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AMPATHCHAT

Ampath Genetics
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Expanded chromosome screening of products of conception (POCs) using next generation sequencing (NGS) technology

Ampath Genetics is excited to announce the introduction of products of conception (POCs) testing using next generation sequencing (NGS) technology. NGS allows analysis of all chromosomes (1-22, X and Y), resulting in significantly increased pick-up rates of chromosomal abnormalities when compared to conventional testing approaches.

Background

The diploid human genome consists of 23 pairs of chromosomes: a pair of each of the autosomes (chromosomes 1–22) and a pair of sex chromosomes (XX or XY). The diploid genome therefore represents 46 chromosomes in total, a state referred to as euploid. One copy of each pair of chromosomes is inherited via the gametes. Gametes therefore contain half the number of chromosomes, and are referred to as being haploid. When a haploid sperm fertilises a haploid egg, a diploid genome containing 46 chromosomes (23 pairs) is generated in the conceptus (zygote).

The production of gametes relies on the process of meiosis to reduce the number of chromosomes present in each gamete to 23 (one pair of each chromosome). However, meiotic segregation of chromosome pairs is prone to errors.

Meiotic non-disjunction occurs when a chromosome pair is not separated correctly, and a gamete receives both copies of the chromosome, while another gamete does not receive a copy. When the gamete that is abnormally carrying two copies of a particular chromosome fertilises a gamete carrying one copy, the resulting conceptus contains three copies of that chromosome (called a trisomy).

Conversely, if the gamete that does not carry a copy of the chromosome fertilises a gamete with one copy of that chromosome, the resulting conceptus has only one copy of that chromosome (called a monosomy). Passing on missing or extra copies of a chromosome via the gametes is associated with adverse pregnancy outcomes – either pregnancy loss (spontaneous abortion) or a baby with congenital abnormalities.

Pregnancy loss associated with chromosomal abnormalities

It is estimated that 15 to 20% of clinically recognised pregnancies end in miscarriage,[1] and approximately 1% of couples experience recurrent (at least two) pregnancy loss. The single-most common cause of early (first-trimester) pregnancy loss is the presence of a major foetal chromosomal abnormality. Such abnormalities account for 65 to 75% of all pregnancy losses.[1]

Chromosomal abnormalities associated with pregnancy loss

Chromosomal abnormalities are responsible for more than 50% of pregnancy losses in the first trimester.[2] Numerical chromosome abnormalities include aneuploidies of whole chromosomes (trisomy or monosomy) and polyploidy (having one or more complete additional sets of chromosomes, which includes triploidy and tetraploidy).

The most commonly occurring chromosomal abnormalities in products of conception are trisomies of the autosomes (chromosomes 1–22), which account for almost two-thirds of all aneuploidies (Figure 1). This is followed by monosomy X (45, X) and triploidy (three full chromosome sets or 69 chromosomes) (Figure 1).

1. Shah et al. 2017. *Fertility and Sterility* 107(4):1028–1033
2. Hardy et al. 2016. *American Journal of Medical Genetics Part A* 170A:2671–2680

For further information, please contact Ampath Genetics on 012 678 1361, ngs@ampath.co.za, or your local Ampath pathologist

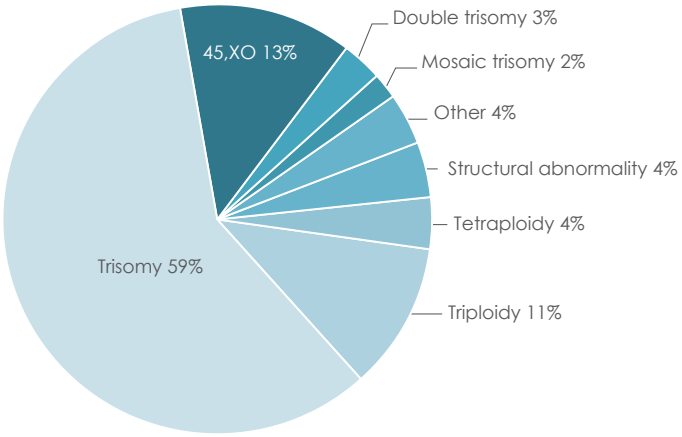

Figure 1: *Aneuploidy distribution in POCs. Adapted from Hardy et al. (2016).*[2]

Autosomal monosomies are very rarely observed in spontaneous abortions. It is suggested that autosomal monosomies present in zygotes generally fail to implant (monosomies of chromosomes 21 and 22 have been observed in POCs, albeit rarely). It has been speculated that reduced expression of certain genes due to monosomy (haploinsufficiency), impacts negatively on cell proliferative capacity (particularly in the cytotrophoblast of the developing embryo), resulting in a lethal effect.[3]

The most frequently occurring trisomies in POCs are trisomy 16 (~30%), trisomy 22 (~15%), trisomy 15 (~10%) and trisomy 21 (~9%) (Figure 2).

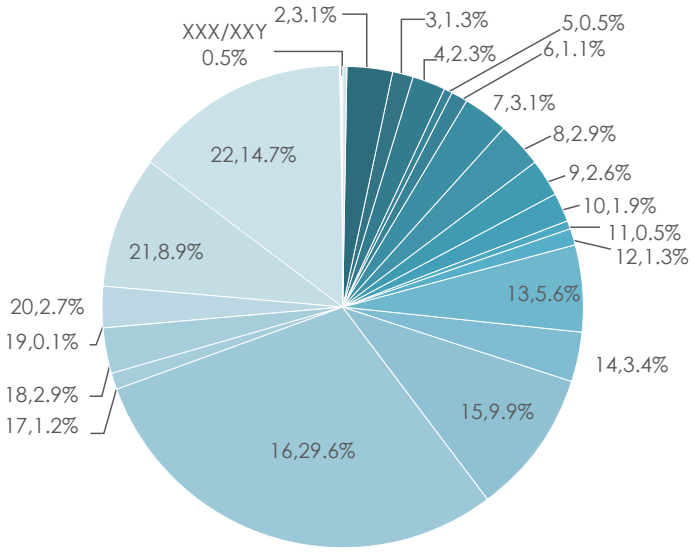

Figure 2: *Frequencies of trisomies in POCs by chromosome. Chromosome, frequency. Adapted from Hardy et al. (2016).*[2]

Collectively, abnormalities of chromosomes 13, 15, 16, 18, 21, 22, X and Y, account for approximately 72% of all chromosomal abnormalities (aneuploidies) observed in POCs.[2, 4]

Aneuploidy rates increase with advancing maternal age

Aneuploidies that arise as a result of errors during meiosis occur at random. Thus, it is difficult to predict the recurrence risk for a patient who has had a previous miscarriage as a consequence of a numerical chromosomal abnormality. However, it is commonly known that the frequency of aneuploidy increases with increasing maternal age. This increase is attributed to increasing rates of nondisjunction during meiosis with advancing maternal age.[5]

Testing for aneuploidies in POCs

There are several techniques that can be used for testing POCs for chromosomal abnormalities. Each technique differs with respect to the material required for testing, and the range of chromosomes screened.

Karyotyping makes use of cultured cells (cultured POC tissue) to stain chromosomes and microscopically analyse a number of nuclei for chromosomal abnormalities. While karyotyping has the advantage of assessing all the chromosomes (1-22, X and Y) simultaneously, it requires viable tissue to culture. Tissue obtained from spontaneous abortions fail to culture in approximately 40% of cases due to non-viable tissue. Further to this, the presence of maternal cell contamination may be propagated and expanded, due to cell culturing, masking any potential aneuploidies present in foetal tissue.[6]

Quantitative fluorescent PCR (QF-PCR) is a molecular technique that does not require viable tissue to screen chromosomes. DNA is extracted from POC tissue (whether viable or not) and selected chromosomes can be quantified using QF-PCR. However, due to the targeted nature of conventional QF-PCR, it does not screen all the chromosomes. Typically, chromosomes 13, 15, 16, 18, 21, 22, X and Y are screened using QF-PCR approaches. While abnormalities of these chromosomes account for approximately 72% of all aneuploidies observed in POCs, 28% are missed (as they are not screened).

Next generation sequencing (NGS) offers low-coverage genome screening to quantify chromosomes. Screening the whole genome allows for coverage of all chromosomes (1–22, X and Y). Additionally, as NGS is a molecular technique, it requires DNA extracted from POCs (and mitigates the need for cell culturing). Together, NGS offers screening of all chromosomes, and can be performed on non-viable tissue. Such testing therefore has a detection rate of >95% and a success rate of >99%.

Expanded chromosome screening of POCs using NGS at Ampath Genetics

With immediate effect, Ampath Genetics has changed its POC testing protocol from QF-PCR-based screening, to NGS.

The NGS technology used by Ampath Genetics to screen POCs allows for the detection of:

1. Trisomies
2. Monosomies
3. Polyploidies[7]
4. Partial deletions and/or duplications[8]

Samples can be collected in saline, and transported at ambient temperature. The turnaround time is seven working days. The cost of the NGS-based test remains the same as the previously offered QF-PCR-based test.

2. Hardy et al. 2016. *American Journal of Medical Genetics Part A* 170A:2671–2680
3. Lebedev et al. 2004. *European Journal of Human Genetics* 12:513–520
4. Segawa et al. 2017. *Reproductive Biomedicine Online* 34:203–210
5. Nagaoka et al. 2012. *Nature Reviews Genetics* 13(7):493–504
6. Van den Berg et al. 2012. *Biochimica et Biophysica Acta* 1822(12):1951–1959
7. The NGS technology Ampath Genetics employs can only detect polyploidies involving the Y chromosome
8. The NGS technology Ampath Genetics employs can only detect partial deletions or duplications of chromosomal material 10 Mb or greater

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