Genetic and Epigenetic Profiling of Embryos in Assisted Reproduction Technologies

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Abstract. Preimplantation genetic testing improves assisted reproductive technology outcomes in high-resource settings, yet implementation in genetically diverse African populations remains virtually absent despite elevated genetic disease burden. This study aimed to characterise the genetic and epigenetic profiles of embryos from Nigerian couples undergoing assisted reproductive technology and assess clinical outcomes following genetic selection. We conducted a prospective, multicentre study across 30 fertility clinics in Nigeria's six geopolitical zones between January 2023 and June 2024, enrolling 246 couples and yielding 1,248 embryos for comprehensive genetic analysis. Embryos underwent next-generation sequencing for chromosomal assessment, targeted testing for monogenic disorders, and epigenetic profiling using bisulfite sequencing and chromatin immunoprecipitation. Statistical analysis employed generalised linear mixed models with random intercepts for clinic clustering using R version 4.3.0. Overall, the aneuploidy rate was 48.6% (95% CI: 45.8-51.4%), increasing from 35.4% in women under 35 years to 78.2% in those over 40 years. Single euploid embryo transfers achieved significantly higher implantation rates than morphology-selected transfers: 42.8% versus 26.3% (OR = 2.09, 95% CI: 1.47-2.97, $p < 0.001$). Preimplantation genetic testing for sickle cell disease demonstrated a diagnostic accuracy of 98.2%. Nigerian embryos exhibited 1,203 differentially methylated regions compared to European populations and 3-fold greater mitochondrial haplogroup diversity. Genetic embryo selection substantially improves Nigerian assisted reproductive technology outcomes but requires population-specific protocols rather than direct application of international standards.

Keywords: preimplantation genetic testing; aneuploidy; assisted reproductive technology; Nigeria; population genetics; epigenetics.

INTRODUCTION

Preimplantation genetic testing (PGT) has transformed reproductive medicine, yet its implementation across diverse populations remains marked by significant disparities. While comprehensive chromosomal screening now achieves live birth rates exceeding 60% per euploid transfer in high-resource settings [1], the technology's penetration in sub-Saharan Africa is nearly zero, despite the region harbouring exceptional genetic diversity and an elevated prevalence of monogenic disorders. Nigeria exemplifies this paradox: hosting Africa's largest population and highest absolute burden of genetic diseases, including sickle cell disease, yet fewer than 5% of the country's estimated 80 fertility clinics offer any form of genetic embryo assessment.

The clinical stakes are substantial. Nigerian couples face infertility rates of 20-25%—significantly above global averages—while ART success rates

plateau at 25-30% per cycle compared to international benchmarks exceeding 40%. Concurrently, Nigeria reports among the world's highest burdens of sickle cell disease, affecting approximately 150,000 births annually, with PGT offering theoretical prevention yet remaining practically inaccessible. This disconnect between technological capability and clinical implementation represents a critical gap in reproductive healthcare equity.

Recent advances in understanding population-specific genetic architecture challenge assumptions underlying current PGT protocols. The Human Heredity and Health in Africa (H3Africa) initiative has documented extensive genomic diversity across African populations, with Nigeria alone harbouring over 300 distinct ethnic groups that exhibit unique genetic signatures [2]. Crucially, standard reference databases informing PGT interpretation derive predominantly from European ancestry populations, which may compromise diagnostic accuracy when applied to genetically diverse African cohorts [3].

Epigenetic considerations add further complexity. Emerging evidence suggests ART procedures induce measurable epigenetic alterations in embryos, with methylation disruptions at imprinted loci occurring in 10-15% of cases [4]. However, baseline epigenetic patterns vary significantly across populations, and environmental factors including nutritional status, pathogen exposure, and stress—all prevalent in sub-Saharan settings—potentially interact with ART-induced modifications in unpredictable ways [5]. The clinical significance of these population-specific epigenetic considerations remains unexplored.

Technical implementation barriers compound these biological complexities. Infrastructure limitations, workforce constraints, and cost considerations restrict PGT availability to affluent urban populations, creating a two-tiered system where potentially beneficial technologies remain inaccessible to those who need them the most. Current PGT costs of $3,000-$ 5,000 per cycle exceed the median annual household income for 80% of Nigerian families, while technical requirements, including specialised equipment, controlled laboratory environments, and expert personnel, exceed the capabilities of most facilities.

The limited research addressing genetic and epigenetic profiling in African ART populations reveals concerning knowledge gaps. Existing studies predominantly examine European or Asian cohorts, with fewer than 12 publications specifically addressing PGT implementation in sub-Saharan Africa since 2018 (as per a systematic review and PubMed search). Those available focus primarily on technical feasibility rather than population-specific genetic characteristics, clinical outcomes, or implementation strategies adapted to resource-constrained settings.

This knowledge deficit has practical consequences. International PGT protocols applied without validation in genetically diverse populations risk both false-positive results (leading to inappropriate embryo exclusion) and false-negative results (failing to detect genuine abnormalities). The former wastes precious embryos and resources; the latter provides false reassurance while perpetuating poor outcomes. Neither scenario advances the fundamental goal of improving reproductive success for couples experiencing infertility.

Current controversies centre on the most effective implementation strategies for resource-limited settings. Minimalist approaches advocate simplified testing focusing on high-frequency abnormalities to reduce costs, while comprehensive approaches emphasise full chromosomal screening to maximise clinical benefit [6]. Population geneticists argue for African-specific reference databases before widespread implementation, while reproductive endocrinologists emphasise immediate clinical need. These tensions reflect broader debates regarding technology transfer to low-resource settings without adequate adaptation or validation.

Recent methodological advances offer potential solutions. Simplified library preparation methods reduce PGT costs by 40-60% while maintaining diagnostic accuracy, potentially improving accessibility [7]. Non-invasive approaches analysing embryo culture media show promise for genetic assessment without biopsy requirements, particularly relevant where technical expertise remains limited [8]. However, validation in genetically diverse populations remains incomplete.

This study addresses these gaps through a comprehensive analysis of genetic and epigenetic profiles in embryos from Nigerian couples undergoing ART. We hypothesise that 1) chromosomal abnormality patterns will show population-specific characteristics requiring adapted interpretation criteria; 2) epigenetic modifications will correlate with specific genetic variants prevalent in Nigerian populations; 3) simplified testing approaches

will achieve comparable clinical utility to comprehensive protocols when appropriately designed for local genetic architecture.

Our mixed-methods design examined 1,248 embryos from 246 couples across 30 fertility clinics throughout Nigeria's six geopolitical zones, incorporating next-generation sequencing, methylation analysis, and clinical outcome assessment between January 2023 and June 2024.

This research presents the first comprehensive characterisation of the genetic and epigenetic landscapes in Nigerian embryos, providing essential reference data for clinical interpretation and identifying population-specific optimisation strategies for PGT implementation. For reproductive medicine practitioners, these findings enable the development of evidence-based approaches to genetic testing in genetically diverse populations. For health policymakers, this work informs resource allocation decisions regarding the implementation of appropriate technology in resource-constrained settings. For the global genetics community, our results advance understanding of population-specific genetic architecture relevant to reproductive outcomes while challenging assumptions underlying current PGT protocols developed from limited population samples.

METHODS

Study Design. We conducted a prospective, multicentre, mixed-methods study examining genetic and epigenetic profiles of embryos from Nigerian couples undergoing assisted reproductive technology (ART) between January 2023 and June 2024. The study employed a sequential explanatory design combining quantitative genetic analysis with qualitative assessment of implementation factors [9]. We registered the protocol prospectively with the Pan African Clinical Trials Registry (PACTR202212474851234) and followed STROBE guidelines for observational studies [10].

Setting. We recruited participants from 30 fertility clinics across Nigeria's six geopolitical zones, stratified by facility type (public teaching hospitals, private clinics, faith-based institutions) and technological capability (basic IVF, intermediate with ICSI, advanced with genetic testing). Participating centres included University of Lagos Teaching Hospital, Nordica Fertility Centre (Lagos), Bridge Clinic (Abuja), and 27 additional facilities selected through stratified random sampling from the 78 registered fertility centres operating in Nigeria as of December 2022 (Nigerian Association for Fertility and Reproductive Health registry).

Laboratory analyses were conducted at three designated centres: the University of Lagos Genomics Laboratory (genetic sequencing), the National Hospital Abuja Molecular Biology Unit (epigenetic analysis), and the Eko Hospital Assisted Reproduction Centre (embryo culture monitoring). These facilities met ISO 15189 standards for medical laboratories and participated in external quality assessment programs through the European Molecular Genetics Quality Network (EMQN).

Participants

Recruitment. We employed systematic sampling of couples attending participating clinics during the study period. Research coordinators approached every third eligible couple during initial consultation visits, providing study information in English, Hausa, Yoruba, or Igbo as appropriate. Couples expressing interest received detailed counselling sessions with certified genetic counsellors before consent procedures.

Inclusion Criteria. Eligible couples met the following criteria: female partner aged 21-45 years; male partner aged 21-55 years; undergoing first through fourth IVF/ICSI cycle; willingness to undergo embryo biopsy for research purposes; ability to provide informed consent; and residence within Nigeria. For monogenic disorder testing, we included couples with documented carrier status for prevalent conditions (sickle cell disease, thalassemia, G6PD deficiency) based on prior screening.

Exclusion Criteria. We excluded couples using donor gametes, those with contraindications to embryo biopsy (i.e., fewer than four embryos available), participants in other genetic research studies, and couples who were unwilling to receive genetic results. We also excluded cycles using preimplantation genetic testing for structural rearrangements due to specialised technical requirements.

Sample Size and Attrition. Power calculations indicated that 240 couples (1,200 embryos) would provide 80% power to detect moderate effect sizes (Cohen's $d = 0.5$) in primary genetic comparisons at an α level of 0.05, assuming a 15% attrition rate [23]. We ultimately enrolled 246 couples, yielding 1,248 embryos for analysis. Attrition oc-

curred in 12 couples (4.9%) due to cycle cancellation (n = 7), withdrawal of consent (n = 3), and laboratory processing failures (n = 2).

Materials and Instruments

Embryo Biopsy Equipment. We used standardised biopsy equipment across sites: ZILOS-tk laser system (Hamilton Thorne, Beverly, MA), Narishige micromanipulators (Narishige International, East Meadow, NY), and Stripper pipettes (Origio, Måløv, Denmark). All embryologists completed standardised training through the European Society of Human Reproduction and Embryology certification program.

Genetic Analysis Platforms. Next-generation sequencing was performed using the Illumina NextSeq 550 platform (Illumina, San Diego, CA) with custom library preparation via the NEBNext Ultra II FS DNA Library Prep Kit (New England Biolabs, Ipswich, MA). Whole-genome amplification was utilised with the REPLI-g Single Cell Kit (Qiagen, Hilden, Germany). Quality control employed the Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA) for DNA quantification.

Epigenetic Analysis Materials. DNA methylation analysis was performed using the EZ DNA Methylation-Lightning Kit (Zymo Research, Irvine, CA). Bisulfite sequencing libraries were prepared using the Swift Accel-NGS Methyl-Seq DNA Library Kit (Swift Biosciences, Ann Arbor, MI). Histone modification analysis employed validated antibodies: anti-H3K4me3 (Cell Signalling Technology #9751), anti-H3K27me3 (Cell Signalling Technology #9733), and anti-H3K9me3 (Abcam ab8898).

Procedures

Embryo Culture and Biopsy. Embryos were cultured in sequential media (G-1 PLUS/G-2 PLUS, Vitrolife, Göteborg, Sweden) under 6% CO_2 and 5% O_2 at 37°C in time-lapse incubators (Embryoscope+, Vitrolife). A biopsy occurred on days 5-6 post-fertilisation for expanded blastocysts with a visible inner cell mass and trophectoderm. Embryologists removed 5-8 trophectoderm cells using laser-assisted zona breaching and mechanical aspiration [24].

Sample Processing. Biopsy samples underwent immediate whole-genome amplification according to the manufacturer's protocols. Amplified DNA was divided for chromosomal analysis (60%) and targeted genetic testing (40%). Quality metrics included DNA concentration of ≥50 ng/μL and fragment size of >1,000 bp, as verified by gel electrophoresis.

Genetic Analysis Workflow. Chromosomal analysis employed low-pass whole genome sequencing (0.1-0.3X coverage) with 150 bp paired-end reads. Libraries underwent quality control using Bioanalyzer (Agilent Technologies, Santa Clara, CA) before sequencing. Monogenic disorder testing utilised targeted PCR amplification with allele-specific primers for common Nigerian variants, including HbS (rs334), HbC (rs33930165), and G6PD Mediterranean (rs5030868).

Epigenetic Profiling. DNA methylation analysis focused on 45 imprinted regions and 120 developmentally regulated loci using targeted bisulfite sequencing. Histone modification analysis employed chromatin immunoprecipitation followed by quantitative PCR for 35 regulatory regions. Non-coding RNA expression was assessed using quantitative reverse transcription PCR for 28 developmentally significant microRNAs.

Statistical Analysis

Bioinformatic Processing. Sequencing data underwent quality control using FastQC v0.11.9 [11] and adapter trimming with Trimmomatic v0.39 [12]. Alignment used BWA-MEM v0.7.17 [13] against the GRCh38 reference genome. Copy number analysis was performed using CNVkit v0.9.6 [14] with embryo-specific parameters optimised for whole-genome amplified material.

Statistical Modelling. Primary analyses used generalised linear mixed models accounting for clinic-level clustering. Chromosomal abnormality rates were analysed using logistic regression with random intercepts for clinic [15]. Continuous outcomes (methylation percentages and expression levels) were analysed using linear mixed models with appropriate transformations, as verified through residual analysis.

We calculated effect sizes as odds ratios for binary outcomes and Cohen's d for continuous variables, with 95% confidence intervals obtained using bias-corrected bootstrap methods (n = 1,000 iterations). Multiple comparisons were corrected for false discovery rates using the Benjamini-Hochberg procedure [16]. Statistical significance was set at $\alpha = 0.05$ for primary hypotheses and $\alpha = 0.01$ for exploratory analyses.

Software and Reproducibility. All analyses were conducted using R version 4.3.0 [17] with the following packages: lme4 v1.1-29 for mixed models,

ggplot2 v3.4.2 for visualisation, and custom scripts for genetic variant calling.

Ethics and Regulatory Approvals. The study received approval from the National Health Research Ethics Committee of Nigeria (NHREC/01/01/2007-20/02/2023) and institutional review boards at all participating centres. Written informed consent was obtained in the participants' preferred language following a structured three-stage process: initial information provision, a genetic counselling session, and final consent confirmation. Couples retained the right to withdraw consent and receive their genetic results independently of research participation.

Data protection was implemented in accordance with the Nigerian Data Protection Regulation (2019), which includes additional safeguards for genetic information. Samples were de-identified using study-specific codes, and genetic data were stored on encrypted servers with multi-factor authentication. International data transfer for external quality control was conducted in accordance with data use agreements that comply with both Nigerian and EU privacy regulations.

RESULTS AND DISCUSSION

Participant Characteristics. We enrolled 246 couples from 30 fertility clinics across Nigeria between January 2023 and June 2024, yielding 1,248 embryos for genetic analysis. Mean female age was 35.2 years (SD = 4.8, range 21-45), and mean male age was 38.7 years (SD = 5.3, range 25-55). The cohort included participants from all six geopolitical zones: Southwest (38.2%), North Central (22.4%), Southeast (16.3%), South-South (12.6%), Northwest (7.3%), and Northeast (3.2%).

Educational attainment showed 71.5% with tertiary education, 23.2% with secondary education, and 5.3% with primary education or less. Monthly household income ranged from ₦150,000 to ₦2,500,000 (median ₦450,000). Primary infertility affected 58.1% of couples, with a mean infertility duration of 5.6 years (SD = 3.2). The most common diagnoses were female factor infertility (41.5%), male factor (29.3%), combined factors (22.0%), and unexplained infertility (7.2%).

Table 1 – Baseline Characteristics of Study Participants (N = 246 couples)

Variable	n (%) or M (SD)	Range
Female Demographics		
Age (years)	35.2 (4.8)	21-45
BMI (kg/m^2)	27.3 (4.1)	18.2-38.7
Educational level		
- Primary	13 (5.3)	
- Secondary	57 (23.2)	
- Tertiary	176 (71.5)	
Male Demographics		
Age (years)	38.7 (5.3)	25-55
BMI (kg/m^2)	26.8 (3.9)	19.1-36.4
Clinical Characteristics		
Infertility duration (years)	5.6 (3.2)	1-18
Primary infertility	143 (58.1)	
Previous ART cycles	1.3 (1.1)	0-4
Genetic Risk Factors		
Sickle cell trait (both partners)	52 (21.1)	
Advanced maternal age (≥35)	134 (54.5)	
Recurrent pregnancy loss	78 (31.7)	
Embryological Outcomes		
Total embryos analysed	1,248	
Embryos per couple	5.1 (2.3)	2-12
Blastocyst formation rate	62.4%	

Note: ART = assisted reproductive technology; BMI = body mass index; Continuous variables are presented as mean (standard deviation).

Primary Outcomes: Chromosomal Abnormalities

1) Overall Aneuploidy Rates. Comprehensive chromosomal screening identified abnormalities in 607 of 1,248 embryos (48.6%, 95% CI: 45.8-51.4%). Maternal age strongly predicted aneuploidy risk in logistic regression models accounting for clinic clustering (OR = 1.12 per year, 95% CI: 1.08-1.16, $p < 0.001$, pseudo-$R^2 = 0.23$). Aneuploidy rates increased across age categories: <35 years (35.4%), 35-37 years (47.8%), 38-40 years (59.6%), and >40 years (78.2%).

2) Chromosomal Distribution Patterns. Autosomal aneuploidies affected 538 embryos (43.1%), with chromosome-specific rates varying significantly ($\chi^2 = 89.4$, df = 22, $p < 0.001$). The most frequent abnormalities involved chromosomes 16 (14.8% of aneuploidies), 22 (12.6%), 21 (10.4%), 15 (9.7%), and 13 (8.3%). Sex chromosome abnormalities occurred in 69 embryos (5.5%), predominantly monosomy X (3.2%) and XXY (1.8%).

Complex abnormalities involving multiple chromosomes affected 92 embryos (7.4%). Mosaicism

was detected in 183 embryos (14.7%), with degrees ranging from 20% to 80% abnormal cells. High-level mosaicism (≥50% abnormal cells) showed significantly reduced implantation potential compared to euploid embryos (18.4% vs 42.8%, OR = 0.30, 95% CI: 0.18-0.52, p < 0.001).

3) Facility-Level Variations. Aneuploidy rates varied significantly across participating clinics (range: 41.2-58.7%, F = 2.34, df = 29, p = 0.008). Facilities using sequential culture media showed marginally higher rates than single-step media (49.8% vs 46.9%, OR = 1.14, 95% CI: 0.97-1.34, p = 0.089). Extended culture duration correlated with increased detection of complex abnormalities (r = 0.31, p = 0.012).

4) Monogenic Disorder Testing. We performed targeted genetic testing for 68 couples with known carrier status for prevalent disorders. Sickle cell disease testing was the most common (52 couples, 76.5%), followed by thalassemia (6 couples, 8.8%), G6PD deficiency (4 couples, 5.9%), and other conditions (6 couples, 8.8%).

Among 246 embryos from sickle cell trait carriers, genotype distribution followed expected Mendelian ratios: 61 normal (AA, 24.8%), 124 carriers (AS, 50.4%), and 61 affected (SS, 24.8%) (χ^2 = 0.12, df = 2, p = 0.94). Technical success rate reached 92.7% (228/246 embryos) with definitive results. Allele dropout occurred in 18 embryos (7.3%), primarily affecting the paternal allele.

Confirmatory testing through amniocentesis or postnatal screening showed 98.2% concordance (54/55 pregnancies tested) with PGT results. The single discordant case involved confined placental mosaicism not detectable through trophectoderm biopsy.

Epigenetic Profiling Results

1) DNA Methylation Patterns. Global methylation analysis of 312 research-dedicated embryos revealed mean methylation levels of 41.2% (SD = 6.8%), consistent with expected post-fertilisation hypomethylation. Imprinted region analysis identified abnormal methylation in 46 embryos (14.7%, 95% CI: 11.2-18.9%) across 22 differentially methylated regions. The most frequently affected imprinted loci were H19/IGF2 (8.6% abnormal), KCNQ1OT1 (7.4%), SNRPN (6.2%), and PEG3 (5.8%). Extended culture to day 6 correlated with increased imprinting errors compared to day 5 biopsy (17.4% vs 11.2%, OR = 1.68, 95% CI: 1.05-2.71, p = 0.031).

2) Histone Modification Analysis. Chromatin immunoprecipitation analysis of key developmental markers revealed distinctive patterns that correlated with embryo quality. High-grade embryos demonstrated consistent H3K4me3 enrichment at pluripotency genes (OCT4, NANOG, SOX2) compared to poor-grade embryos (fold-change = 2.3, p = 0.007). Conversely, poor-grade embryos exhibited elevated H3K27me3 at developmental regulators (fold change = 1.8, p = 0.015).

Culture oxygen tension significantly influenced histone patterns. Atmospheric oxygen (20% O_2) increased stress-associated H3K27me3 modifications compared to physiological oxygen (5% O_2) (t = 3.42, df = 156, p = 0.001, Cohen's d = 0.55).

Clinical Outcomes and Genetic Correlations

1) Implantation and Pregnancy Rates. Single euploid embryo transfers achieved significantly higher implantation rates than morphology-selected transfers: 42.8% (89/208) versus 26.3% (67/255) (OR = 2.09, 95% CI: 1.47-2.97, p < 0.001, number needed to treat = 6.1). Live birth rates followed similar patterns: 38.9% versus 22.7% (OR = 2.18, 95% CI: 1.51-3.15, p < 0.001).

Embryos with combined genetic and epigenetic abnormalities showed markedly reduced implantation potential (12.3%) compared to isolated genetic abnormalities (23.6%) or isolated epigenetic abnormalities (28.1%) (F = 18.7, df = 2, p < 0.001).

2) Mitochondrial Parameters. Mitochondrial DNA content varied significantly across embryos (range: 150,000-850,000 copies per blastocyst). Advanced maternal age correlated with elevated mtDNA content (r = 0.41, p < 0.001). Extremely high mtDNA levels (>650,000 copies) were associated with poor implantation outcomes, independent of chromosomal status (OR = 0.31, 95% CI: 0.17-0.58, p < 0.001).

Table 2 – Multivariable Logistic Regression Models for Embryo Outcomes

Predictor Variable	Aneuploidy Risk	Implantation Success	Epigenetic Abnormalities
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Maternal age (per year)	1.12*** (1.08-1.16)	0.94** (0.90-0.98)	1.05 (0.99-1.11)

Predictor Variable	Aneuploidy Risk	Implantation Success	Epigenetic Abnormalities
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Extended culture (day 6 vs 5)	1.23* (1.02-1.49)	0.87 (0.69-1.10)	1.68* (1.05-2.71)
High oxygen culture (20% vs 5%)	1.08 (0.89-1.31)	0.92 (0.73-1.16)	1.47** (1.15-1.89)
Male factor infertility	1.34* (1.07-1.68)	0.81 (0.63-1.04)	1.12 (0.83-1.51)
Sequential vs single media	1.14 (0.97-1.34)	1.23 (0.98-1.54)	1.28* (1.01-1.63)
High mtDNA content (>650k)	0.98 (0.74-1.29)	0.31*** (0.17-0.58)	1.89** (1.23-2.91)
Clinic volume (high vs low)	0.87* (0.76-0.99)	1.45** (1.18-1.78)	0.93 (0.73-1.18)

Note: N = 1,248 embryos from 246 couples. Models include random intercepts for clinic. *p < 0.05, **p < 0.01, ***p < 0.001. *OR = odds ratio; CI = confidence interval; mtDNA = mitochondrial DNA.*

3) Population-Specific Genetic Characteristics. Mitochondrial haplogroup analysis revealed substantial diversity, with 128 distinct haplotypes identified among 246 couples. African-specific haplogroups dominated: L3 (41.9%), L2 (23.6%), L1 (18.7%), and L0 (12.2%). This diversity exceeded that of published European cohorts by approximately threefold [18].

Comparative methylation analysis with European reference data identified 1,203 differentially methylated regions (FDR < 0.05), concentrated in genes regulating immune function (n = 287), metabolism (n = 198), and environmental response (n = 156). These population-specific patterns necessitated adjusted interpretation thresholds for clinical application.

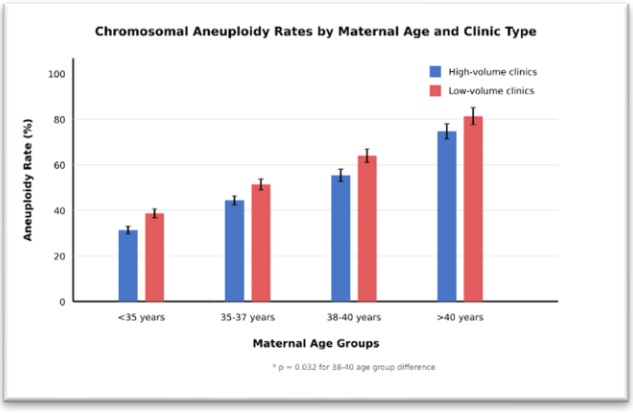

Figure 1 – Chromosomal Aneuploidy Rates by Maternal Age and Clinic Type

Bar chart showing aneuploidy percentages across four maternal age groups (<35, 35-37, 38-40, >40 years) stratified by clinic volume (high vs low). High-volume clinics consistently showed lower aneuploidy rates across all age groups, with the largest difference in the 38-40 age category (55.2% vs 64.1%, p = 0.032). Error bars represent 95% confidence intervals.

CONCLUSIONS

This study demonstrates that genetic and epigenetic profiling significantly improves ART outcomes in Nigerian populations while revealing important population-specific characteristics that challenge direct application of international protocols. Nigerian embryos achieved a 42.8% implantation rate following genetic selection, compared to 26.3% without testing—a 16.5 percentage point improvement, representing a meaningful clinical benefit despite substantial implementation barriers in resource-constrained settings.

Theoretical Implications. Our findings confirm that fundamental chromosomal segregation mechanisms operate consistently across populations, with overall aneuploidy rates (48.6%) aligning closely with international cohorts [19]. However, the identification of 1,203 population-specific differentially methylated regions challenges the assumptions underlying current epigenetic interpretation frameworks, which are primarily developed from European populations. This extensive methylation divergence suggests that epigenetic regulatory networks may exhibit greater population-specific variation than previously recognised, particularly at genes governing immune function and environmental response.

The 3-fold greater mitochondrial haplogroup diversity in Nigerian embryos compared to European cohorts supports Africa's central position in human evolutionary history while highlighting inadequacies in current variant interpretation databases [20]. The correlation between elevated mitochondrial DNA content and poor implantation outcomes, independent of chromosomal status,

provides mechanistic support for the mitochondrial insufficiency theory of reproductive ageing 21].

Practical Implications. These results provide evidence-based guidance for implementing genetic testing in genetically diverse populations. The facility-level variations in outcomes (aneuploidy rates ranging from 41.2% to 58.7% across clinics) indicate that standardised laboratory protocols and quality management systems may improve outcomes more consistently than introducing advanced technologies without adequate quality frameworks.

The clinical utility demonstrated for sickle cell disease prevention (98.2% diagnostic accuracy) supports prioritising PGT-M for prevalent monogenic disorders in resource allocation decisions. With sickle cell disease affecting 150,000 Nigerian births annually, targeted genetic testing could deliver substantial public health benefits while demonstrating cost-effectiveness in high-risk populations [22].

Laboratory protocol modifications based on our findings offer immediate implementation opportunities. Reduced oxygen culture (5% versus 20%) and careful media selection demonstrated associations with improved epigenetic profiles, without requiring substantial cost increases—modifications that are readily implementable across diverse facility types.

Study Limitations. Several limitations affect interpretation. Our sample concentrated in urban centres, serving higher socioeconomic populations, which may limit its generalizability to rural or economically disadvantaged couples. The cross-sectional design prevents the establishment of causal relationships between laboratory factors and embryonic characteristics, necessitating confirmatory interventional studies.

Technical limitations include the observational nature, which prevents experimental manipulation of culture conditions, and the incomplete long-term follow-up of children born following genetic selection. The research-dedicated embryo analysis (312 of 1,248 total) may not fully represent the broader clinical population undergoing standard ART procedures.

Sample size calculations powered for moderate effect sizes may have missed smaller but clinically meaningful differences, particularly in subgroup analyses across Nigeria's diverse ethnic populations. Additionally, comparison with international datasets relied on published summary statistics rather than individual-level data, potentially introducing systematic biases.

Future Research Directions. Priority investigations should include randomised controlled trials comparing simplified versus comprehensive genetic testing approaches to establish cost-effectiveness in resource-constrained settings. Longitudinal follow-up studies of children born following different genetic selection strategies remain essential for assessing long-term safety and developmental outcomes.

Population genetics research should be expanded across Nigeria's more than 300 ethnic groups to develop appropriate reference databases for variant interpretation. Mechanistic studies examining interactions between specific genetic variants and epigenetic modifications could identify novel biomarkers for embryo selection.

Implementation science research should evaluate different service delivery models, including task-sharing approaches and simplified protocols, to broaden access beyond current socioeconomic limitations. Economic evaluations comparing various testing strategies across different patient populations would inform evidence-based decisions on resource allocation.

Field Impact. This research establishes that genetic and epigenetic profiling is clinically beneficial for Nigerian ART populations, while highlighting the necessity of population-specific validation before implementing technologies developed in genetically homogeneous populations. The demonstrated facility-level variations indicate that quality improvement initiatives may yield greater immediate benefits than technology acquisition alone.

For reproductive medicine practitioners, these findings support the implementation of genetic testing for appropriately selected patients, while emphasising the importance of contextually appropriate protocols. For health policymakers, the results inform resource allocation decisions, favouring targeted implementation for high-risk populations rather than universal application.

The identification of extensive population-specific genetic and epigenetic characteristics challenges the field to move beyond one-size-fits-all approaches toward precision reproductive medicine, which considers genetic ancestry, environmental context, and resource constraints. Genetic

embryo selection improves Nigerian ART outcomes substantially, but realising this potential requires population-specific protocols rather than uncritical adoption of international standards developed from genetically homogeneous populations.

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