ASIAN J TROP BIOTECHNOL
Volume 22, Number 1, June 2025
Pages: 16-22

ISSN: 0216-6887
E-ISSN: 2301-8658
DOI: 10.13057/biotek/c220103

Genomic, hormonal, and anthropometric factors as crucial contributors to male infertility in Abeokuta, Southwestern Nigeria

ADEBAYO A. AMBALLI[1,♥], WASIU E. OLOOTO[1], OLAYINKA T. KOSOKO[2], FOLARIN O. OWAGBORIAYE[3]

[1]Department of Chemical Pathology and Immunology, Faculty of Basic Medical Sciences, Olabisi Onabanjo University. Hospital Road, Ogun State, Nigeria. Tel.: +234-803-353-4252, ♥email: amballi.adebayo@oouagoiwoye.edu.ng
[2]Department of Health Services, Federal University of Agriculture Abeokuta, Nigeria. Abeokuta 110111, Ogun State, Nigeria
[3]Department of Plant Science and Applied Zoology, Faculty of Science, Olabisi Onabanjo University. Ogun State, Nigeria

Manuscript received: 12 February 2025. Revision accepted: 2 Mei 2025.

Abstract. *Amballi AA, Olooto WE, Kosoko OT, Owagboriaye FO. 2025. Genomic, hormonal, and anthropometric factors as crucial contributors to male infertility in Abeokuta, Southwestern Nigeria. Asian J Trop Biotechnol 22: 16-22.* The failure to get pregnant despite regular, unprotected sexual intercourse among couples has psychosocial and socioeconomic impacts. This study aims to investigate the contributions of anthropometric, genomic, and hormonal factors to male infertility. Hence, 48 infertile men and 12 men with proven fertility, aged from 26 to 55 years, were selected as cases and controls, respectively. Anthropometric measurements (height and weight) were done, Body Mass Index (BMI) was computed, and serum Follicle-Stimulating Hormones (FSH), Luteinizing Hormones (LH), and testosterone were measured. Semen was obtained for seminal fluid analysis and phenotyping assay. There is no significant change in the anthropometric indicators (height, weight, BMI); 4.2% of the infertile men were normospermic, 20.8% were azoospermic, and 75% were oligospermic. There was an increase in FSH, LH, and testosterone in all the infertile subgroups and a significant decrease ($p < 0.05$) in sperm count in infertile males. While 70% of sperm cells exhibited fast movement in the controls, sperm movement was 9.5, 5.5, and 15% normal, slow, and non-motile, respectively, among infertile men. The percentage of deletions in Azoospermia Factor a (AZFa) region using sY84 and sY86 was 66.7%, and none in the control group. It can be concluded that high plasma levels of gonadotrophins, low sperm count, and high levels of testosterone are pathognomonic of male infertility. While the deletion of AZFa region is conclusively indicative of infertility, its non-deletion does not conclusively indicate fertility; thus, factors other than hormonal abnormalities are associated with male infertility.

Keywords: Obesity**,** phenotyping, semen quality, testicle

INTRODUCTION

The inability to achieve pregnancy despite regular, adequate (3-4 times per week) unprotected sexual exposure over 2 years is a complex disorder with both genetic and environmental causes (Gunes et al. 2016). The acceptability of identifiable underlying causative factors and sharing of contributory factors between men and women has eroded the primitive African taboo and beliefs that infertility is mainly a female problem rather than male or both. These beliefs were extended to outcomes of fertility in which case the blames of having female children was placed on the woman as being a weaker partner with resultant matrimonial neglect and option of taking up new wives. While in some cases taking up another wife results in having a male child, in other cases it results in having more female children, pointing to the superior role of fathers in determining the sex of children rather than mothers.

Factors of etiological importance in male infertility include congenital abnormalities, prolonged exposure of testes to excessive heat, chronic alcoholism, drugs (cocaine, marijuana, cimetidine, spironolactone, nitrofurantoin, etc.), endocrine disorders, erectile or ejaculatory dysfunction, infection (urinary tract infection, sexually transmitted infection), lifestyle choices, such as diet and exercise, obesity, testicular failure, varicocele, poor semen quality, and idiopathic (ASRM 2015; Barak et al. 2016). Idiopathic causes represent unexplained causes of azoospermia or oligospermia and constitute majority of cases. More studies of the idiopathic group revealed that infertility can be secondary to genetic abnormalities involving the sex chromosomes such as chromosomal translocations, presence of an extra X chromosome as observed in Klinefelter syndrome (XXY), cystic fibrosis, Noonan syndrome, and partial deletions of portions of Y-chromosome primarily affecting the Azoospermia Factor (AZF) regions, resulting in abnormal spermatogenesis and male infertility (Sokol 2001; Chen 2007; Dhanoa et al. 2016; Kuroda et al. 2020). Interactions between genetic factors and environmental factors influence sensitivity to environmental stressors like exposure to Endocrine-Disrupting Chemicals (EDCs) and alter gene expression thereby impairing reproduction and fertility. The importance of the human Y-chromosome in male fertility was revealed by result from several studies indicating that one or more genes located on the long arm of the Y-chromosome are or are most probably involved in spermatogenesis. Deletions of small portions of Y-chromosome responsible for spermatogenesis are believed to be an important azoospermia factor (He et al. 2017).

Partial deletion within the male-specific region of the Y-chromosome, known as Y-chromosome microdeletions (YCMs), is present in as many as 5 and 10% of severe oligospermic and azoospermic men, respectively (Rabinowitz et al. 2021). These microdeletions are distinguished by the segment of the Y-chromosome that is absent. Four subsidiary non-overlapping sub-regions were identified as AZFa (located in the most proximal segment of Yq11, AZFb (located in the middle segment of Yq11), AZFc (located in the distal segment of Yq11), and AZFd (located between AZFb and AZFc). Patients with complete AZFa deletion present with Sertoli cell-only syndrome (Foresta et al. 2001; Ferlin et al. 2003), while those with AZFd microdeletions may have mild oligospermia or normal sperm count but abnormal sperm morphology (Ceylan et al. 2009).

The reported prevalence of YCMs within the world's populations of infertile men displays vast heterogeneity based on region and ethnicity (Rabinowitz et al. 2021). In Africa and Asia, YCMs are a common cause of male infertility, with prevalence of between 10 and 15%, but less than 5% in other populations. The most common deletions occurring in the AZFc region of the Y-chromosome (Trinh et al. 2023). A study reported that complete Y-chromosome microdeletions are rare in men with sperm count > 1 million sperm/mL (Khon et al. 2019). Routine screening for Y-chromosome microdeletions should be done only if sperm concentration is ≤ 1 million sperm/mL (Khon et al. 2019). Both Y-chromosome microdeletion and chromosomal abnormalities increase in prevalence as sperm concentrations decrease and azoospermic men have been reported to have the greatest frequency of genetic abnormalities (Liu et al. 2020).

Nigeria has many regions and ethnic groups. Thus the frequency of AZF microdeletions is expected to vary among Nigerians in different regions. Therefore, it is important to understand the frequency and pattern of Y-chromosome microdeletion, in addition to the effect of body mass index and hormonal disruption, among infertile men in southwestern Nigeria. This is important because idiopathic causes are also important to be investigated in a country with diverse ethnic groups.

MATERIALS AND METHODS

Study area

The study was carried out in Abeokuta, Ogun state, Nigeria, among male subjects attending the infertility clinic of the State Hospital, Sokenu, Abeokuta, Ogun state, Nigeria.

Procedure

Study design

The study is a case-control and cross-sectional study that included diagnosed male infertility, while control subjects were male with proven fertility.

Study population

In this study period, 48 infertile males and 12 men who have proven evidence of fatherhood and whose wives have given birth to 2 or more children were selected as cases and controls, respectively. The test subjects were randomly recruited among people that attended the Infertility Clinic of Ogun State Hospital, Ijaye, Abeokuta, for the purpose of impaired fertility. In contrast, the control subjects were recruited, in a similar manner, among male staff of the hospital. Structured questionnaires were administered to both test and control subjects to obtain their biodata such as age, social status, and dietary habits.

Inclusion and exclusion criteria

Subjects included in the study were male subjects diagnosed with infertility (primary or secondary), age range 26-55 years. Excluded from the study were male subjects less than 26 years and above 55 years in age; those having physical abnormalities (testicular atrophy, hypoplastic testes, varicocoele); those diagnosed with other chronic conditions such as coronary heart disease, diabetes mellitus, hypertension, and stroke; heavy cigarette smokers; chronic alcoholics; and drug addicts.

Medical history and clinical examination

Medical history was taken for demographic and other characteristic purposes, and clinical examination was carried out on all the tests and controls to detect physical abnormalities in their genitalia and scrotum.

Anthropometric measurement

Anthropometric measurements (height and weight) were done using standard methods (Olooto et al. 2016). Body Mass Index (BMI) was computed using the formula BMI = weight in kilograms/height in metres squared (kg/m^2). Tests and controls were categorized based on BMI as underweight (<18.5); healthy weight (18.5-24.9); overweight (25-29.9); class I obesity (30.0-34.9); class II obesity (35-39.9); and class III obesity (≥40.0) respectively (WHO 2004).

Sample collection and storage

Seminal fluid

The participants were advised to abstain from sexual intercourse (oral, anal, vaginal) for a period of 2-7 days prior to sample collection in order to improve sperm concentration. Semen was collected from the test and control subjects by masturbation, in a private room close to the laboratory in order to shorten the time intervals between collection and analysis, hence, to limit the exposure of the semen to temperature fluctuations. The collection was done in a clean, dry, wide-mouthed glass bottle that is non-toxic to spermatozoa. The collected semen was analyzed within one hour of collection. Neubauer hemocytometer chamber was used to estimate the sperm count. Sperm analysis (total sperm cell count, cellular morphology, viscosity, white blood cell count and percentage motility) was carried out according to the World Health Organization guidelines (WHO 2010). The seminal fluid was categorized as aspermia (no ejaculate), azoospermia (no sperm cell), oligospermia ($<20 \times 10^6$ sperm/mL), and severe oligospermia ($<5 \times 10^6$ sperm/mL) (WHO 2010).

Blood sample

Next, 10 mL of venous blood sample was aseptically collected from each participant's antecubital vein (tests and controls), transferred into a clean, plain, labelled bottle; allowed to clot; and then centrifuged at 6000 rpm for 5 minutes at room temperature to obtain the serum. The clear serum was separated and kept at -20°C till assayed.

Hormonal assays

The hormones (LH, FSH and testosterone) were estimated by radioimmunoassay (RIA) method using Enzyme-Linked Immunosorbent Assay (ELISA) kits supplied by Immunometrics (UK) Limited. FSH, LH and testosterone assessments were done using the methods of Fahie-Wilson (Fahie-Wilson et al. 2000) and genomic analysis was done using the method described by Wang et al. (2012).

Phenotyping assay

Aberrant sperm cells were examined after smearing of semen and staining with New Zealand methylene blue to detect abnormalities of germ cells using the Diff Quick Method laid down (Lee et al. 2002).

PCR amplification of the AZFa gene (SY84 and SY86)

DNA was extracted by a salting-out method from leukocytes of peripheral blood samples and amplified using PCR (Miller et al. 1988). The PCR reaction was performed with the Solis Biodyne 5X HOT FIREPol Blend Master mix. Thermal cycling was conducted in a Peltier thermal cycler (PTC100) (MJ Research Series) at 95°C for 15 minutes to carry out initial denaturation. This is followed by 35 amplification cycles at 95°C for 30 seconds; at 61°C for 60 seconds for annealing, and at 72°C for 90 seconds to carry out extension; and at a temperature of 72°C for a period of 600 seconds to carry out final extension. The amplification product was separated on a 1.5% agarose gel, electrophoresis was carried out at 80V for 1 h 30 mins, and DNA bands were visualised by ethidium bromide staining using 100bp DNA ladder (Solis Biodyne) as DNA molecular weight marker. Two Sequence-Tagged Sites (STS) markers on the long arm of Y-chromosome were used for the detection of interstitial microdeletions for AZFa (Krausz et al. 2014). The markers used are SY84 and SY86, with the sequences and sizes of their base pair shown in Table 1.

Data analysis

Data were analysed using SPSS version 21.0. The variables were all expressed as the mean and standard error of mean (Mean ± SEM). A bar chart was used to describe variables. An independent student t-test was performed to compare means of variables between the test and control groups. Differences amongst variables were considered significant at $p < 0.05$.

Ethical consideration

Ethical approval was achieved from the ethical committee of State Hospital, Sokenu, Abeokuta, Ogun state, Nigeria, based on the recommendations Declaration of Helsinki. The approval number is SHA/RES/VOL 2/187. After explaining the procedures involved in the research to each participant, informed consent was also obtained from them before the commencement of the study.

RESULTS AND DISCUSSION

The results of this study are presented in the following tables and figures. Table 2 shows the anthropometric measurements of both test and control subjects. There was no statistically significant difference ($p > 0.05$) between the two groups. Table 3 shows the serum hormone levels in both test and control subjects. The values of LH, FSH and testosterone were observed to be non-significantly ($p > 0.05$) higher among the test subjects than among the control subjects.

Table 2. Anthropometric measurements of both test (infertile men) and control subjects

Parameters	Test	Control	t-value	p-value
Age (Yrs)	42.89 ± 6.03	42.0 ±4.71	0.476	0.64
Weight (Kg)	64.79 ±4.76	64.33 ±3.34	0.310	0.76
Height (m)	1.60 ±0.04	1.63 ±0.04	0.233	0.82
BMI (kg/m^2)	24.35 ±1.81	24.10 ±1.62	0.439	0.66

Note: Values are mean ± SEM, level of statistical significance was considered at $p < 0.05$

Table 3. Serum hormone levels in both test (infertile men) and control subjects

Parameter	Test N=48	Control N=12	t-value	p-value
LH (miu/mL)	8.27±4.86	6.6±3.08	2.74	0.48
FSH (miu/mL)	3.60±1.73	3.25±0.78	3.00	0.21
Testosterone (ng/mL)	4.30±0.81	3.65±0.07	3.75	0.39

Note: Values are mean ± SEM, level of statistical significance was considered at $p < 0.05$

Table 1. STS markers of AZF regions with sequences and their product size

STS marker	Locus (region)	Sequence 5'-3'	Size of PCR product Base Pair (BP)
SY84	AZFa	F- AGA AGG GTC TGA AAG CAG GT R- GCC TAC TAC CTG GAG GCT TC	326
SY86	AZFa	F- GTG ACA CAC AGA CTA TGC TTC R- ACA CAC AGA GGG ACA ACC CT	320

Note: STS: Sequence Tagged Sites; F: Forward primer; R: Reverse primer

Figure 1 shows sperm count among the test group and the control group. It shows a higher sperm count among the control group (79.0 × 10^6 cells/mL) than values obtained in the test group (15.05 × 10^6 cells/mL)

Figure 2 shows sperm cell viability among the control and test subject groups. It shows that 85 and 15% of the sperm cells produced by the control group are viable sperm cells and dead cells, respectively, as compared to the 40% and 60% living and dead cells, respectively, observed in infertile test group.

Figure 3 shows morphological abnormalities among the tests group and the control group. Abnormal sperm cells were observed in 45% of the tests (infertile men) compared to 20% in the control group.

Table 4 shows the motility profile of sperm cells among the test and control subjects. Hence, 70% of the control group exhibited significant fast motility among while 11% of the tests exhibited significant fast motility. Table 4 in the Sperm Cell Motility Profile shows the same p-value (at 0.001), but the t-value varies, as in the results in the test and control columns.

Table 5 shows the proportion of the AZFa gene that had a microdeletion among the control and test groups. No microdeletion of the AZFa gene was observed in the control group (0.0%), while 33.3% of the test group had it.

Figure 4 shows the electrophoresis results of the blood cells of subjects for the analysis of microdeletions in the AZFa region using sY84 Amplifying at 326bp. Lane M consists of a 100bp DNA marker base-pair ladder as a negative control, lanes A and B represent the control group, while lanes C, D, and E represent the test group.

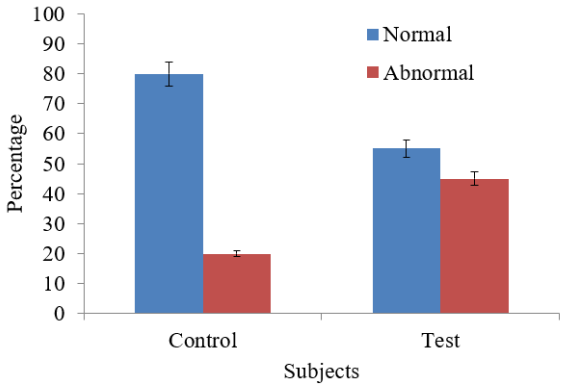

Figure 3. Bar chart showing the difference in sperm cell morphology among the test and control subject groups. Values are expressed as mean ± SEM

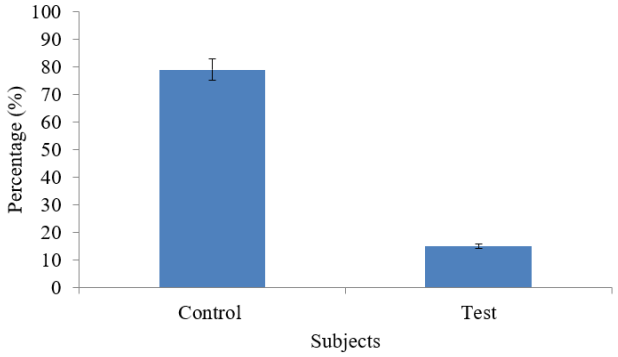

Figure 1. Bar chart showing total sperm count in the test and control subject groups. Values are expressed as mean ± SEM

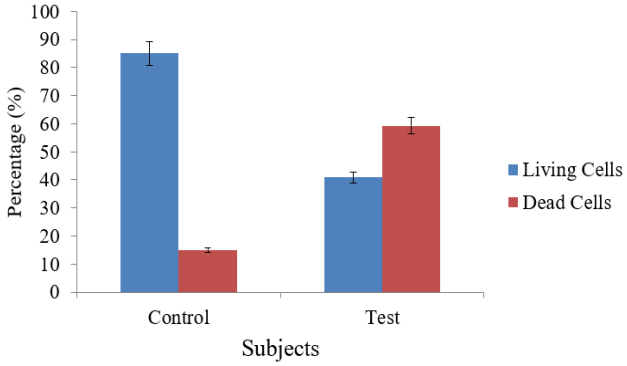

Figure 2. Bar chart showing comparative sperm cell viability among the control and the test subjects group. Values are expressed as mean ± SEM

Table 4. Motility profile of sperm cells among the control and subject groups

Motility	Test	Control	t- value	p-value
Rapidly progressively motile (%)	11.00±10.00	70.00±10.00	2.34	0.0007
Slowly progressively motile (%)	13.3±9.83	9.5±3.53	1.74	0.0010
Non-progressive motile (%)	16.7±16.02	5.50±3.54	1.26	0.0013
Non motile (%)	59.0±1.45	15.0±1.62	2.96	0.0006

Note: Values are mean ± SEM, level of statistical significance was considered at $p < 0.05$

Table 5. Proportion of micro-deletions of AZFa gene among the control and the subject groups

AZFa gene	Control				Test			
	Present		Absent		Present		Absent	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)	Number (n)	Percentage (%)	Number (n)	Percentage (%)
sY84	12	100.0	0	0.0	16	33.3	32	66.7
sY86	12	100.0	0	0.0	16	33.3	32	66.7

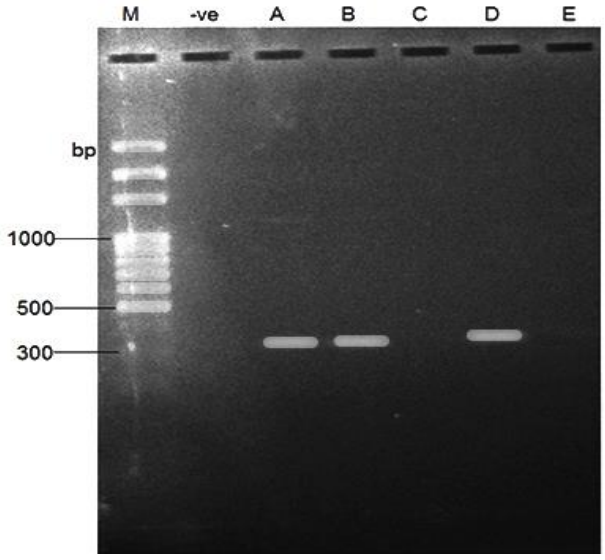

Figure 4. Electrophoresis pattern of blood cells of subjects for microdeletions in the AZFa region using sY84 amplifying at 326bp

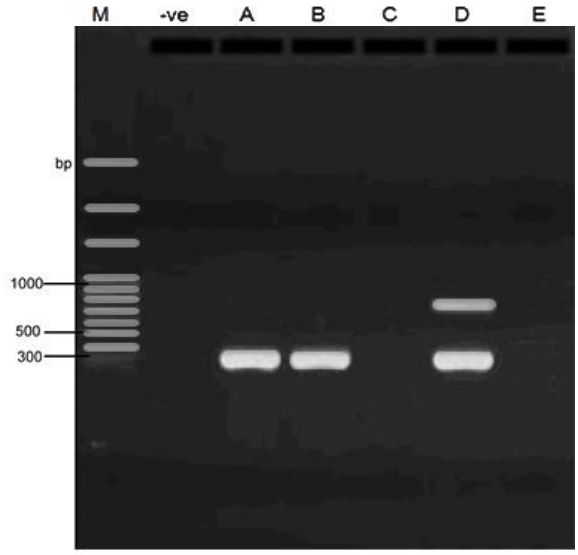

Figure 5. Electrophoresis pattern of blood cells of subjects for microdeletions on AZFa region using sY86 amplifying at 320bp

Figure 5 shows the electrophoresis results of blood cells of subjects for the analysis of microdeletions in the AZFa region using sY86 Amplifying at 320bp. Lane M consists of a 100bp DNA marker base-pair ladder as a negative control; lanes A and B represent the control group, while lanes C, D, and E represent the test group.

Discussion

Results obtained in this study revealed no significant difference in the anthropometric indices between the test and controls (Table 2), corroborating earlier reports (Oztekin et al. 2020). There are controversial reports on the impact of obesity on male infertility. Contrasting reports are being made; while some report impaired semen parameters and impaired fertility, others do not show an impact (Ameratunga et al. 2023). A study reported that obesity impacts male fertility by causing multifaceted reproductive disruptions (Chaudhuri 2022), while another study reported that body mass index has no effect on semen parameters but correlates negatively with hormones (Oztekin et al. 2020). These variations in hormones were due to fat accumulation in adipose tissues. The obtained results in this study indicate that infertility is not related to anthropometric variations in the test group but rather due to hormone and semen qualities.

The importance of hormonal evaluation in male infertility cannot be overemphasized. Some researchers had reported this in a study where male sex hormones, especially testosterone, were found to be the key players in male-related infertility (Nunes et al. 2023). Determining the levels of testosterones and gonadotropins (FSH, LH) in infertile men presenting with oligospermia and azoospermia has been emphasized (Concepción-Zavaleta et al. 2022), to provide evidence-based information on the functionality status of the hypothalamic-pituitary-testicular axis. This leads to possible need to evaluate the adrenal, thyroid, and lactotrophs in patients with central hypogonadism. Another study on the importance of hormonal evaluation in infertile men with non-obstructive azoospermia recommended that estradiol should be measured when there is an alteration in testosterone concentration (Salama and Blgozah 2020). Elevated levels of serum prolactin have a detrimental effect on male reproduction through inhibition of the pulsatile release of gonadotropins from the anterior pituitary gland (Dabbous and Atkin 2018). From this study, a non-significant ($p > 0.05$) increase in the serum levels of LH, FSH and testosterone was observed in the test group when compared to the control group (Table 3). A similar finding was reported in a previous study (Deventhiran et al. 2017). The observed increase in LH, FSH and testosterone levels indicates physiologic or compensatory responses to azoospermia and oligospermia and reflects problems at the gonadal level.

The total sperm count recorded among the control and the test groups revealed a higher count among the control group (79.0×10^6 cells/mL) than values obtained in the test group (15.05×10^6 cells/mL) (Figure 1). Also, 4.2% (2 out of 48) of the studied infertile males were observed to be normospermic, 20.8% (10 out of 48) were azoospermic, while 75% (36 out of 48) were oligospermic. This finding is supported by a previous study where sperm count was found to decline significantly in infertile men and adversely affect fertility (Levine et al. 2023).

Sperm vitality (the percentage of viable sperm cells) has also been described as an important factor in male infertility. The result obtained from this study revealed that 85% of the sperm cells produced by the control group are living and viable as compared to the 40% of living sperm cells observed in the infertile test group (Figure 2). Also, 60% of the sperm cells produced by the test group were observed to be dead, as compared to 15% of dead sperm cells observed among the control group. This corroborates a previous study, which reported a high rate of abnormal

semen quality in male partners of infertile couples (Bani et al. 2023).

A study (Agarwal et al. 2022) has emphasized the importance of sperm morphology in semen analysis for evaluating male fertility. For sperm morphology, results from this study show abnormal sperm cells in 45% of the tests (infertile men) compared to 20% observed among the control group (Figure 3). The observed morphologic abnormalities will assist in deciding the assisted reproductive treatment modalities to be adopted for the affected couples in order to achieve pregnancy in their female partner.

Semen analysis for motility assessment classifies spermatozoa as “rapidly progressive”, “slowly progressive”, “non-progressive”, and “immotile” (Sikka and Ayaz 2018). While the most common cause of male infertility is low sperm count, some men are infertile because of poor sperm motility. In this study, it was observed that 11% of infertile men exhibited rapidly progressive spermatozoa motility, 13.3% exhibited slowly progressive spermatozoa motility, 16.7% exhibited non-progressive spermatozoa motility, and 59% exhibited non-motility among the test group. In comparison, 70% exhibited rapidly progressive motility, 9.5% exhibited slowly progressive motility, 5.5% exhibited non-progressive motility, and 15% exhibited non-motility among the controls (Table 4). The observed differences in motility between the two groups were significant ($p < 0.05$) and thus constituted a major factor responsible for the observed infertility in this group of people. The significance of the mobility of sperm in semen analysis has been reported in a previous study (Dcunha et al. 2020).

Accumulating molecular studies have shown that the deletion of AZFa, AZFb, and AZFc is the most common genetic microdeletion in Y-chromosome of infertile males throughout the world (Liu et al. 2019). In this study, the percentage of Y-chromosome deletions in AZFa region using sY84 and sY86 was observed to be 33.3% (Table 5). This result corroborates that of Ilango and Poongothai (2017), who reported Y-chromosome deletion at AZFa portion in 53% of the studied infertile male subjects. This finding indicates that problems associated with male infertility are not limited to structural and hormonal abnormalities but are also due to genetic factors. Small Y-chromosome microdeletions have been reported among 2% of men with normal fertility and 7% of infertile men (16% in azoospermia or severe oligozoospermia) (Liu et al. 2019). This suggests that the presence of Y-chromosome microdeletion is not an absolute indicator or marker of infertility.

In conclusion, based on the findings from this study, it could be concluded that male infertility is not limited to hormonal and sperm quality abnormalities, but Y-chromosome abnormalities. Still, Y-chromosome deletion is a factor to be considered, especially in idiopathic male infertility. Screening for Y-chromosome microdeletions in infertile men as a routine diagnostic tool will be of immense assistance in the management options available for azoospermic and oligozoospermic patients.

ACKNOWLEDGEMENTS

This study was prompted by our quest to contribute to finding solutions to the increasing prevalence and incidence of male infertility. We want to appreciate the patients of State Hospital, Sofekun, Abeokuta, Ogun State, who volunteered to take part in this study and the hospital's staff for their cooperation.

REFERENCES

Agarwal A, Sharma R, Gupta S, Finelli R, Parekh N, Selvam MK, Henkel R, Durairajanayagam D, Pompeu C, Madani S, Belo A. 2021. Sperm morphology assessment in the era of intracytoplasmic sperm injection: Reliable results require focus on standardization, quality control, and training. World J Mens Health 40 (3): 347-360. DOI: 10.5534/wjmh.210054.

Ameratunga D, Gebeh A, Amoako A. 2023. Obesity and male infertility. Best Pract Res Clin Obstet Gynaecol 90: 102393. DOI: 10.1016/J.bpobgyn.2023.102393.

ASRM. 2015. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: A committee opinion. Fertil Steril 103 (3): e18-25. DOI: 10.1016/j.fertnstert.2014.12.103.

Bani SB, Anachem R, Banyeh M, Dapare PPM, Adams Y, Nkansah C, Mensah K, Appiah SK, Bani F, Osumanu H, Abbam G, Daud S. 2023. Study on semen quality among men seeking infertility treatment in Tamale, Ghana. Asian J Res Rep Urol 6 (1): 27-36.

Barak S, Baker HWG. 2016. Clinical management of male infertility. In: Jameson JL, De Groot LJ (eds.). Endocrinology: adult and pediatric E-Book. Elsevier Health Sciences. DOI: 10.1016/B978-0-323-18907-1.00141-4.

Ceylan GG, Ceylan C, Elyas H. 2009. Genetic anomalies in patients with severe oligozoospermia and azoospermia in eastern Turkey: A prospective study. Genet Mol Res 8 (3): 915-922. DOI: 10.4238/vol8-3gmr616.

Chaudhuri GR, Das A, Kesh SB, Bhattacharya K, Dutta S, Sengupta P, Syamal AK. 2022. Obesity and male infertility: Multifaceted reproductive disruption. Middle East Fertil Soc J 27: 8. DOI: 10.1186/S43043-022-00099-2.

Chen CP. 2007. Chromosomal abnormalities associated with omphalocele. Taiwan J Obstet Gynecol 46 (1): 1-8. DOI: 10.1016/S1028-4559(08)60099-6.

Concepción-Zavaleta M, Paz Ibarra JL, Ramos-Yataco A, Coronado-Arroyo J, Concepción-Urteaga L, Roseboom PJ, Williams CA. 2022. Assessment of hormonal status in male infertility. An Update. Diabetes Metab Syndr 16 (3): 102447. DOI: 10.1016/J.dsx.2022.102447.

Dabbous Z, Atkin SL. 2018. Hyperprolactinaemia in male infertility: Clinical case scenarios. Arab J Urol 16 (1): 44-52. DOI: 10.1016/j.aju.2017.10.002.

Dcunha R, Hussein RS, Ananda H, Kumari S, Adiga SK, Kannan N, Zhao Y, Kalthur G. 2020. Current insights and latest updates in sperm motility and associated applications in assisted reproduction. Reprod Sci 29 (1): 7-25. DOI: 10.1007/s43032-020-00408-y.

Deventhiran R, Ramanathan K, Nandakumar N. 2017. Prevalance of male infertility in India: Studies on the effects of gonadotropin releasing hormones. Asian J Pharm Clin Res 10 (8): 208-210. DOI: 10.22159/ajpcr.2017.v10i8.18254.

Dhanoa JK, Mukhopadhyay CS, Arora JS. 2016. Y-chromosomal genes affecting male fertility: A review. Vet World 9 (7): 783-791. DOI: 10.14202/vetworld.2016.783-791.

Fahie-Wilson M. 2000. Detection of macroprolactin causing hyperprolactinemia in commercial assays for prolactin. Clin Chem 46 (12): 2022-2023. DOI: 10.1093/clinchem/46.12.2022.

Ferlin A, Moro E, Rossi A, Dallapiccola B, Foresta C. 2003. The human Y chromosome’s Azoospermia Factor b (AZFb) region: Sequence, structure, and deletion analysis in infertile men. J Med Genet 40 (1): 18-24. DOI: 10.1136/jmg.40.1.18.

Foresta C, Moro E, Ferlin A. 2001. Y chromosome microdeletions and alterations of spermatogenesis. Endocr Rev 22 (2): 226-239. DOI: 10.1210/edrv.22.2.0425.

Gunes S, Arslan MA, Hekim GNT, Asci R. 2016. The role of epigenetics in idiopathic male infertility. J Assist Reprod Genet 33 (5): 553-569. DOI: 10.1007/s10815-016-0682-8.

He T, Zhang X, Deng H, Zhou W, Zhao X, Zhao H, Lu J, Zheng Y, Zhang C, Zhang L, Yin A. 2017. A novel Y chromosome microdeletion potentially associated with defective spermatogenesis identified by custom array comparative genome hybridization. Reprod Biomed Online 34 (1): 75-81. DOI: 10.1016/j.rbmo.2016.09.010.

Ilango L, Poongothai J. 2017. Screening of Y-chromosomal microdeletions using 8 STS primers in idiopathic infertile men from Coimbatore District, Tamilnadu. Intl J Innovative Sci Res Technol 2 (10): 614-620.

Khon TP, Khon JR, Owen RC, Coward RM. 2019. The prevalence of Y-chromosome microdeletions in oligozoospermic men: A systematic review and meta-analysis of European and North American Studies. Eur Urol 76 (5): 626-636. DOI: 10.1016/J.eururo.2019.07.033.

Krausz C, Hoefsloot L, Simoni M, Tüttelmann F. 2013. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: State-of-the-art. Andrology 2 (1): 5-19. DOI: 10.1111/j.2047-2927.2013.00173.x.

Kuroda S, Usui K, Sanjo H, Takeshima T, Kawahara T, Uemura H, Yumura Y. 2020. Genetic disorders and male infertility. Reprod Med Biol 19 (4): 314-322. DOI: 10.1002/rmb2.12336.

Lee RK, Hou JW, Ho HY, Hwu YM, Lin MH, Tsai YC, Su JT. 2002. Sperm morphology analysis using strict criteria as a prognostic factor in intrauterine insemination. Intl J Androl 25 (5): 277-280. DOI: 10.1046/j.1365-2605.2002.00355.x.

Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, Pinotti R, Swan SH. 2023. Temporal trends in sperm count: A systematic review and meta-regression analysis. Hum Reprod Update 23 (6): 646-659. DOI: 10.1093/humupd/dmx022.

Liu JL, Peña V, Fletcher SA, Kohn TP. 2020. Genetic testing in testing in male infertility – reassessing screening thresholds. Curr Opin Urol 30 (3): 317-323. DIO: 10.1097/MOU.0000000000000764.

Liu T, Song YX, Jiang YM. 2019. Early detection of Y chromosome microdeletions in infertile men is helpful to guide clinical reproductive treatments in southwest of China. Medicine (Baltimore) 98 (5): e14350. DOI: 10.1097/MD.0000000000014350.

Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16 (3): 1215. DOI: 10.1093/nar/16.3.1215.

Nunes DC, Ribeiro JC, Alves MG, Oliveria PF, Bernardino RL. 2023. Male sex hormones, metabolic syndrome, and aquaporins: A triad of players in male (in) fertility. Intl J Mol Sci 24 (3): 1960. DOI: 10.3390/ijms24031960.

Olooto WE, Olawale OO, Amballi AA, Ezima Nkechi E, Ogundahunsi AO. 2016. Plasma lipid profile and hepatic transaminase activities as indicator of cardiovascular disorder and hepatocellular damage in type 2 diabetes mellitus patients. Afr J Sci Nat 3: 10-16.

Oztekin U, Caniklioglu M, Sari S, Gurel A, Selmi V, Isikay L. 2020. The impact of BMI on reproductive hormones, testosterone/estradiol ratio and semen parameters. Cent Eur J Urol 73 (2): 226-230. DOI: 10.5173/ceju.2020.0020.

Rabinowitz MJ, Huffman PJ, Haney NM, Kohn TP. 2021. Y-chromosome microdeletions: A review of prevalence, screening, and clinical considerations. Appl Clin Genet 14: 51-59. DOI: 10.2147/TACG.S267421.

Salama N, Blgozah S. 2020. Serum estradiol levels in infertile men with non-obstructive azoospermia. Ther Adv Reprod Health 14:2633494120928342. DOI: 10.1177/26334941.20928342.

Sikka CS, Ayaz A. 2018. Standardized semen analysis and quality control management for multicenter male reproductive toxicology clinical trials. Bioenvironmental Issues Affecting Men's Reproductive and Sexual Health. Academy Press. DOI: 10.1016/B978-0-12-801299-4.00023-2.

Sokol RZ. 2001. Infertility in men with cystic fibrosis. Curr Opin Pulm Med 7 (6): 421-426. DOI: 10.1097/00063198-200111000-00011.

Trinh ST, Nguyen NN, Le HTT, Pham HTM, Trieu ST, Tran NTM, Ho S, Tran V, Trinh V, Nguyen HT, Pham MN, Dang TD, Dinh VH, Doan HT. 2023. Screening Y Chromosome Microdeletion in 1121 Men with Low Sperm Concentration and the Outcomes of Microdissection Testicular Sperm Extraction (mTESE) for Sperm Retrieval from Azoospermic Patients. Appl Clin Genet 16: 155-164. DOI: 10.2147/TACG.S420030.

Wang J, Fan HC, Behr B, Quake SR. 2012. Genome-wide single-cell analysis of recombination activity and de novo mutation rates in human sperm. Cell 150 (2): 402-412. DOI: 10.1016/j.cell.2012.06.030.

World Health Organization. 2004. WHO Classification of obesity by BMI.

World Health Organization. 2010. WHO laboratory manual for the examination and processing of human semen, 5th edition. Cambridge University Press, Cambridge.