Importance of Chromosomal Aberration Screening in Assisted Pregnancy

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Abstract

The interface between assisted pregnancy and chromosomal aberration screening accommodates numerous sensitive and critical issues that have an impact on infertile couples. Infertility is a major public health issue, and approximately 1 out of 6 people worldwide are considered to suffer from infertility during their reproductive lifespans. The aim of this review is to emphasize the importance of chromosomal aberration screening in assisted pregnancy. With technological advances, genetic tests are becoming increasingly relevant in reproductive medicine. It is important to identify the cause of infertility and this requires genetic testing whether it male and/or female including Identifying carriers of inherited diseases and planning antenatal testing. Genetic conditions can additionally be transmitted to the offspring and in consequence create trans-generational infertility or specific serious fitness problems. Furthermore, genetic tests provide direction towards the most appropriate assisted reproductive techniques. There is therefore a need for Government to formulate a policy on the need to conduct genetic testing on the zygote of donated egg cells before implantation.

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Introduction

Infertility is a prevalent condition in many countries with its impact more prominent in the developing countries of Africa and Asia (Omokanye *et al.*, 2018). The burden of infertility as shown by its prevalence varies across the world; studies from Nigeria, Ghana recorded the prevalence of 30.3% and 11.8%, respectively, while a study involving 27 African countries found a range of between 10% and 20% (Fadare and Adeniyi, 2015). These values are higher when compared with results from studies carried out in more developed nations of the North; studies conducted in Scotland and the USA found the prevalence of 9.1% and 10%, respectively (Silber, 2011; Barseghyan *et al.*, 2015). To date, more than 5 million babies have been born worldwide through assisted reproduction technologies (Omokanye *et al.*, 2017). In Nigeria, Oladapo Ashiru pioneered the in vitro fertilization (IVF) program in 1984 and his team successfully delivered the first IVF baby in 1989 (Okwelogu *et al.*, 2012). This was subsequently followed by reported birth of IVF babies in private and public‐funded facilities within the country. This includes Orhue at University of Benin Teaching Hospital; 2007, Joseph Ikechebelu at Life Specialist Hospital, Nnewi, 2011 and Omokanye and his team at University of Ilorin Teaching hospital, Ilorin; Nigeria (Omokanye *et al.*, 2018).

Chromosomal Aberrations

A chromosomal disorder occurs when there is a change in the number or structure of the chromosomes. This change in the amount or arrangement of, the genetic information in the cells may result in problems in growth, development and/or functioning of the body systems (Swati *et al*., 2018).

According to Swati *et al*. (2018); Chromosome abnormality, namely aneuploidy (having abnormal number of chromosomes in a cell) and structural abnormalities (including deletions, duplication, translocation, inversion, insertions rings, and isochromosome) may cause genetic disorder such as Down's syndrome

Methods of Detecting Chromosomal Aberrations

Different types of methods have been developed for chromosomal aberration detection, which are given below:

Cytogenetic Testing

Karyotype analysis

Karyotype is the size, number and appearance of chromosomes of a eukaryotic cell. Such characteristics, along with morphological and molecular data, can be used to learn about patterns and mechanisms of evolution and speciation (Braeuchler, 2015). Karyotype analysis is a cytogenetic study evaluating the number and appearance of chromosomes using light microscopy for structural defects. Although karyotype analysis remains in routine use for the detection of structural chromosomal abnormalities in infertile men, a major shortcoming is the inability to detect DNA changes smaller than 4 Mb, and it is also labor intensive and time consuming (Pastuszak *et al.*, 2012).

Molecular Test

Fluorescence in situ hybridization (FISH)

FISH was initially introduced into the clinic in 1992 using X- and Y-chromosomal probes to treat families at risk of transmitting sex-linked disorders for the determination of the sex of the embryos as an alternative to the polymerase chain reaction (PCR) based approach (Griffin and Oğur, 2018). Later in 1993, the first applications of FISH for aneuploidy screening were carried out, assessing chromosome copy numbers of chromosomes X, Y, 13, 18 and 21 – the most common aneuploidies associated with live birth defects (Griffin and Oğur, 2018)

array-CGH (aCGH)

aCGH (array comparative genome hybridization) entails processes in common with chromosomal CGH such as whole Gene amplification and fluorescent labeling of samples and a chromosomally normal reference (Griffin and Oğur, 2018). However, rather than hybridization to metaphase chromosomes, the hybridization process is carried out on microarrays (bacterial artificial chromosomes or synthetic oligonucleotides) and the outcome analyzed with specialized software to give quality results (Griffin and Oğur, 2018)

Next-Generation Sequencing

NGS has currently been introduced into the medical institution for aneuploidy screening to substitute aCGH owing to its potential for scalability (Zheng *et al.*, 2015). Following whole genome amplification (in common with aCGH), a barcoding step follows to enable the identification of embryo-specific sequences after which the amplified product is broken down into small sequence-ready fragments. Those fragments are then subjected to massively parallel sequencing with low coverage for the purpose of aneuploidy screening (Knapp *et al.*, 2012).

Real-Time Quantitative Pcr (Rt-Qpcr)

RT-qPCR technique is a robust, rapid, accurate and economical CCS method. It has been developed and validated on TE biopsies (Dahdouh *et al.*, 2015). Briefly, first, TaqMan copy number assays are used to perform a pre-amplification step involving the multiplex amplification of 96 loci. The pre-amplified products are then quantified using RT-qPCR in a 384-well plate, and whole chromosome aneuploidies are determined. The complete procedure lasts about 4h and can additionally be combined with mutation detection (Griffin and Oğur, 2018).

Snp Arrays and Karyomapping

Single-nucleotide polymorphism microarrays (SNParrays) detect genetic variation throughout the genome and have been utilized for the detection of chromosome abnormalities in human IVF embryos (Dahdouh *et al.*, 2015). Primarily developed to study genome wide association (GWA), SNP arrays contain features of biallelic loci where every allele is of not dissimilar frequency (Dahdouh *et al.*, 2015). Taking the SNP array output of each parent and a genetic relation of known disease status (typically an affected child), four distinct sets of markers can be identified across each parental chromosome. Karyomapping determines inheritance from parental/grandparental haploblocks (inherited chromosomal segments). First, it entails identification of ‘ informative’ loci for parental haplotype, that is where one parent is homozygous and the other heterozygous, like a classical back-cross (Griffin and Oğur, 2018)

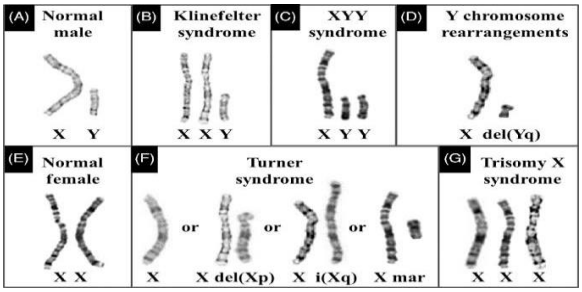

Figure 1: Chromosomal causes of infertility; Sex chromosome numerical and structural abnormalities associated with human infertility (Yatsenko *et al.*, 2014).

Normal male (A) and normal female (E) chromosomal complement is shown. The common sex chromosome aneuploidies detected by classical karyotype analyses are shown for (B) Klinefelter syndrome (47,XXY), (C) XYY men, (F) Turner syndrome (45,X), and (G)

trisomy X women (47,XXX). (F) Gross structural X and Y chromosome rearrangements include deletions of the short arm of chromosome X (del(Xp)), isochromosome composed of the two long arms of chromosome X (i(Xq)), small marker (mar) chromosome containing X-specific or Y-specific DNA. The X- and Y-chromosome structural rearrangements can be observed by chromosome analysis, such as deletions of the long arm (del(Yq)) (D). However, high resolution techniques (fluorescence in situ hybridizations (FISH) or ochromosomal microarray analyses) are essential for better characterization

Assisted Methods of Reproduction

Assisted reproductive technology (ART) includes all treatments and techniques that encompass the in vitro handling of human gametes or embryos with the intention of achieving a pregnancy (Bhandari *et al.*, 2018). 35 years of assisted reproductive technology (ART) use has become one of the standard procedures of infertility treatment. More than 5 million babies are born after ART (15% births). Therefore, it is necessary to monitor, evaluate and trace the modifications of procedures with growing technical development of medical practice in the field of reproductive medicine (kissin *et al.*, 2014).

In vitro fertilization (IVF)

In vitro fertilization (IVF) is a process in which ovum and sperm are allowed to fertilize in vitro. It is a complex procedure, which includes a number of different steps: ovarian stimulation, egg retrieval, embryo culture, and finally embryo transfer (Chronopoulou and Harper, 2015). Its success depends on multiple factors, namely, embryo status (genetic complement), endometrial receptivity, and an adequate embryo transfer technique (Dahdouh *et al.*, 2015). Unfortunately, a high proportion of embryos may be aneuploid, and the transfer of these is associated with decreased implantation rates (IRs), high miscarriage rates, and decreased live birth rates. To bypass the high embryo aneuploidy rate, reproductive endocrinologists have traditionally transferred multiple embryos with the aim of achieving at least one single live birth (Chronopoulou and Harper, 2015).

Purpose of Chromosomal Aberration Screening in Reproductive Medicine

The overall fertility rate is decreasing; for example, in the US, 12% of women receive fertility treatment over the course of their lifetimes, so it is important to emphasize the fertility journey of couples. The reproductive systems of both partners function in a combined and precisely coordinated way to conceive a child; for this reason, evaluation of both members of the couple is mandatory.

In particular, genetic tests are carried out for three main purposes in reproductive medicine: identification of the infertility causes, identification of genetic diseases transmissible to offspring and Optimization of the assisted reproductive technology (ART) (Cariati *et al.*, 2019).

Identification of Infertility Cause

A medical evaluation is indicated when the couple fails to achieve pregnancy after 12 -months of regular, unprotected sexual intercourse. Currently, the diagnostic timeline of infertile couples includes biochemical and instrumental analyses that allow for a diagnosis in 65% of cases; in the remaining 35% of cases, which are undiagnosed, genetic tests are performed. Considering that approximately 15% of genetic disorders are associated with infertility and that similar clinical signs can have genetic and nongenetic causes, it is important that an infertility diagnosis be determined by the combination of an accurate medical history and instrument- and laboratory-based evaluations, including targeted genetic tests (Ferlin *et al.*, 2006). Confirmation of the clinical diagnosis through genetic evaluation can lead to more specific and targeted medical management.

Table 1: The chromosome aberrations related to pretesticular male infertility

Genetic Condition	**Test**	**Chromosome/ Genetic alteration**	**Assisted reproduction technique**	**Inheritance**
Klinefelter syndrome	Karyotype	47,XXY (85– 90%) 46,XY/47,XXY mosaicism (6– 7%)46,XX/47,XXY	testicular sperm retrieval + ICSI	De novo mutation
De la Chapelle syndrome	FISH	SRY+ XX (80–90%)	heterologous Fertilization	Autosamal Dominant
Microdeletion Y chromosome AZFc	Molecular diagnosis by PCR	Interstitial deletion of AZFc Y region.	testicular sperm retrieval+ICSI	Y linked

Gravholt *et al.,* 2018; Omokanye *et al.,* 2018

Table 2: The genetic causes related to ovarian female infertility

GENETIC CONDITION	TEST	CHROMOSOME/ GENETIC ALTERATION	ASSISTED REPRODUCTION TECHNIQUE	INHERITANCE
Turner (45,X) (other names monosomy X,	Karyotype	Monosomy X: 45,X0	-donor	Not applicable
Trisomy X	Karyotype	47XXX or mosaic	Donor	Not applicable

Omokanye *et al.,* 2018; Rossetti *et al.,* 2017

Identifcation of Genetic Diseases Transmissible to Offspring

It is well known that in 20– 25% of cases, perinatal mortality is caused by inherited chromosomal or genetic alterations (Chronopoulou and Harper, 2015). Testing is available for more than 2000 genetic disorders, including common diseases, such as sickle-cell anemia, cystic fibrosis, and spinal muscular atrophy, or more complex conditions, such as mental retardation and congenital heart disease.

Currently, during the antenatal period, a variety of techniques is available to identify a transmissible disorder to the offspring in the presence of carrier or affected couples. Each of these techniques can be applied only during a specific time period of pregnancy or at different embryo stages in the IVF protocol (Omokanye *et al.,* 2018).

Invasive prenatal diagnosis (PND)

Invasive PND is usually performed on DNA extracted from fetal cells obtained by chorionic villus sampling (CVS) (between the 11th and 13th weeks of gestation) or from amniocytes (from the 15th to the 20th week), and the result is obtained in 7 or 15days, respectively (Linan *et al.,* 2018).

An increasing amount of interest has been shown regarding the noninvasive prenatal diagnosis (NIPD) of monogenic disease that is able to detect fetal genetic alterations in maternal blood at an early gestational age (approximately 10 weeks). However, although noninvasive prenatal testing (NIPT) of cell-free fetal DNA (cfDNA) for the screening of chromosomes 21, 18, 13, X and Y has been clinically adopted, NIPD remains a challenge. Very recently, NIPD for clinical use has been adopted in cases of sex-linked disorders and RHD (Chiu *et al.,* 2018). Several studies have tested the application of NIPD in monogenic diseases, such as β-thalassemia, congenital adrenal hyperplasia, and Duchenne and Becker muscular dystrophy (Linan *et al.,* 2018).

Preimplantation genetic testing (PGT)

PGT has the same diagnostic motivation as the traditional PND, with the advantage of advancing the timing of diagnosis at the embryo stage. Only disease-free embryos are transferred to the mother, avoiding recourse to therapeutic abortion. Even for couples who are able to conceive naturally, PGT requires the application of IVF techniques, including (a) the collection of gametes from both partners; (b) the

fertilization of the oocyte by intracytoplasmic sperm injection (ICSI); (c) the embryo biopsy, which allows one or more cells from the blastomere or trophectoderm to be taken 3 or 5 days, respectively, post fertilization; (d) molecular analysis and (e) the embryo transfer (Griffin and Oğur, 2018).

Preimplantation Genetic Diagnosis (PGD)

All variants of preimplantation genetic diagnosis (PGD) are designed to minimize the chances of transferring genetically abnormal embryos formed after in vitro fertilization (IVF). The process involves referral and counseling of the couples, standard IVF treatment, oocyte pick-up, embryo culture and biopsy, genetic diagnosis and finally selective transfer of unaffected embryos (Griffin and Oğur, 2018).

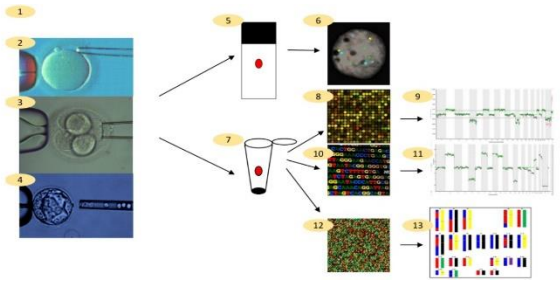

Figure B: Diagram showing the process of preimplantation genetic screening from biopsy to diagnosis (Griffin and Oğur, 2018).

1. Three types of biopsy to sample genetic material for PGS: (2). Polar body, (3). cleavage stage (blastomere), and (4). blastocyst (trophectoderm). (5.) Cells were initially fixed to glass slides for diagnosis. (6). FISH (fluorescence in-situ hybridization). (7). Whole genome amplification (8). Array CGH (comparative genomic hybridization) microarray. (9). Output of a-CGH analysis showing multiple chromosome gains and losses. (10). NGS (next generation sequencing). (11). Output of NGS analysis showing the same multiple chromosome gains and losses as a-CGH but with a greater dynamic range. (12). SNP chip microarray. (13). Karyomapping analysis showing the origin of chromosome abnormalities, chromosome exchange events and extra/missing chromosomes.

Molecular Approaches for the Optimization of Art Techniques

Human embryos that are developed in vitro show a great deal of acquired chromosomal abnormalities; for this reason, PGT for aneuploidy (PGT-A) has been developed to select euploid embryos that are suitable for transfer. PGT-A is primarily indicated for couples with advanced maternal age, recurrent implantation failure, recurrent abortions, or severe male infertility. Meiotic errors are one of the main causes of the low success rate (~30%) of in vitro fertilization techniques. Randomized studies and meta-analyses have shown that the PGT-A technique does not increase the live birth rate but decreases the miscarriage rate and increases the efficiency of IVF techniques (Omokanye *et al.*, 2018).

The evolution of PGT-A techniques started with a limited number of chromosomes analyzed by fluorescence in situ hybridization (FISH) in 1995 It was soon overcome by the analysis of the whole chromosome set by using different genetic platforms, such as metaphase Comparative Genomic Hybridization (mCGH), array-based Comparative Genomic Hybridization (aCGH), single nucleotide polymorphism (SNP) microarray, quantitative polymerase chain reaction (qPCR), and, most recently, NGS (Van der Aa *et al.*, 2018).

Currently, the most commonly used technique is NGS. This method involves the amplification of the genome from a single cell by WGA, the preparation of a DNA library, starting directly from the amplified DNA, and the subsequent sequencing of a pool of libraries in parallel, each identified by a specific " barcode" sequence (Van der Aa *et al.*, 2018).

Finally, an analysis software that compares the sequences obtained in each sample with respect to the human hap map reference genome" allows the identification of the possible presence of chromosome aneuploidies. Literature data confirm that NGS can be successfully applied to the diagnosis of a variety of genetic abnormalities, even in single cells isolated from human embryos following WGA, and has numerous advantages over the technologies traditionally used for PGT-A (Omokanye *et al.*, 2018). However, it was soon clear that the gold standard was to develop a method forthe analysis of both monogenic diseases and PGT-A at the same time. Indeed, as previously discussed, the very recent innovation for this purpose is the use of NGS to analyze single gene mutations and chromosomal copy number variations to select euploid disease-free embryos (Sermon, 2017). Currently, only a novel mutation continues to be a challenge (Munné, 2018).

Conclusion

Unexplained infertility may lead to problems in pregnancy and for the future child. Thorough investigation of the reasons behind infertility is hence desirable including identifying carriers of inherited diseases and planning antenatal testing. Genetic conditions can additionally be transmitted to the

offspring and in consequence create trans generational infertility or specific serious fitness problems. Furthermore, genetic tests provide direction toward the most appropriate assisted reproductive techniques. PGD has become widely practiced throughout the world for various indications, and it helps to restore reproductive confidence. PGD can substantially decrease the eventual risks of passing a genetic undesired condition to the offspring.

Recommendation

Health service providers be encouraged to conduct genetic testing on the zygote before implantation particularly those resulting from donated sperm cells and ova. Also, Government should help subsidize the Cost of assisted reproduction procedures in order to encourage genetic testing.

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