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# Y127-AZFb Gene Expression and its Association with Systemic Biomarkers in Infertile Men Undergoing ART

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**ABSTRACT**

**Background & Objective:** Male infertility contributes to nearly half of global infertility cases and is often linked to Y-chromosome microdeletions. Beyond genetic defects, thyroid hormones and systemic inflammation influence spermatogenesis and ART outcomes. This study was conducted with aim to evaluate the association between Y127-AZFb gene expression and systemic biomarkers in infertile men.

**Materials & Methods:** This case-control study included 120 infertile men undergoing ART and 120 fertile controls. Serum T3, T4, TSH, and IL-6 levels were measured using CLIA and ELISA. Y127-AZFb expression was quantified via qRT-PCR. Non-parametric analyses, ROC curves, Spearman correlations, and logistic regression assessed associations and predictive value. $P<0.05$ was considered statistically significant.

**Results:** Infertile men showed lower T3, T4, and Y127-AZFb expression and higher TSH and IL-6 ($P<0.05$). Y127-AZFb correlated positively with T3 and T4, and negatively with TSH and IL-6. ROC analysis demonstrated high accuracy for T3 (AUC=0.912), TSH (0.880), IL-6 (0.851), and Y127-AZFb (0.803). Y127-AZFb dysregulation was strongly associated with adverse ART outcomes ($P<0.01$), while logistic regression identified reduced Y127-AZFb, low T3, and elevated IL-6 as independent predictors of infertility.

**Conclusion:** Male infertility is characterized by thyroid dysfunction, systemic inflammation, and decreased Y127-AZFb expression. Integrated assessment of endocrine, inflammatory, and genetic markers which may impair spermatogenic function and is associated with ART outcomes and may aid in risk stratification; however, causal relationships cannot be established. Y127-AZFb expression may serve as a prognostic indicator of reproductive success beyond conventional semen analysis.

**Keywords:** Male infertility, Y127-AZFb, Thyroid hormones, Interleukin-6, Assisted reproductive technology, Spermatogenesis

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## 1. Introduction

Clinical infertility is defined as "the failure to achieve pregnancy after 12 months of regular, unprotected intercourse", which affects 8-12% of couples globally, with male factors contributing to nearly half of cases **(1)**. Assisted Reproductive Technologies (ART) are essential for infertility due to age-related decline, genetic factors, idiopathic causes, and severe oligospermia or azoospermia **(2)**. However, the impact of male infertility on embryo development,

implantation, chromosomal abnormalities, and genetic, epigenetic, and obstetric outcomes remains unclear **(3)**.

Male factor infertility constitutes a substantial proportion of infertility cases and is often linked to genetic abnormalities, particularly Y-chromosome microdeletions, with AZF regions (AZFa, AZFb, and AZFc) essential for spermatogenesis. Among these, AZFb is critical for meiotic development, and its deletions are associated with spermatogenic arrest at the primary spermatocyte stage. The Y127 Sequence-Tagged Site (STS), a key AZFb marker, is widely used to assess its integrity due to its functional significance. Beyond structural defects, altered AZF gene expression may impair function even in structurally intact Y chromosomes **(4)**. In addition to genetic causes, systemic conditions such as inflammation and hormonal regulation are key determinants of male reproductive health. Elevated pro-inflammatory cytokines, including Interleukin-6 (IL-6), are linked to sperm dysfunction through oxidative stress and reduced motility **(5)**. Although thyroid disorders are well known to affect female fertility, emerging evidence suggests thyroid hormones may also influence male reproduction by regulating Sertoli and Leydig cell function and supporting sperm maturation, though mechanisms remain unclear **(6)**. These inflammatory and endocrine disturbances may contribute to transcriptional dysregulation of AZFb-region genes rather than structural loss. Unlike AZFb microdeletions, which cause irreversible spermatogenic failure, altered AZFb gene expression may reflect functional dysregulation in structurally intact Y chromosomes influenced by endocrine or inflammatory factors.

While AZF microdeletion screening detects irreversible structural defects, many infertile men undergoing ART lack such deletions yet exhibit impaired sperm quality and poor outcomes, suggesting transcriptional dysregulation. As ART does not guarantee implantation or pregnancy, combined assessment of Y127-AZFb expression with inflammatory and endocrine markers may better predict outcomes by capturing functional impairments. This study was conducted with aim to evaluate the association between Y127-AZFb gene expression and systemic biomarkers in infertile men.

## 2. Materials and Methods

This case–control study, evaluated Y127-AZFb gene expression in relation to inflammatory and endocrine markers in men undergoing ART. It included 240 participants: 120 infertile men undergoing ART (cases) and 120 age-matched fertile men with prior natural conception (controls), recruited from ART clinics following standardized evaluation.

Inclusion criteria were infertile men aged 20-50 years with primary infertility, defined as failure to conceive after 12 months of unprotected intercourse. All participants were scheduled for ART procedures such as IVF or ICSI and provided informed consent for genetic and molecular analyses.

Age-matched healthy men with at least one naturally conceived child were included as controls. All control participants had normal semen parameters according to WHO 2021 criteria and were willing to provide informed consent for study participation.

Cases included a history of testicular surgery, trauma, or infections that could affect fertility, chromosomal abnormalities other than Y-chromosome microdeletions, systemic conditions such as diabetes or significant endocrine disorders, and the use of medications known to impair sperm production. Controls were excluded if they had a history of infertility or reproductive disorders, chronic illnesses or medications affecting fertility, or semen parameters outside the normal range according to WHO 2021 guidelines.

Fasting venous blood samples (6-8 mL) were collected in plain and EDTA tubes, and serum and plasma were separated for biochemical and hormonal analyses using standardized protocols. Thyroid hormones (T3, T4, TSH) were measured by CLIA, and Interleukin-6 (IL-6) by ELISA. RNA was isolated from EDTA samples, and cDNA was synthesized to assess Y127-AZFb gene expression using qRT-PCR.

Serum IL-6 was measured by sandwich Enzyme-Linked Immunosorbent Assay (ELISA) using the Origin Diagnostics & Research, Kerala, India kit (Cat: OPK 1156) according to the manufacturer's instructions.

Serum T3, T4, and TSH were measured using Chemiluminescent Immunoassay (CLIA) on the IMMULITE 1000 analyzer (Siemens, Germany) with commercial kits: T3 (Cat. No: LKT31, 100 tests), T4 (Cat. No: LKT41, 100 tests), and TSH (Kit Code: LKTS1, 100 tests).

RNA was isolated using an RNA extraction kit, quantified using a biospectrometer, and then reverse-transcribed into cDNA using a cDNA synthesis kit.

Y127-AZFb gene expression was quantified by real-time PCR using gene-specific primers (Forward: 5′-GGCTCACAAACGAAAAGAAA-3′; Reverse: 5′-CTGCAGGCAGTAATAAGGGA-3′), with GAPDH as the internal reference. Primers (20 bp) were obtained from Eurofins Genomics India Pvt. Ltd., and amplification was performed on a CFX Opus 96 system. Reactions (20 µL) contained 2× Master Mix, primers, cDNA, and nuclease-free water. Cycling included initial denaturation at 95°C for 5 minutes, followed by 30-40 cycles of 94°C for 1 minute, 57°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 10 minutes and melt-curve analysis. Relative expression was calculated by normalizing to GAPDH using the $2^{-}\Delta\Delta Ct$ method.

Normality of data was assessed using the Shapiro-Wilk test, and differences between cases and controls were analyzed using the Mann-Whitney U test. ROC curve analysis evaluated the discriminatory ability of biomarkers, while the associations between hormonal, inflammatory, and genetic parameters were examined using Spearman's rank correlation. Multivariable logistic regression identified independent predictors of infertility. $P<0.05$ was considered statistically significant.

## 3. Results

A total of 240 participants (120 infertile men and 120 fertile controls) were analyzed for thyroid hormones (T3, T4, TSH), interleukin-6, and Y127-AZFb gene expression using real-time PCR. Statistical analyses included group comparisons, correlation, ROC curve analysis, and logistic regression to identify predictors of infertility and gene dysregulation.

As shown in Table 1, T3, T4, TSH, IL-6, and Y127-AZFb expression were non-normally distributed in both groups ($P<0.05$), justifying the use of non-parametric analyses for comparisons and correlations.

Figures 1 and 2 showed that infertile men showed significantly lower serum T3 and T4 levels, indicating impaired thyroid function. Figure 3 showeds elevated TSH levels in cases, suggesting compensatory thyroid stimulation. Figure 4 revealed higher IL-6 levels, reflecting increased systemic inflammation. Figure 5 showed reduced Y127-AZFb gene expression, indicating potential disruption in spermatogenesis-related genetic regulation. Overall, these findings suggest that male infertility is associated with altered thyroid function, heightened inflammation, and decreased expression of a key gene involved in sperm production.

Figure 6 presented the diagnostic performance of hormonal, inflammatory, and genetic markers in distinguishing infertile men undergoing ART from fertile controls using ROC analysis. All markers showed good performance. T3 demonstrated strong discrimination (AUC=0.912), followed by TSH (AUC=0.880) and IL-6 (AUC=0.851) (Figure 6). T4 also showed robust performance (AUC=0.813) with the highest positive likelihood ratio, supporting case identification at low levels. Y127-AZFb expression exhibited good accuracy (AUC=0.803) (Figure 7) with complete specificity at the selected cut-off. Overall, these markers showed high predictive value, supporting their combined utility in identifying male infertility cases.

The scatter plot (Figure 8) showed a moderate positive correlation between T3 levels and Y127-AZFb gene expression ($\rho=0.3815$, $P<0.01$). The simultaneous decrease in both variables suggests that lower levels of T3 are associated with reduced expression of Y127-AZFb, which may reflect impaired spermatogenic function.

The scatter plot (Figure 9) revealed a strong correlation between T4 levels and Y127-AZFb gene expression ($\rho=0.3327$, $P<0.01$). The decrease in T4 levels is accompanied by lower Y127-AZFb expression, which may reflect impaired spermatogenic function.

The scatter plot (Figure 10) revealed a strong negative correlation between Thyroid-Stimulating Hormone (TSH) levels and Y127-AZFb gene expression in cases ($\rho= -0.5819$, $P<0.01$). As TSH levels increase, Y127-AZFb gene expression decreases. This suggests that thyroid disorders could be associated with the downregulation of the spermatogenesis-related gene Y127-AZFb.

The scatter plot (Figure 11) showed a strong negative correlation between the levels of interleukin and the gene expression of Y127-AZFb in cases ($\rho= -0.5874$, $P<0.01$). The higher the levels of interleukin, the lower the levels of Y127-AZFb gene expression, indicating that high levels of inflammation are related to low levels of Y127-AZFb gene expression.

**Table 1. Normality assessment of continuous variables among cases and controls using Shapiro-Wilk test**

| Variable | Cases P-value | Controls P-value | Distribution summary |
|---|---|---|---|
| **Tri-iodothyronine (T3)** | <0.01 | <0.01 | Non-normal in both groups |
| **Tetra-iodothyronine (T4)** | <0.01 | 0.002 | Non-normal in both groups |
| **Thyroid-stimulating hormone (TSH)** | <0.01 | 0.008 | Non-normal in both groups |
| **Interleukin-6** | <0.01 | <0.01 | Non-normal in both groups |
| **AZF gene (Y127-AZFb)** | <0.01 | <0.01 | Non-normal in both groups |

As shown in Table 2, Y127-AZFb dysregulation was significantly associated with adverse ART outcomes. Failed implantation occurred exclusively in the dysregulated group (100%), while all cases with normal expression showed successful implantation (P<0.01). Miscarriage was higher in the dysregulated group (87.8% vs. 12.2%), and poor embryo quality was predominantly observed with dysregulation (89.8%) (P<0.01). Adverse pregnancy outcomes were also largely confined to the dysregulated group (100%), whereas favorable outcomes were mainly seen with normal expression (95.2%).

In the unadjusted model (Table 3), Y127-AZFb expression showed a strong inverse association with infertility (OR=0.00061, P<0.01) and remained an independent predictor after adjustment (Adjusted OR=0.00044, 95% CI: 0.00001-0.02989, P<0.01). Lower T3 was also independently associated (Adjusted OR=0.939, P<0.01), while IL-6 showed a positive association (Adjusted OR=1.464, P=0.001). Other variables, including T4, TSH, testosterone, FSH, metabolic parameters, hsCRP, and NT-proBNP, were not significant.

**Table 2. Association of Y127-AZFb Gene Expression with ART and Pregnancy Outcomes**

| | | Y127-AZFb Gene Expression | | |
|---|---|---|---|---|
| **Outcome Category** | **Outcome Measure** | **Normal Expression n (%)** | **Dysregulation n (%)** | **P-value** |
| **Implantation** | No failed implantation | 40(67.8) | 19(32.2) | <0.01 |
| | Failed implantation | 0(0.0) | 61(100.0) | |
| **Miscarriage** | No miscarriage | 29(96.7) | 1(3.3) | <0.01 |
| | Miscarriage | 11(12.2) | 79(87.8) | |
| **Embryo Quality** | Poor | 5(10.2) | 44(89.8) | <0.01 |
| | Average | 14(28.0) | 36(72.0) | |
| | Good | 21(100.0) | 0(0.0) | |
| **Pregnancy Outcome** | Good | 40(95.2) | 2(4.8) | <0.01 |
| | Bad | 0(0.0) | 78(100.0) | |

**Table 3. Unadjusted and Multivariable Logistic Regression Analysis for Predictors of Infertility**

| **Variable** | **Model 1: Unadjusted OR (95% CI)** | **P-value** | **Model 2: Adjusted OR (95% CI)** | **P-value** |
|---|---|---|---|---|
| **Y127AzFb gene expression** | 0.00061(0.00007–0.00550) | <0.01 | 0.00044(0.00001–0.02989) | <0.01 |
| **Fasting blood sugar (mg/dL)** | | | 0.997(0.974-1.021) | 0.820 |
| **Total cholesterol (mg/dL)** | | | 1.015(0.992-1.039) | 0.210 |
| **Triglycerides (mg/dL)** | | | 1.001(0.978-1.025) | 0.904 |
| **Tri-iodothyronine (T3)** | | | 0.939(0.912-0.967) | <0.01 |
| **Tetra-iodothyronine (T4)** | | | 1.188(0.900-1.567) | 0.224 |
| **Thyroid-stimulating hormone (TSH)** | | | 1.265(0.796-2.012) | 0.320 |
| **Testosterone (ng/dL)** | | | 1.000(0.998-1.003) | 0.812 |
| **Follicle-stimulating hormone (FSH)** | | | 1.093(0.977-1.222) | 0.119 |
| **Interleukin-6** | | | 1.464(1.166-1.838) | 0.001 |
| **hsCRP** | | | 1.043(0.266-4.092) | 0.951 |
| **NT-proBNP** | | | 1.020(0.998-1.043) | 0.078 |

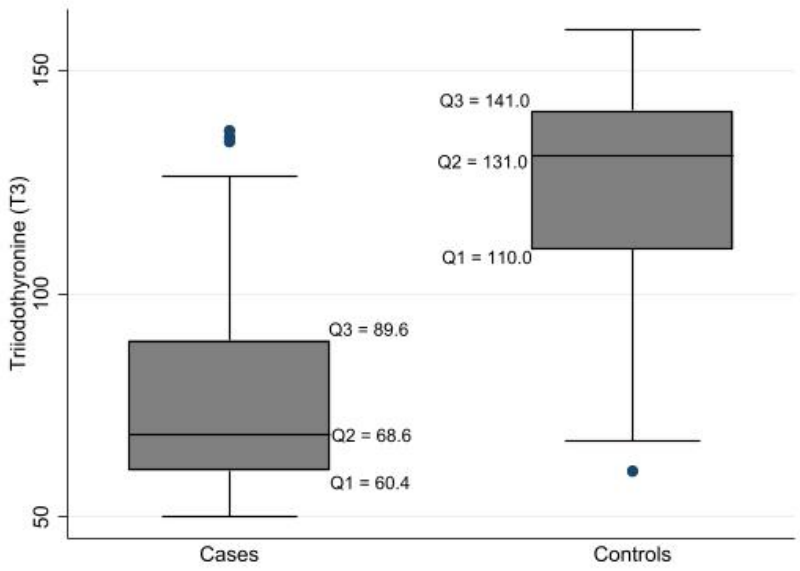



**Figure 1. Comparison of Serum T3 between infertile cases and fertile controls.**

The box plot illustrates the distribution of serum T3 levels among infertile men (cases) and fertile controls. Median values (Q2), interquartile ranges (Q1-Q3), and outliers are depicted. Cases show lower T3 levels (Q1=60.4, median=68.6, Q3=89.6) compared to controls (Q1=110.0, median=131.0, Q3=141.0), indicating reduced thyroid hormone levels in infertile individuals.

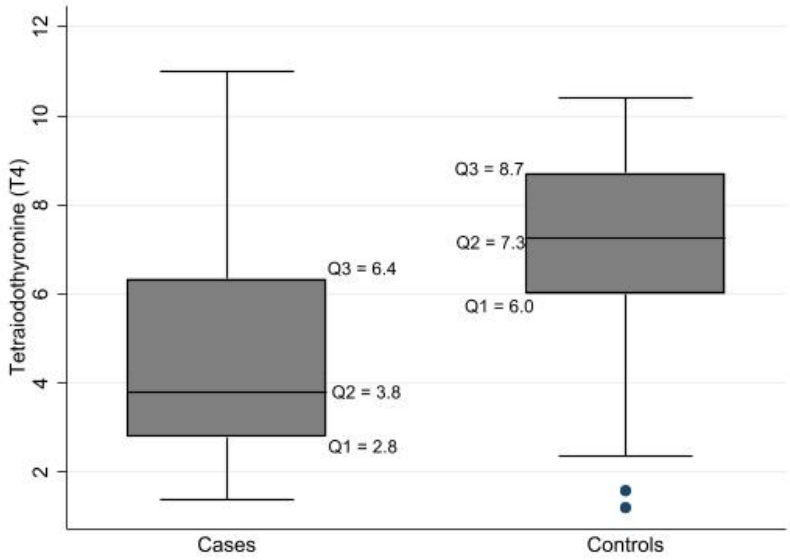



**Figure 2. Comparison of Serum T4 between infertile cases and fertile controls**

The box plot illustrates serum T4 levels among infertile men (cases) and fertile controls. Median values (Q2), interquartile ranges (Q1-Q3), and outliers are shown. Cases exhibit lower T4 levels (Q1=2.8, median=3.8, Q3=6.4) compared to controls (Q1=6.0, median=7.3, Q3=8.7), indicating reduced thyroid hormone levels in infertile individuals.

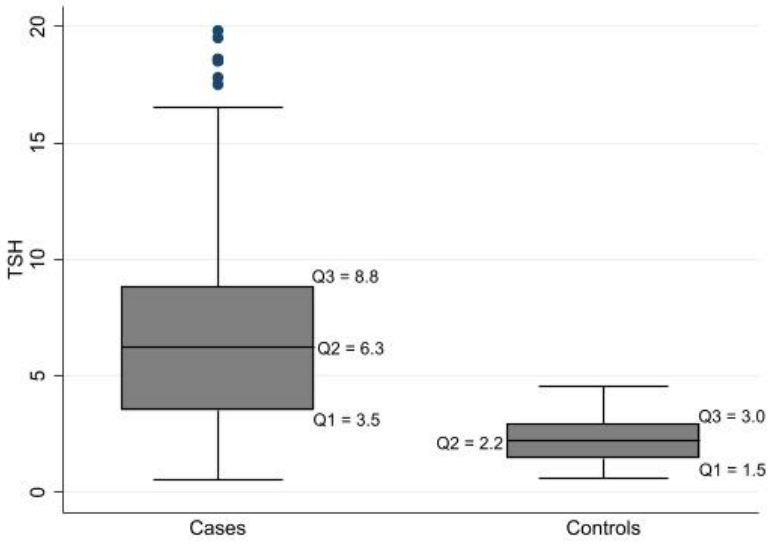



**Figure 3. Comparison of Serum TSH between infertile cases and fertile controls**

The box plot illustrates serum TSH levels among infertile men (cases) and fertile controls. Median values (Q2), interquartile ranges (Q1-Q3), and outliers are depicted. Cases show higher TSH levels (Q1=3.5, median=6.3, Q3=8.8) compared to controls (Q1=1.5, median=2.2, Q3=3.0), indicating elevated TSH levels in infertile individuals.

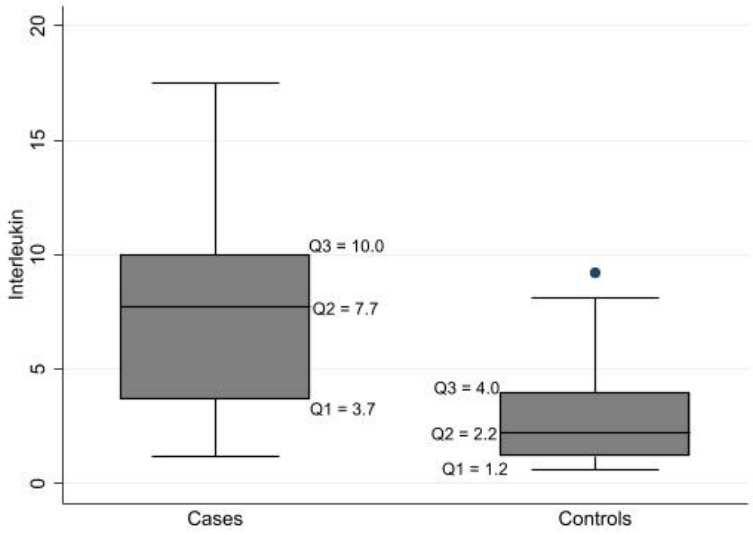



**Figure 4. Comparison of Serum IL-6 between infertile cases and fertile controls**

The box plot illustrates serum IL-6 levels among infertile men (cases) and fertile controls. Median values (Q2), interquartile ranges (Q1-Q3), and outliers are shown. Cases exhibit higher IL-6 levels (Q1=3.7, median=7.7, Q3=10.0) compared to controls (Q1=1.2, median=2.2, Q3=4.0), indicating increased systemic inflammation in infertile individuals.

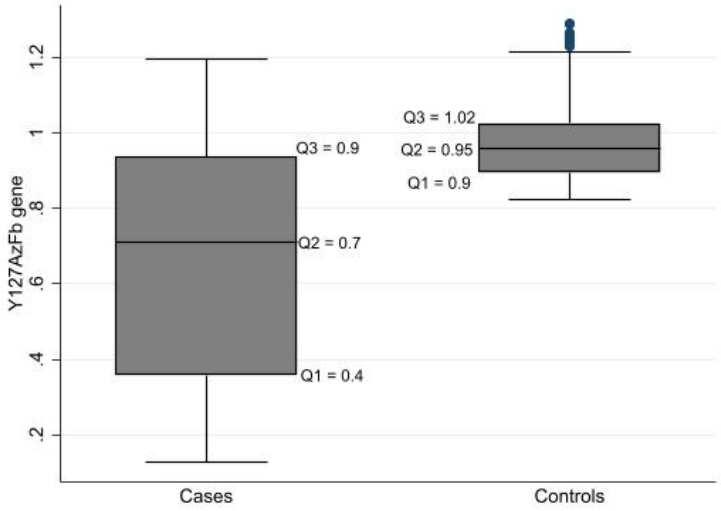



**Figure 5. Comparison of Y127-AZFb expression between infertile cases and fertile controls**

The box plot illustrates relative Y127-AZFb gene expression among infertile men (cases) and fertile controls. Median values (Q2), interquartile ranges (Q1-Q3), and outliers are shown. Cases exhibit lower gene expression (Q1=0.4, median=0.7, Q3=0.9) compared to controls (Q1=0.9, median=0.95, Q3=1.02), indicating reduced Y127-AZFb expression in infertile individuals.

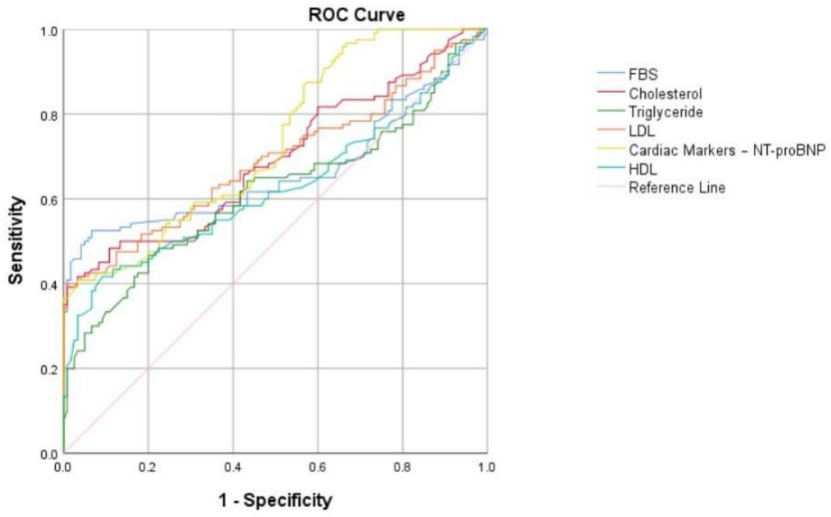



**Figure 6: ROC curves illustrating the diagnostic performance of T3, T4, TSH, IL-6, in distinguishing infertile men from fertile controls**

The ROC curves illustrate the diagnostic performance of Fasting Blood Sugar (FBS), cholesterol, triglycerides, LDL, HDL, and NT-proBNP in distinguishing infertile men from fertile controls. Sensitivity is plotted against 1-specificity, with the reference diagonal line indicating no discriminatory ability. Among the markers, NT-proBNP and lipid parameters show moderate discriminatory performance, while others demonstrate lower accuracy in differentiating cases from controls.

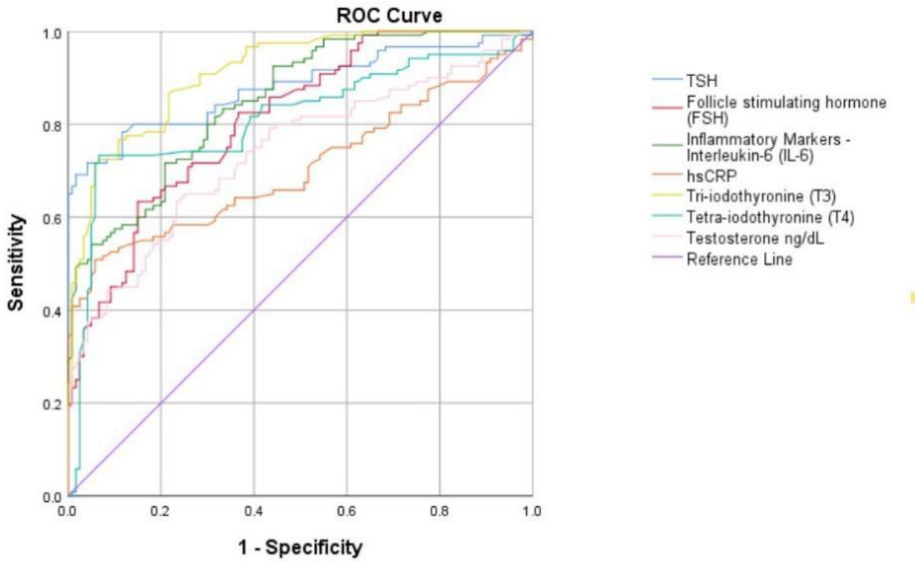



**Figure 7: ROC curves illustrating the diagnostic performance of Y127-AZFb expression in distinguishing infertile men from fertile controls**

The ROC curves illustrate the diagnostic performance of Thyroid-Stimulating Hormone (TSH), Follicle-Stimulating Hormone (FSH), Interleukin-6 (IL-6), High-Sensitivity C-Reactive Protein (hs-CRP), Triiodothyronine (T3), Tetraiodothyronine (T4), and testosterone in distinguishing infertile men from fertile controls. Sensitivity is plotted against 1-specificity, with the reference diagonal indicating no discrimination. T3 shows the highest discriminatory performance, followed by TSH and IL-6, while other markers demonstrate moderate to lower accuracy.

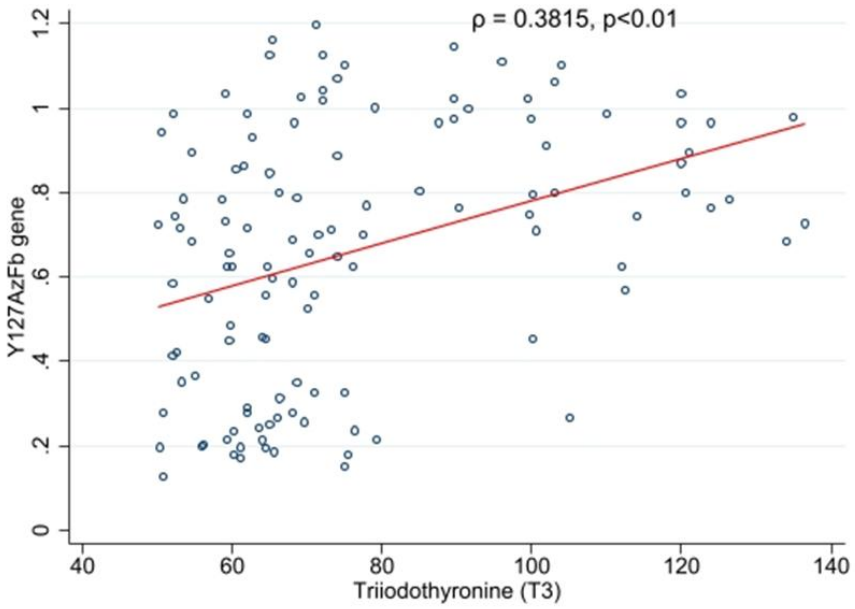



**Figure 8: Relationship between triiodothyronine (T3) and Y127-AZFb gene expression among cases**

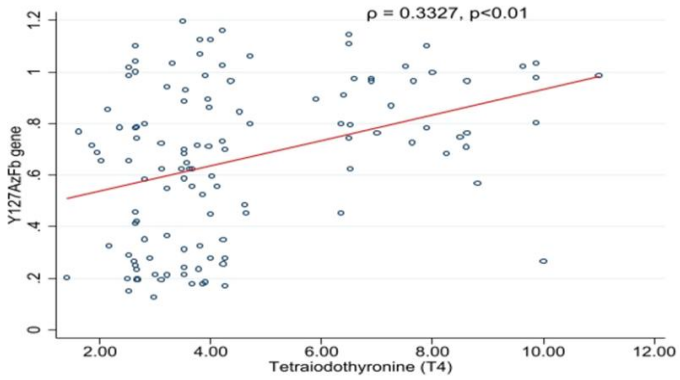



**Figure 9:** **Relationship between tetraiodothyronine (T4) and Y127-AZFb gene expression among cases**

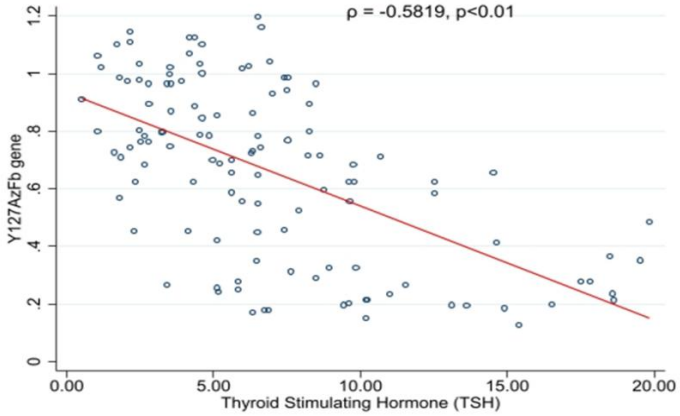



**Figure 10.** **Relationship between thyroid stimulating hormone (TSH) and Y127-AZFb gene expression among cases**

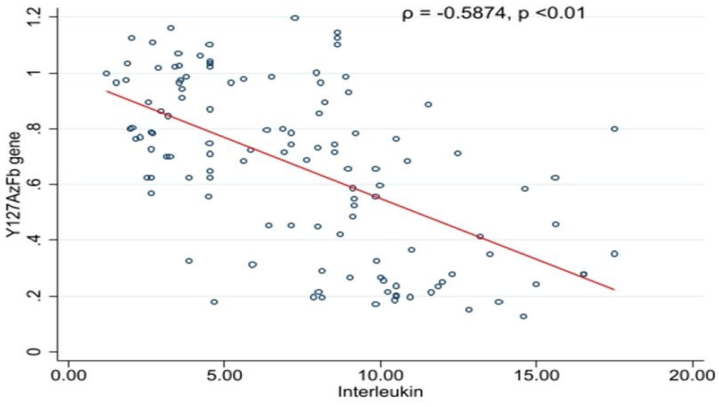



**Figure 11.** **Relationship between interleukin levels and Y127-AZFb gene expression among cases**

## 4. Discussion

The results of the present study showed that endocrine imbalance, systemic inflammation, and genetic dysregulation act in concert to contribute to male infertility in men undergoing Assisted Reproductive Technology (ART). Non-normal distributions of serum T3, T4, TSH, IL-6, and Y127-AZFb gene expression necessitated non-parametric

analyses. Infertile men exhibited reduced T3 and T4 with compensatory TSH elevation, indicating thyroid dysfunction, alongside increased IL-6 levels suggestive of heightened inflammatory activity. Concurrently, reduced Y127-AZFb gene expression points to impaired genetic control of spermatogenesis. Together, these findings suggest an association between endocrine imbalance, systemic inflammation, and altered AZFb gene expression in male infertility.

Our cohort exhibited a distinct profile of primary hypothyroidism, characterized by elevated TSH with reduced T3 and T4, reinforcing the role of thyroid hormones as critical regulators of male reproductive physiology. Increasing evidence suggests that thyroid dysfunction adversely affects spermatogenesis, sperm motility, and overall testicular function **(7)**. Although clinical practice emphasizes TSH as the primary screening marker, our ROC analysis showed strong discriminatory performance of Tri-iodothyronine (T3) in distinguishing infertile men (AUC=0.912). This suggests that peripheral thyroid hormone availability, rather than pituitary response alone, may be more relevant to reproductive impairment. This is supported by multivariate logistic regression identifying T3 as the most significant endocrine predictor of male infertility, surpassing TSH. Although experimental studies indicate that T3 influences germ cell development, our findings demonstratesd an association rather than direct regulatory effects. However, our study demonstrates an association between circulating T3 levels and infertility status, not a direct regulatory effect on spermatogenesis. The elevated TSH levels observed in our infertile cohort may reflect a compensatory response to reduced thyroid hormone activity and could impair testicular function through dysregulation of Sertoli and Leydig cells, consistent with endocrine alterations reported in other subfertile populations.

In the present study, IL-6 levels were significantly elevated in infertile men and were independently associated with increased odds of infertility (adjusted OR=1.464), supporting the role of chronic inflammation in male reproductive failure. IL-6 is a key pro-inflammatory cytokine implicated in oxidative stress, disruption of the blood-testis barrier, and germ-cell apoptosis **(8)**. Consistent with this mechanism, prior studies have demonstrated that persistent low-grade inflammation adversely affects sperm concentration, motility, and DNA integrity **(9)**. The ROC analysis in our study showed that IL-6 had good discriminatory performance for infertility status (AUC=0.851), supporting its potential as a systemic surrogate marker of testicular inflammatory burden. Notably, the inverse correlation between Y127-AZFb expression and IL-6 levels suggests an association between Y-chromosome gene activity and systemic inflammation; however, a direct causal or suppressive relationship cannot be established, warranting further mechanistic investigation.

A key finding of this study is the significant downregulation of Y127-AZFb gene expression in infertile men compared to fertile controls. AZFb region genes are essential for meiotic progression and spermatid maturation, and their disruption is a known cause of severe spermatogenic failure **(10)**. Although classical AZFb Y-chromosome microdeletions are well-established causes of such failure, fewer studies have examined quantitative expression changes in men without complete deletions **(11)**. Our findings suggest that, even without chromosomal loss, transcriptional downregulation of Y127-AZFb may represent functional impairment contributing to infertility. Its potential as a reliable molecular marker is supported by strong inverse odds ratios in both unadjusted and adjusted models, with ROC analysis showing high specificity at the selected cut-off, indicating utility in confirming infertility status. This is consistent with transcriptomic evidence linking idiopathic male infertility to altered expression of Y-linked spermatogenesis genes.

As presented in the current study, Y127-AZFb gene dysregulation was consistently associated with poor ART outcomes including implantation failure, higher miscarriage rates, reduced embryo quality, and adverse pregnancy outcomes while normal expression was linked to favorable outcomes across these parameters. The exclusive occurrence of implantation failure in the dysregulated group supports a potential association with early embryo development and embryo–endometrial interaction. Previous studies have shown that AZFb microdeletions are strongly associated with spermatogenic failure and meiotic arrest **(12)**. Extending this, our findings indicate that altered Y127-AZFb expression, even without structural deletions, is associated with impaired reproductive outcomes.

The higher miscarriage rates and poorer embryo quality in the dysregulated group support an association between Y127-AZFb expression and embryo viability **(13)**, although a direct mechanistic role cannot be inferred. Unlike conventional AZF microdeletion screening, which detects irreversible structural defects, these findings highlight the clinical relevance of functional gene expression changes within a structurally intact Y chromosome. Collectively, Y127-AZFb expression may serve as a promising prognostic biomarker for predicting ART outcomes, warranting further validation. Given the cross-sectional case–control design of our study, all observed relationships are associative, and causal inferences regarding endocrine, inflammatory, and genetic interactions cannot be established.

A key strength of the present study is the analysis of Y127-AZFb gene expression, offering functional insight beyond routine Y-chromosome microdeletion screening. The study further links Y127-AZFb activity with ART and pregnancy outcomes, highlighting its potential in both identifying infertility and predicting treatment success. Additionally, the use of appropriate

non-parametric tests, ROC analysis, and multivariable logistic regression strengthens the robustness, reliability, and clinical relevance of the findings.

Nevertheless, certain limitations should be acknowledged. The case-control design limits causal inference and precludes assessment of temporal relationships, and residual confounding may persist due to unmeasured factors such as BMI, smoking, alcohol use, environmental exposures, and lifestyle variables. The lack of longitudinal follow-up limits the evaluation of dynamic biomarker changes and their predictive value over time. Additionally, ART outcomes may be influenced by unmeasured female factors, embryo transfer protocols, and laboratory conditions not controlled in this study. Embryo quality was assessed morphologically without genetic evaluation, limiting interpretation of chromosomal competence. Furthermore, focusing solely on Y127-AZFb expression without assessing AZFa and AZFc or performing functional validation restricts broader understanding of Y-chromosome transcriptional dynamics. Future longitudinal, multicenter studies with functional assays and comprehensive confounder adjustment are needed to validate these findings.

## 5. Conclusion

This study showed that male infertility is associated with altered thyroid hormones, increased inflammation, and reduced Y127-AZFb expression, reflecting interplay between endocrine, inflammatory, and genetic factors. Y127-AZFb dysregulation was linked to poor ART outcomes, including reduced embryo quality, miscarriage, and adverse pregnancy outcomes. These findings suggest that Y127-AZFb may serve as a functional biomarker for ART prognosis, although further mechanistic studies and validation in larger cohorts are required. Moreover, integrating Y127-AZFb gene expression with thyroid and inflammatory markers may enhance risk stratification in men undergoing ART. Such a combined biomarker approach could help identify individuals at higher risk of poor embryo quality or implantation failure, potentially guiding clinical decision-making, including early intervention, optimization of endocrine status, or personalized ART strategies. However, prospective validation is required before routine clinical implementation.

## 6. Declarations

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### Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki (1964 and later amendments) and approved by the Institutional Ethics Committee of Genetika (Ref No: 09/2023/IECG),

### Authors' Contributions

S.P.P contributed toconceptualization, methodology, investigation, data curation, and drafting. T.K.D, J.I., and D.R.D provided supervision, project administration, and resources. J.V, J.J.S.N, and D.R.D contributed to analysis and validation. A.EV, J. J, and A.D.C assisted with data curation and investigation. Review and editing were conducted by T.K.D and D.R.D. All authors read and approved the final version of the manuscript.

### Conflict of Interest

None declared.

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