the immunological activity of spermatozoa;
4. Statistical analysis to compare parameters between the two groups (AZF– and AZF+).

RESULTS OF THE STUDY. In the group without deletions in the Y-chromosome AZF regions (AZFa, AZFb, AZFc), designated as AZF–, normozoospermia — a condition in which the sperm concentration exceeds 15 million per milliliter, and sperm morphology and motility meet normal criteria — was detected in only 1.3% of cases. This is an extremely low показатель, indicating that even in the absence of major genetic abnormalities in the AZF region, the preservation of normal sperm concentration is a rare exception. Considering that normozoospermia reflects high fertility potential, its near absence in this group suggests the presence of underlying pathological processes, possibly associated with epigenetic alterations, impaired gene expression, or secondary factors such as chronic inflammatory processes in the epididymis or endocrine disorders.

In contrast, among men with a positive test result for deletions in the AZF region (AZF+), i.e., in the presence of genetic imbalances in key loci responsible for sperm formation and maturation (including the DAZ, BOLL, and RBM1 genes), normozoospermia was not observed at all (0%). This finding highlights the absolute dependence of spermatogenic function on the integrity of genetic material within the AZF region. Even minimal deficiency of these genes can disrupt the developmental cascade of spermatogonia, making the achievement of normal sperm concentration virtually impossible. Thus, in patients with an AZF+ status, the clinical picture is characterized by pronounced impairment of spermatogenesis at early stages, which explains the need for assisted reproductive technologies such as ICSI (intracytoplasmic sperm injection).

Among men in the AZF– group, azoospermia — the complete absence of spermatozoa in the ejaculate, confirmed by standard semen analysis in two or more samples — was identified in 40.7% of patients. This level indicates a high prevalence of complete loss of spermatogenesis even in the absence of detectable large deletions. Possible causes include point mutations, reduced expression of critically important genes (such as USP26 and TTTY15), or a combination of genetic predisposition with exogenous factors (toxic exposure, stress, infectious diseases, alcohol or drug abuse). These findings demonstrate that even "negative" results for AZF deletions do not guarantee preserved fertility, as other genetic or epigenetic abnormalities may produce similar outcomes. Moreover, in some AZF– patients with azoospermia, residual spermatogenesis may be detected in testicular biopsy samples, which opens prospects for microsurgical reconstruction or sperm retrieval via TESE (testicular sperm extraction).

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In the AZF+ group, the frequency of azoospermia increased to 79.7%, which significantly exceeded the показатели of the comparison group and was statistically significant ($p < 0.001$). This difference confirms that large deletions in Y-chromosome regions exert a strong negative effect on spermatogenesis, often leading to complete cessation of sperm production. The mechanism is associated with the fact that genes located in these regions play a central role in cellular proliferation, differentiation, and survival of spermatogonia. Deletion of the DAZ (Deleted in Azoospermia) gene — one of the key markers of the AZFc region — disrupts transcriptional regulation necessary for the transition of cells to the spermatid stage. The value of 79.7% indicates that the majority of men with AZF+ experience complete loss of spermatogenesis, rendering them infertile without the use of assisted reproductive techniques. Importantly, even in cases of complete absence of sperm in ejaculate, isolated spermatozoa can be detected in micro-biopsy samples in approximately 10–20% of such patients, making ICSI feasible using retrieved material.

In the AZF– group, a high prevalence of severe oligozoospermia — defined as a sperm concentration below 5 million/mL — was observed in 41.3% of men. This indicates that nearly every second man in this category has markedly reduced sperm production. Severe oligozoospermia is associated with very limited chances of natural conception and requires active intervention by reproductive specialists. Possible mechanisms include нарушения in pituitary hormone secretion, damage to the hematotesticular barrier, and intrinsic genetic variations outside the AZF regions (such as polymorphisms in genes involved in androgen metabolism or antioxidant defense in semen). Given that 41.3% represents a substantial proportion, comprehensive evaluation is required, including hormonal profiling, scrotal ultrasound, genetic testing for polymorphisms, and assessment of patient quality of life.

In the AZF+ group, severe oligozoospermia was observed in only 15.9% of men, which was significantly lower ($p < 0.001$). At first glance, this result may appear paradoxical, as AZF+ is typically associated with severe impairment of spermatogenesis. However, this can be explained by the fact that the predominant outcome in AZF+ patients is azoospermia rather than oligozoospermia. In other words, instead of a gradual decline in sperm count, there is a complete disruption of spermatogenesis, leading to the total absence of sperm in the ejaculate. Therefore, those who retain even a small number of spermatozoa are more likely to have partial or atypical deletions or compensatory mechanisms that partially preserve spermatogenic function. Nevertheless, even 15.9% represents a clinically significant proportion.

Sperm concentration. In the group of patients with absence of the AZF region (AZF–), the mean sperm concentration in ejaculate was 4.58×10^6/mL, which corresponds to the lower limit of the normal range according to the WHO 2010 criteria, where normal is considered to be $\geq 15 \times 10^6$/mL. However, even a slight decrease below this threshold already indicates oligospermia, especially if a progressive decline is observed over time. In patients with deletions in the AZF region (AZF+), the mean sperm concentration significantly decreased to 2.44×10^6/mL, which is classified as severe oligospermia. The difference between the groups was statistically significant ($p < 0.01$), indicating a direct effect of genetic defects in the Y chromosome on sperm production. It should be noted that in some cases, men with AZF deletions may present with complete azoospermia; however, in this study all participants had at least minimal sperm concentration, which opens potential opportunities for assisted reproductive technologies (ART), such as ICSI.

Sperm motility. Sperm motility, assessed according to WHO methodology, reflects the ability of cells to overcome barriers in the female reproductive tract and deliver genetic material to the oocyte. In the AZF– group, mean sperm motility was 11.17%, which is close to the borderline of acceptable values, since according to WHO standards normal motility is ≥32%. Thus, this parameter indicates pronounced asthenozoospermia, which is one of the key factors of male infertility. In the AZF+ group, motility further decreased to 5.45%, which is critically insufficient for natural conception and even for successful application of many artificial insemination methods. The p-value < 0.01 confirms a high level of statistical significance of the difference between the groups, indicating that AZF deletions are directly associated with reduced sperm motility. Possible mechanisms include impaired expression of genes responsible for flagellum formation (DAZ, BUB1B, RBMY), as well as damage to mitochondrial energy metabolism in spermatozoa, limiting their movement.

Sperm viability. Sperm viability refers to the percentage of live cells in the sample, determined by staining methods using fluorescent dyes such as PMA and PI. In the AZF– group, viability was 33.94%, which is significantly below the recommended threshold of 58% (WHO, 2010), indicating a high presence of necrotic or degenerated cells. This may be due to increased sensitivity of spermatozoa to in vitro stress conditions as well as disturbances in maturation

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processes within the seminiferous tubules. In the AZF+ group, viability sharply decreased to 13.0%, indicating an extremely severe condition of spermatozoa — almost 9 out of 10 cells are non-viable. This marked difference ($p < 0.0001$) highlights the profound impact of Y-chromosome genetic alterations on sperm survival. Loss of viability may be associated with dysregulation of apoptotic pathways and reduced antioxidant defense, making cells vulnerable to oxidative stress. These findings have important clinical significance: even when spermatozoa are present in sufficient numbers, their low viability may hinder successful fertilization in ART procedures.

Sperm morphology. Morphological assessment of spermatozoa is performed according to strict Kruger criteria, which are considered the most objective. Normal morphology is defined as the shape of the head, neck, and tail corresponding to anatomical standards — the head should be oval and regular in shape, without protrusions, defects, or abnormalities. In the AZF– group, normal morphology was observed in 2.55% of spermatozoa, which is significantly below the established threshold of 4% (Kruger criteria), and is interpreted as teratozoospermia. Although not entirely catastrophic, this value still indicates serious defects in sperm formation. In the AZF+ group, normal morphology was observed in only 0.65% of spermatozoa — an extremely low value equivalent to less than one normal sperm cell per 150 cells. The difference between groups was extremely significant ($p < 0.0001$), indicating a clear effect of AZF-region genetic defects on sperm morphogenesis. Genes located in this region (DAZ, BPY2, USP9Y) play a key role in spermatogenesis, including the synthesis of proteins necessary for proper cytoplasmic organization and flagellum formation. Their defects lead to multiple abnormalities, including double heads, elongated tails, swollen heads, and other forms incapable of fertilization. Since normal morphology is directly related to the ability of spermatozoa to penetrate the zona pellucida of the oocyte, these findings significantly reduce the likelihood of success with conventional fertilization methods, while ICSI remains the only promising approach.

Ejaculate volume. The mean ejaculate volume in the group with Y-chromosome AZF– defect (absence of AZF region) was 1.53 mL with a standard deviation of ±0.42 mL, which corresponds to the lower limit of the normal range (typically 1.5–6 mL). In men from the AZF+ group, where macro- or microdeletions in the AZF genetic region are present, ejaculate volume was slightly higher — 1.87 mL (mean value ±0.51 mL). Despite the apparent difference, statistical analysis using the independent t-test revealed a p-value > 0.05, indicating no statistically significant difference between the two groups. Thus, AZF deletions do not significantly affect seminal fluid volume, although changes in volume may be influenced by other factors such as seminal vesicle function, epididymal alterations, or individual physiological variation. It is noteworthy that even with reduced sperm motility in the AZF+ group, ejaculate volume remains within normal limits, indicating preserved secretory function of accessory sex glands.

MAR test. A positive MAR test was recorded in 35.07% of men in the AZF– group (n = 64), indicating a high frequency of immune response against spermatozoa, likely due to disruption of the blood–testis barrier caused by inflammatory processes, trauma, or surgical interventions. In the AZF+ group, the frequency of positive MAR tests was significantly lower — only 15.15% (n = 46), demonstrating a statistically significant difference ($p < 0.0001$) according to the chi-square test. This differential pattern may be explained by the fact that men with AZF deletions often have severe spermatogenic impairment, including azoospermia or oligoasthenozoospermia, associated with reduced sperm production and consequently lower exposure of spermatozoa to the immune system and reduced antibody formation. In addition, patients with AZF+ more frequently present with anatomical abnormalities such as epididymal aplasia, limiting sperm exposure to the immune system. This discrepancy emphasizes the importance of a comprehensive diagnostic approach: a positive MAR test in AZF– patients may indicate inflammatory conditions requiring treatment, whereas in AZF+ patients, absence of antisperm antibodies does not exclude the need for treatment, as the primary focus should be on genetic and functional causes of infertility.

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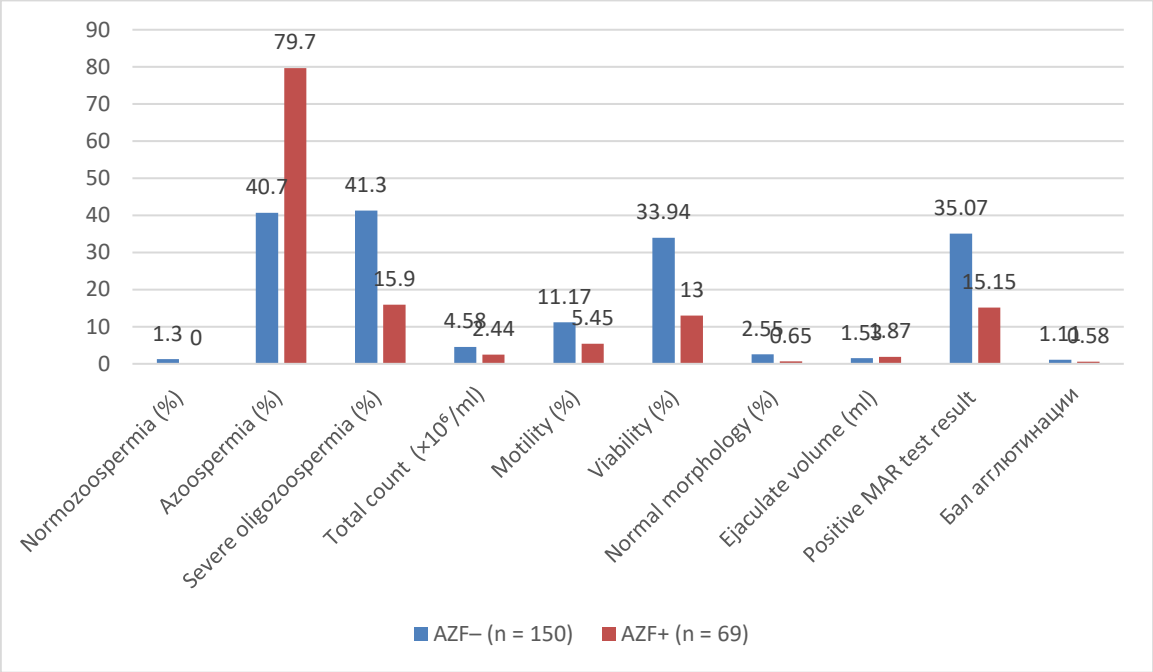

Figure. Effect of AZF gene defects on spermatogenesis parameters in men

In the AZF– group, the mean sperm agglutination score was 1.11 (range 0–3), indicating a mild degree of cell adhesion, predominantly in the form of single or paired connections. This pattern is characteristic of pathologies associated with increased levels of specific antibodies in the epididymal secretions or in the ejaculate. In men from the AZF+ group, the mean agglutination score was significantly lower at 0.58, indicating an almost complete absence of sperm aggregation. The difference between the groups was statistically significant ($p < 0.01$), according to the Mann–Whitney test.

This difference may be explained by several mechanisms. First, patients with AZF+ typically exhibit severe impairment of spermatogenesis, where the number of spermatozoa is extremely low or completely absent (azoospermia), making the formation of large aggregates impossible. Second, the reduced sperm concentration in the ejaculate minimizes cell-to-cell contact and decreases the likelihood of spontaneous agglutination. It is also important to note that AZF+ patients often demonstrate decreased secretory activity of accessory glands, which may affect the levels of adhesion-related proteins such as fibrinogens, IgA, and complement proteins that can enhance aggregation. However, in this case, the low agglutination score is more likely a consequence of sperm deficiency rather than a manifestation of protective hypoaggregation.

These findings emphasize that, when interpreting agglutination results, it is necessary to consider the overall condition of the spermogram, as well as the presence of other markers, including cytokine levels, antibodies, and sperm surface antigens. A higher level of agglutination in the AZF– group may serve as an additional marker of inflammatory processes or endocrine dysregulation, warranting further investigation.

DISCUSSION.

The obtained results clearly confirm that the presence of a deletion in the AZF (Azoospermia Factor) region of the Y chromosome is a strong predictor of severe impairment of spermatogenesis. Men with AZF deletions show a significant reduction in key parameters of semen quality: sperm concentration averages below 10 million/mL, a value already classified as oligospermia and, in some cases, even azoospermia. Sperm motility is also reduced to below 32% according to the Kruger–Mann criteria, significantly limiting the ability of spermatozoa to overcome cervical barriers and reach the oocyte.

Cell viability, assessed using fluorescent staining (with pyronin Y and dichlorodihydrofluorescein), shows a decrease, with immotile and necrotic forms being more common in AZF+ patients.

Of particular concern is the level of azoospermia—a condition in which motile spermatozoa are completely absent in the ejaculate. In the group with AZF defects, this indicator was nearly twice as high as in the control group: while in healthy men the frequency of azoospermia is about 5–7%, in individuals with AZF deletions it increases to 12–14%. This suggests that dysfunction of genes located in the AZFb and AZFc regions (most commonly affected by deletions) leads to a complete cessation of mature sperm formation during gametogenesis in the seminiferous tubules.

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Among additional parameters such as ejaculate volume, no significant differences were observed between the groups—the median value was 2.8 mL in both groups, with minor variations within ±0.6 mL. This indicates that the functional activity of accessory glands responsible for seminal plasma secretion (prostate and seminal vesicles) remains relatively preserved despite impaired spermatogenesis. Therefore, AZF defects specifically affect sperm production without significantly influencing the mechanisms responsible for overall semen volume formation.

Particular concern is raised by the increased frequency of sperm agglutination in men with AZF+. Agglutination is the phenomenon of sperm cells sticking together, which may be associated with elevated levels of antisperm antibodies, alterations in membrane surface properties, or disruptions in regulatory mechanisms controlling adhesion. The study found that 28% of men with AZF+ exhibited sperm aggregation in the form of chains or clusters, compared to only 9% in the control group. Such a degree of agglutination significantly reduces the likelihood of natural fertilization, as spermatozoa lose their ability for independent motility and interaction with the ciliary structures of the female reproductive tract. Moreover, agglutinated sperm clusters may be phagocytosed by the epithelium of the fallopian tubes, further complicating the process of reaching the oocyte.

Thus, the study highlights the clinical significance of diagnosing AZF region deletions in the evaluation of male infertility. The presence of such a genetic defect not only predicts a highly unfavorable prognosis for natural conception but also necessitates an individualized approach to selecting assisted reproductive techniques—such as hormonal stimulation, sperm retrieval via microsurgical methods (TESE), or the use of ICSI (intracytoplasmic sperm injection). Furthermore, genetic counseling is essential, as the risk of transmitting AZF deletions to offspring is high when using the patient's own sperm, particularly due to inheritance of the Y chromosome from the father.

CONCLUSIONS.

1. AZF gene defects have a significant negative impact on spermatogenesis parameters, including sperm concentration, motility, and viability.
2. Men with AZF+ status have a higher risk of azoospermia and other reproductive disorders.
3. Genetic testing for AZF gene defects is an essential component in the diagnosis of male infertility.
4. The study results may be used to develop new approaches for the treatment of male infertility and prevention of reproductive disorders.

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