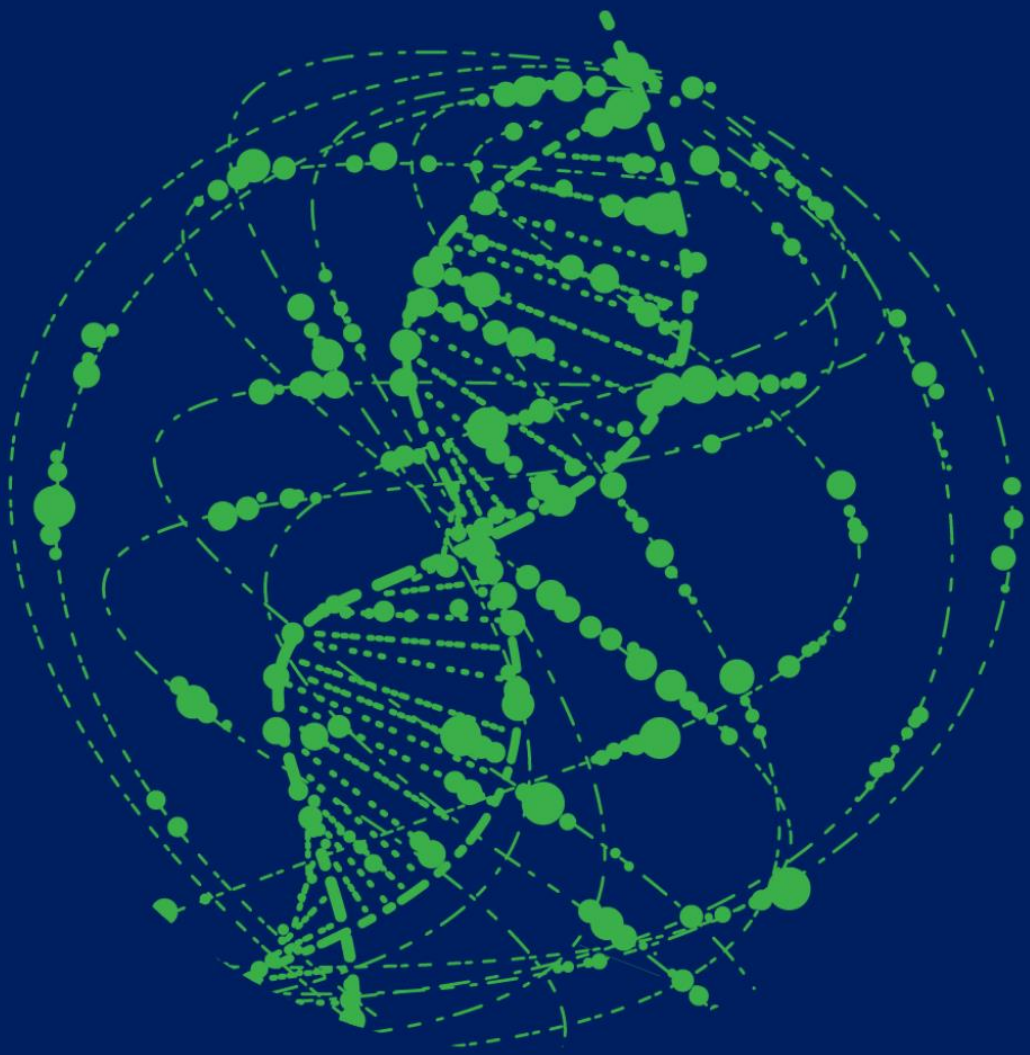
NEXT BIOSCIENCES

POCScreen

Products of Conception Statistics

January 2021-December 2021



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Introduction

Dear Colleague,

Recurrent miscarriage continues to be a challenging reproductive problem for the patient and clinician. It is a traumatic event and has psychological implications.

Although many chromosomal abnormalities that cause miscarriages are sporadic and have a low recurrence risk, some abnormalities (such as translocations) are expected to significantly increase the risk of recurrence - which may necessitate parental karyotyping. By identifying the approximate 50% of women whose pregnancy loss was due to chromosomal abnormalities, comprehensive chromosome screening will prevent a large proportion of patients undertaking unnecessary and costly evaluations. If a fetal chromosomal abnormality is excluded, there may be a possible treatable cause for a given miscarriage, and investigations can be focused on identifying this. Genetic testing outcomes can therefore be used to guide counselling for future pregnancies. Furthermore, the psychological benefit of identifying the aetiology of a fetal loss, cannot be understated.

The POCScreen test offered by Next Biosciences employs next generation sequencing (NGS) technology to rapidly and accurately screen products of conception (POC) for abnormal chromosome numbers (aneuploidies), and large deletions and/or duplications of chromosomal material. We have compiled the following statistics on tests done in our laboratory. We hope that you will find these informative with regards to the trends seen in POC screening in the private sector in South Africa.

Warm Regards,

Dr Yvonne Holt

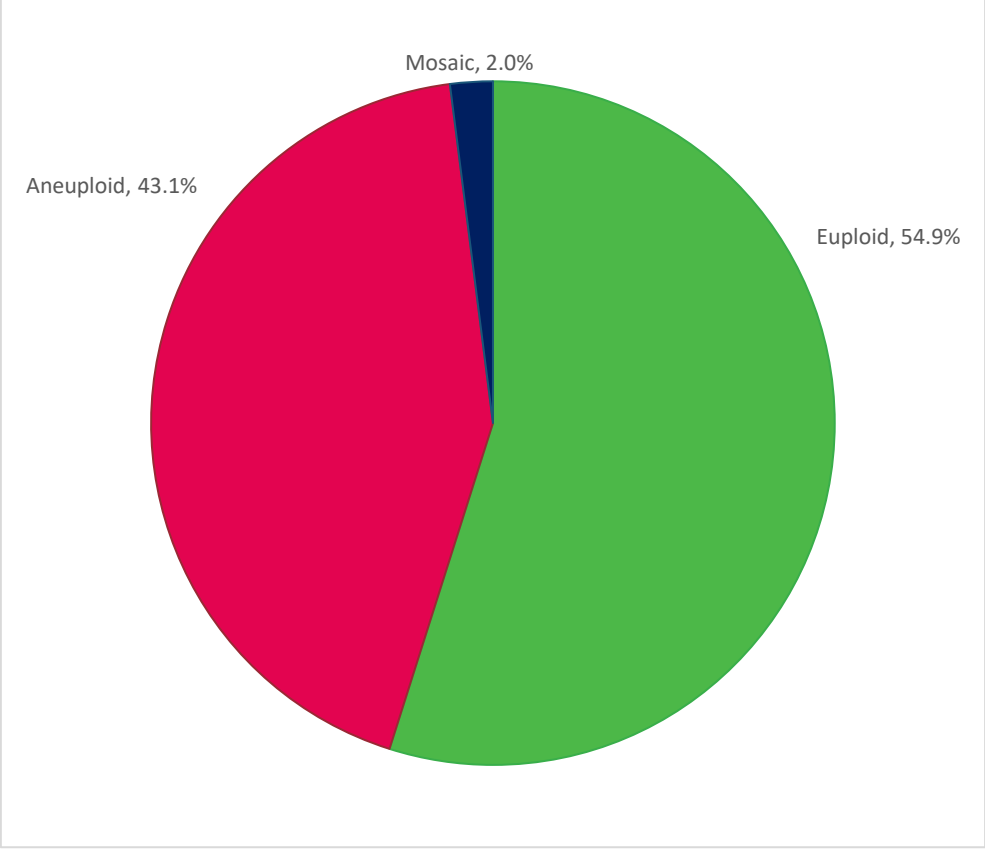
Chief Medical Officer

Overall Results

Aneuploidy is defined as an atypical number of chromosomes, meaning a cell nucleus has more or less than the expected 46 chromosomes or segments of a chromosome deleted or duplicated. We detected an aneuploidy in 43.1% of POC samples tested, whereas 54.9% of samples had a normal chromosome complement. An aneuploidy rate of 43.1% is consistent with current literature which reports that approximately 50% of pregnancy losses, especially in the first trimester, are caused by chromosomal abnormalities[1].

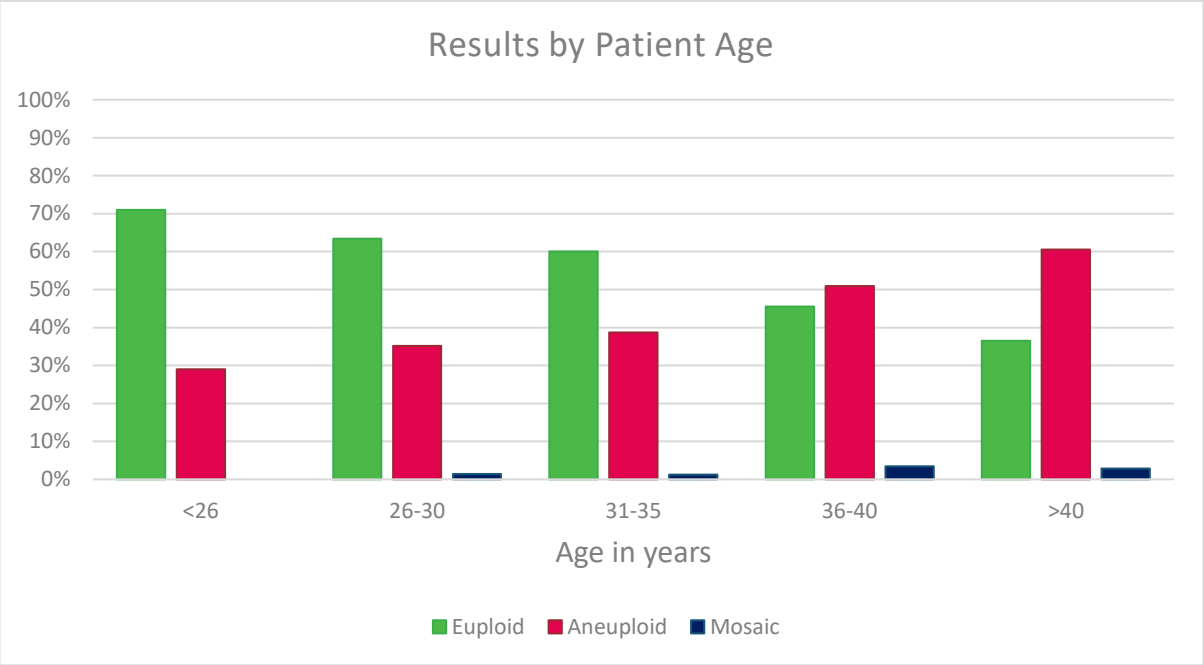
Mitotic errors post-fertilization, or meiotic errors pre-fertilization which is corrected in the early embryo through trisomic rescue, can give rise to two distinct cell populations within a single pregnancy, this phenomenon is called mosaicism. The association between mosaicism and pregnancy loss is not well documented, however, the rate of mosaicism seen in prenatal diagnosis ranges from 1 to 2%[2]. We found 2.0% of the POC samples tested revealed mosaicism. The clinical consequences are dependent on the chromosome(s) involved and the level of mosaicism.

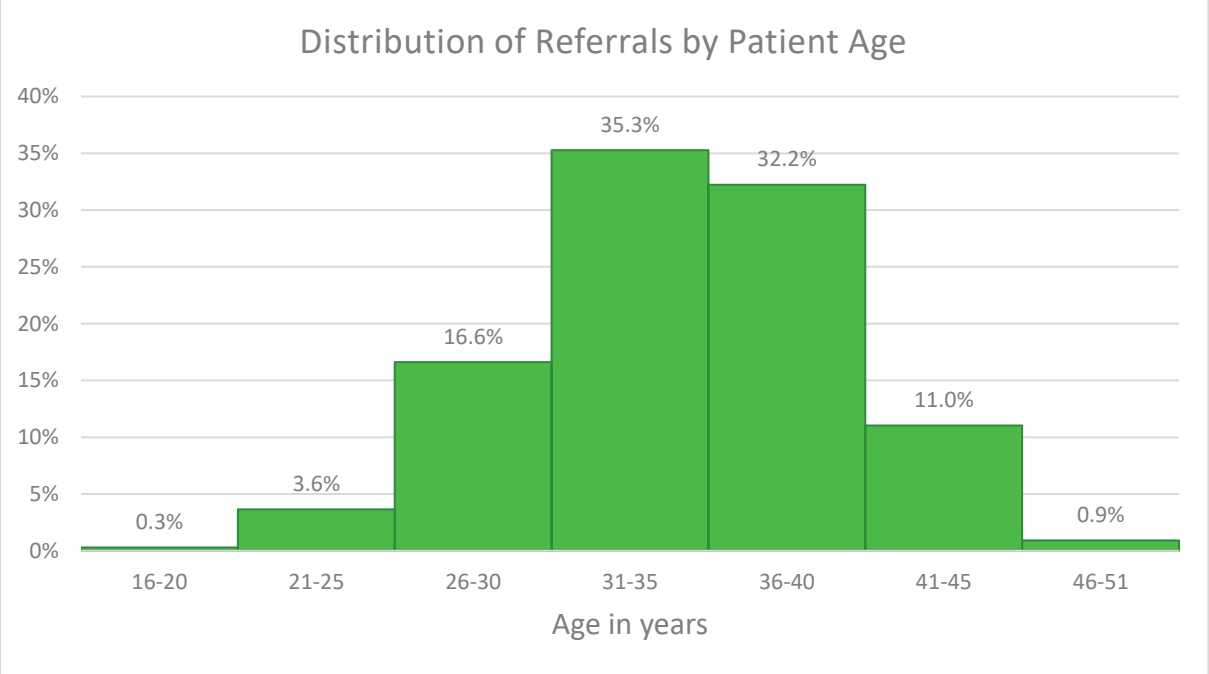
The failure rate of conventional karyotyping is recorded to be between 10% to 40%[3]. POCScreen testing uses next-generation sequencing (NGS) technology to obtain a molecular profile on DNA extracted from POC tissue, which mitigates the need for cell culture. This allowed us to report no test failures for 2021 and highlights the advantage of using NGS technology for POC testing.



Results Per Maternal Age Group

As expected, aneuploidy rates increase with maternal age. The overall aneuploidy rate observed in patients 35 years and younger was 36.7% compared to 53.3% in older patients. The incidence of chromosome segregation errors are higher in aging oocytes; consequently, the number of good quality oocytes in older mothers is fewer than in younger mothers, which increases the frequency of miscarriage[4]. The paternal age effect on aneuploidy is still unclear.

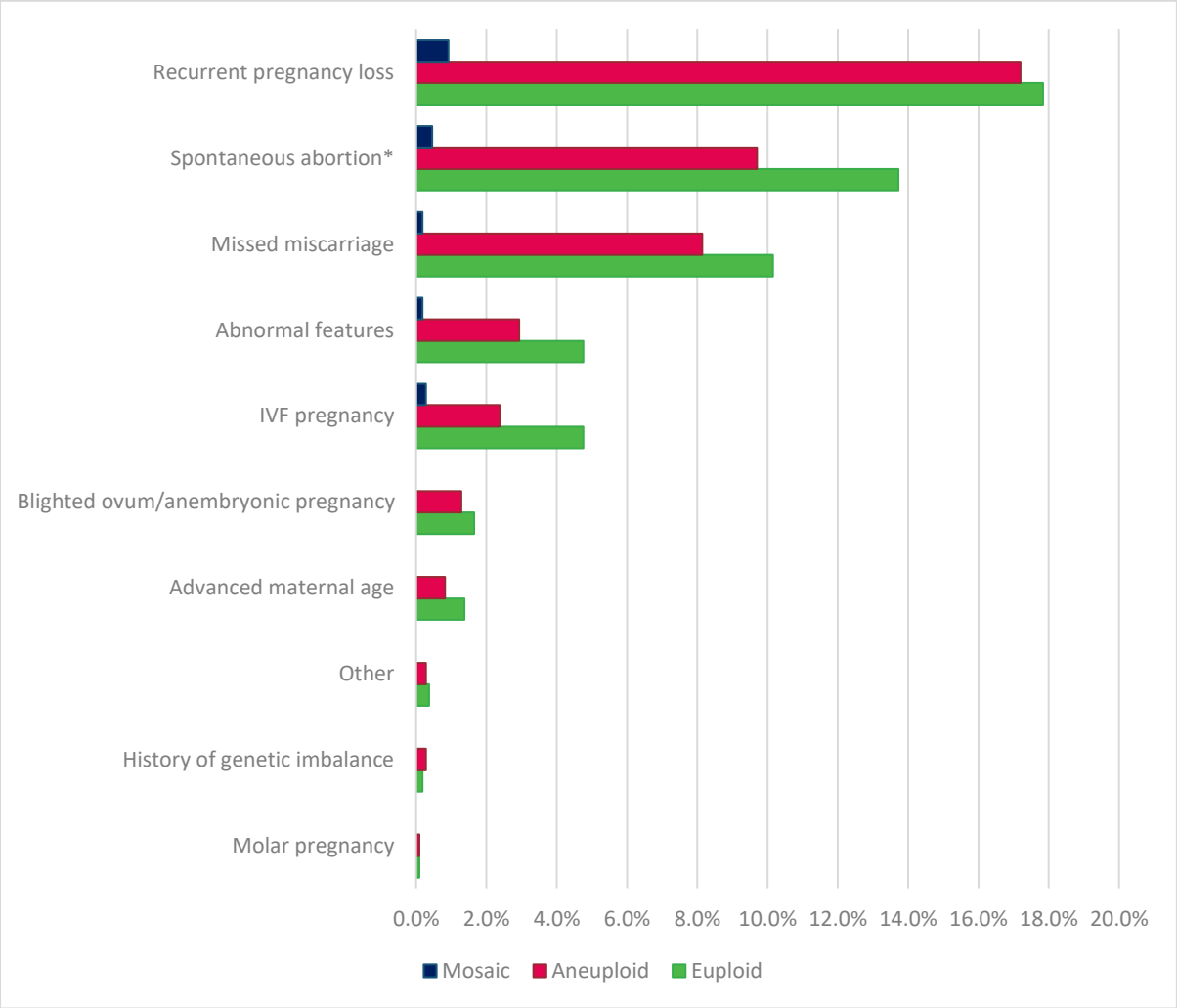




Reasons for Referral

POCScreen is requested after an unexplained pregnancy loss, with recurrent miscarriage as the most common referral reason. Recurrent miscarriage is usually defined as the occurrence of two or more consecutive losses of pregnancy. For the purpose of this document, we include two or more losses and did not take live births into account, as this information is generally not provided. The contribution of aneuploidy reported for recurrent miscarriage patients varies[5]. Our data showed that a numerical chromosomal anomaly is identified in 47.8% of POCs referred after unexplained recurrent pregnancy loss, illustrating the value it could add for these presumably chance events. This information may aid clinicians in focusing their investigations.

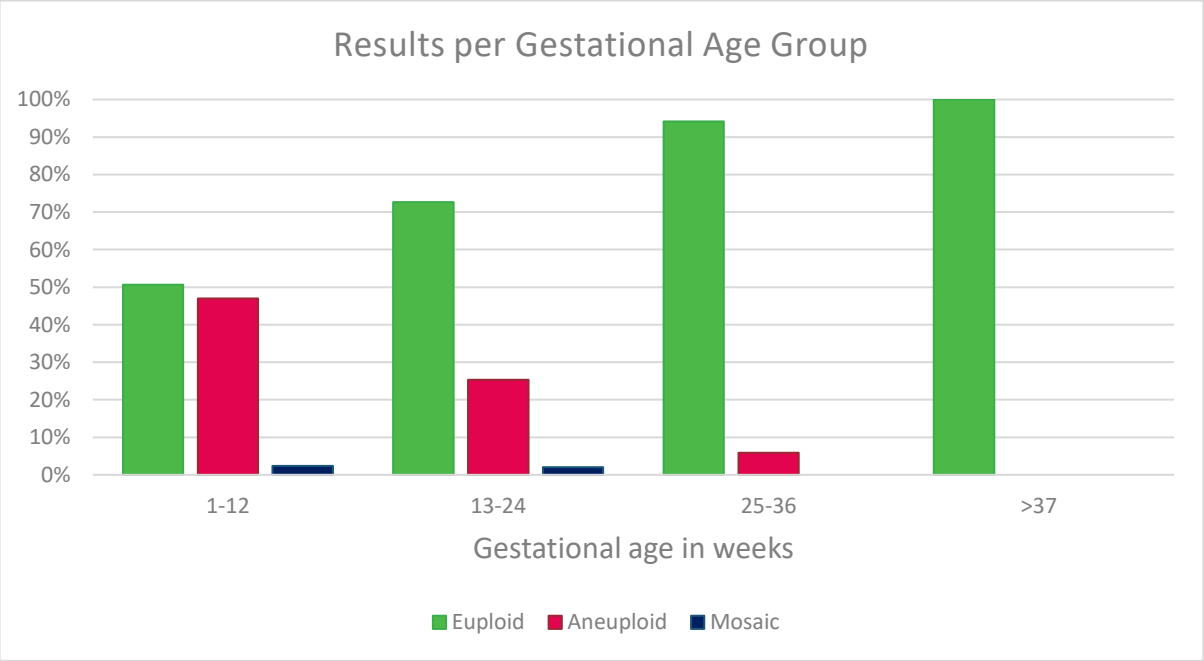
Complete hydatidiform moles (CHM) have no identifiable fetal tissue and abnormal growth of the placenta. CHM result from receiving two paternal genomes with no maternal contribution and will therefore present as euploid since there is no change in chromosome number. Partial moles are triploid, with two paternal and one maternal genome. As with non-molar triploidy, certain types cannot be detected with POCScreen.

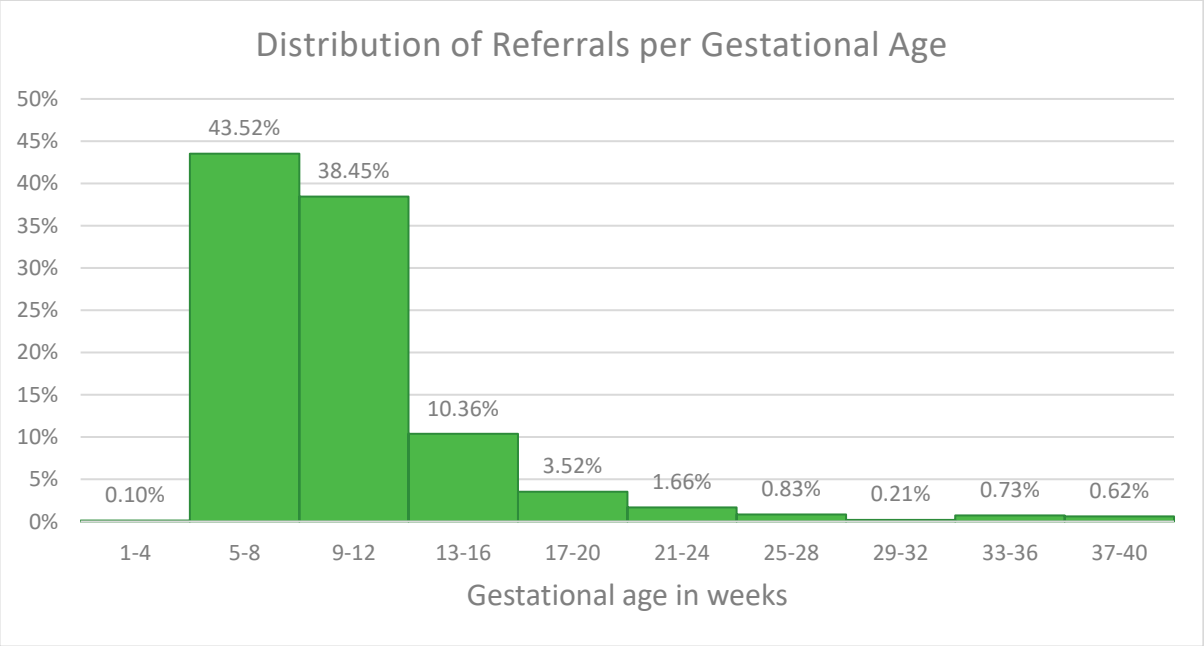


* Spontaneuous abortion/loss – no additional information provided.

Results Per Gestational Age Group

As discussed, it is accepted that about 50% of early pregnancy loss is caused by chromosomal abnormalities, this was also observed in the current dataset where 47.0% of POCScreen results with a gestational age less than 12 weeks showed an aneuploidy. As gestational age increases the frequency of aneuploidy decreases. Most trisomies and monosomies are not compatible with life, therefore, chromosomal abnormalities are less likely to be the cause of miscarriage further along in the pregnancy. Alternative or higher resolution testing is recommended in late trimester pregnancy losses if no aneuploidies are identified, especially if abnormal features are reported.

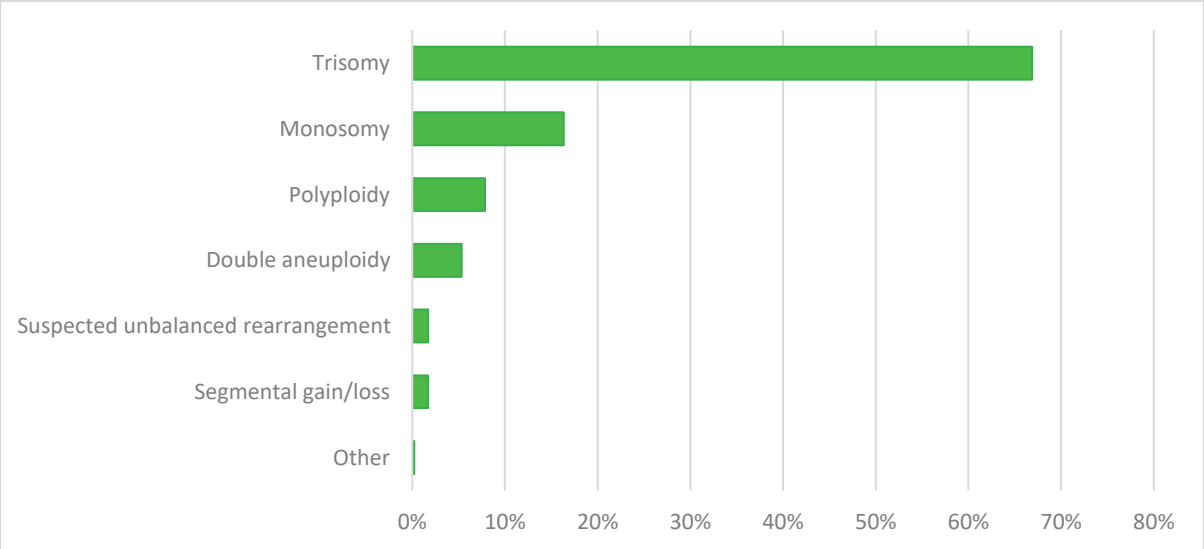




Types of Abnormalities

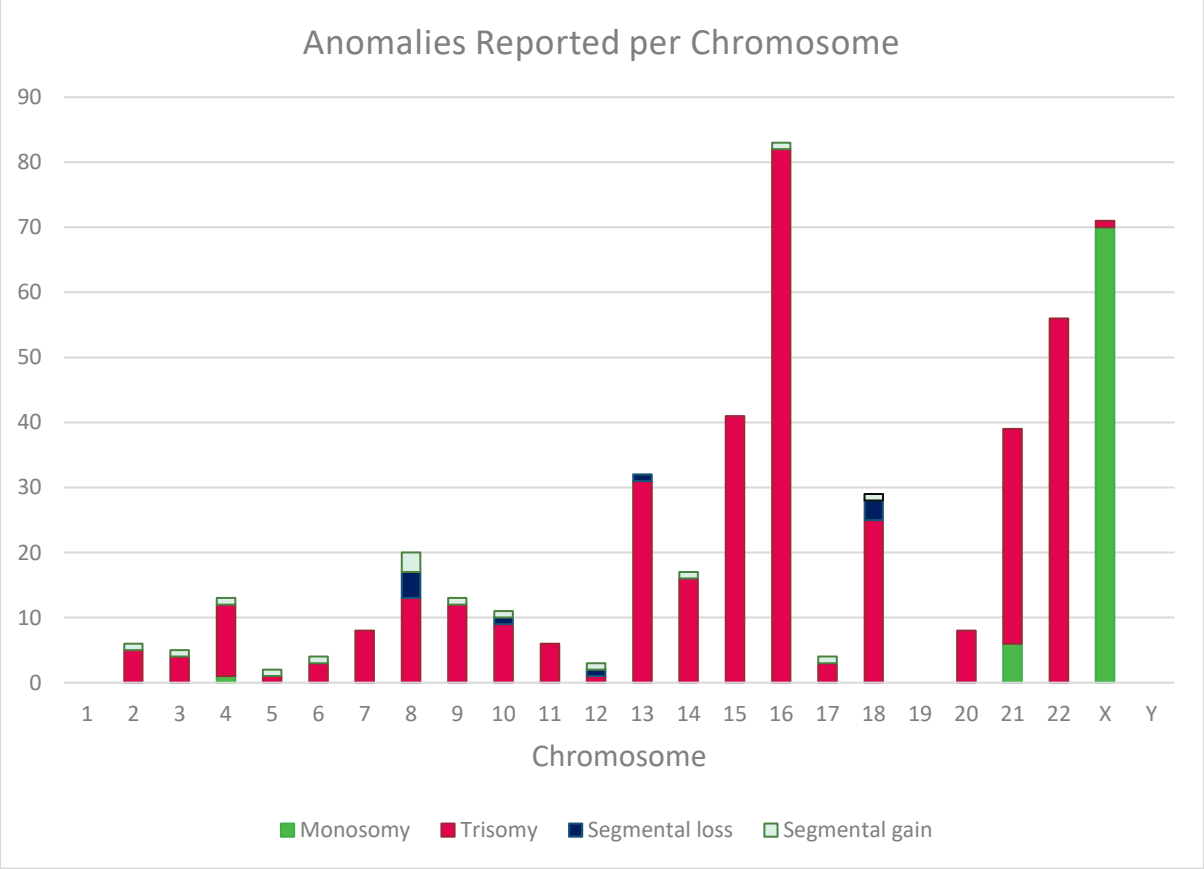
The spectrum of detected chromosomal abnormalities in our dataset was represented by numeric events, ploidy changes and segmental/partial chromosomal events. Numeric events include single trisomy, monosomy and double aneuploidy (which refers to two respective trisomies or monosomies, or both) which comprises 88.5% of anomalies detected. Ploidy changes refers to the gain of an additional full set of chromosomes and comprises 7.9% of anomalies detected. It is important to note that the technology does not allow for the detection of certain types of polyploids, e.g., 69, XXX. Partial chromosomal gain and loss events comprise 3.4% of anomalies detected, and include single segmental gains or losses, or multiple events within the same profile.

Segmental chromosomal gains or losses are reported when smaller, sub-chromosomal regions are duplicated or deleted. NGS allows for the identification of gains or losses larger than 10 mega base pairs (Mbp) with an associated probability of detection related to the sensitivity and specificity of the technology. Smaller gains/losses may be detected based on the quality of the sample. The detection limit can be explained in relation to the size of chromosome 21, the smallest chromosome, which is 47 Mbp. The molecular profile observed for 1.7% of POC samples showed a gain and loss of genetic material from one or more chromosomes, which can be indicative of an unbalanced structural chromosomal rearrangement. In these cases, parental karyotyping is recommended to determine if this is an inherited or *de novo* chromosomal abnormality. It is reported that in approximately 4% of couples with multiple miscarriages, one or both parents have an unknown inherited balanced translocation[6]. NGS does not allow for the distinction between a balanced translocation and a euploid profile.

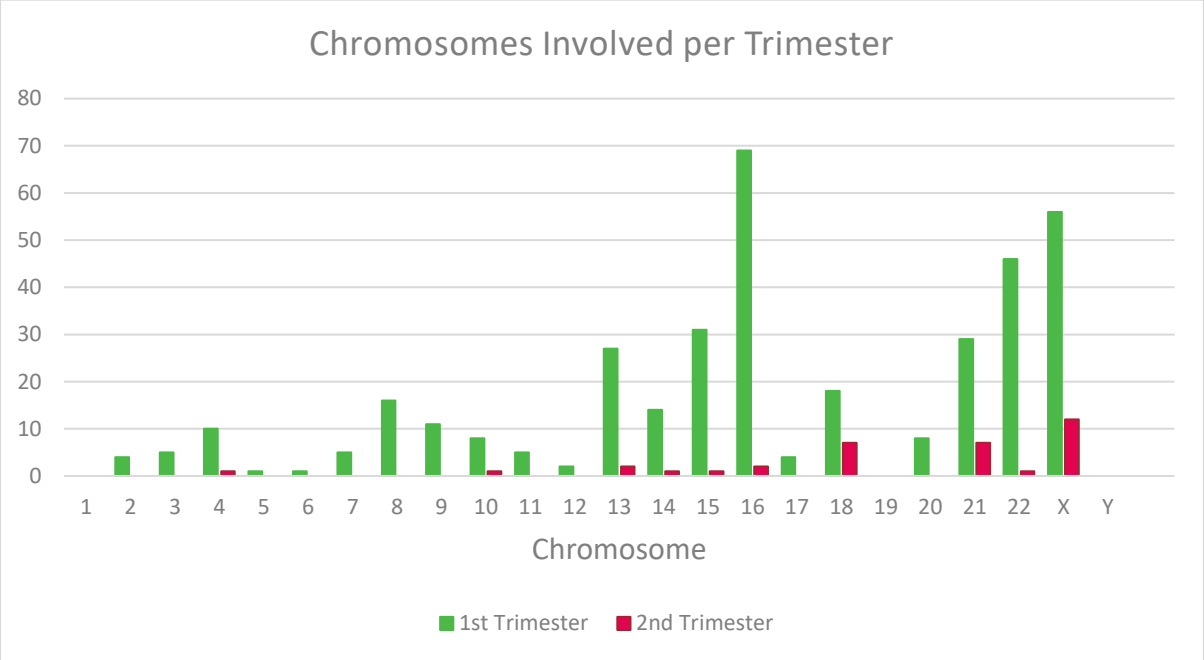


Abnormalities Per Chromosome

Chromosome 16 was the most frequently observed trisomy which is consistent with published studies[1]. Interestingly, it has been recently shown that levels of chromosome 16 nondisjunction in paternal meiosis might have similar relative influence on fetal aneuploidy when compared to nondisjunction rates in maternal meiosis[7]. Other common aneuploidies in POCs included trisomies of chromosomes 13, 15, 18, 21 and 22. Most of these are described as the most frequent both in preimplantation embryos at blastocyst stage and POCs[8,9]. Monosomy X was the only type of whole chromosome loss observed, apart from a few cases of monosomy 21, and is consistent with the clinical diagnosis of Turner syndrome in live born individuals. Monosomy X is one of the most common cytogenetic abnormality in spontaneous abortions, only 1% of conceptuses survive to term[10]. There is no increased risk of recurrence of this abnormality. However, if recurrent pregnancy loss is observed maternal karyotyping would be recommended to rule out mosaic monosomy X, which is associated with a higher risk of adverse obstetric outcomes.

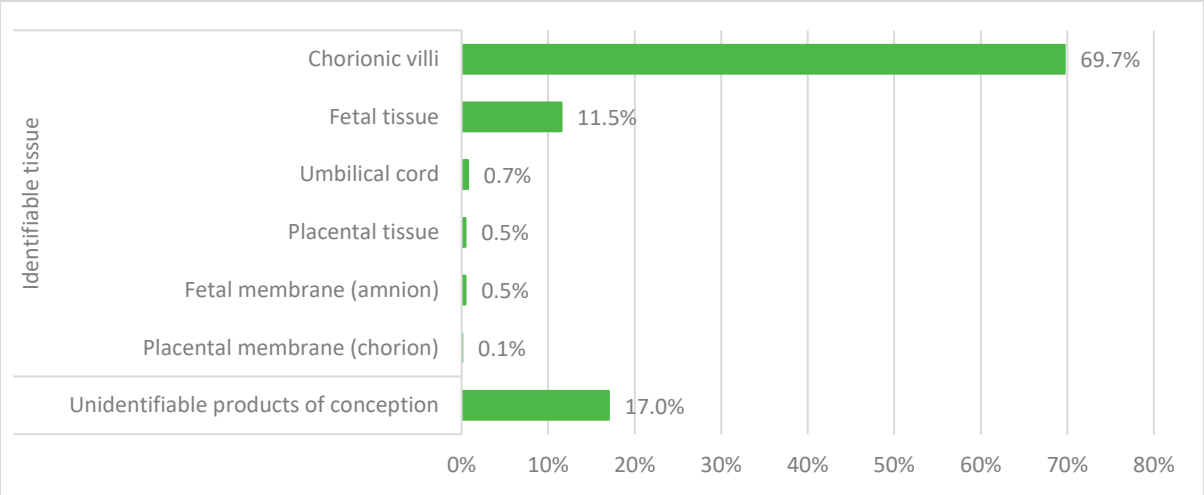


The graph below shows involvement of nearly all the chromosomes in first trimester miscarriages. As shown in the previous section, these can be numerical and structural in nature. In the second trimester, aneuploidies involving chromosomes 13, 18, 21 and X were most prevalent, as opposed to anomalies involving other chromosomes. As some cases of trisomy 13, 18 ,21 and monosomy X may result in live birth, these pregnancies may progress further before a miscarriage occurs.

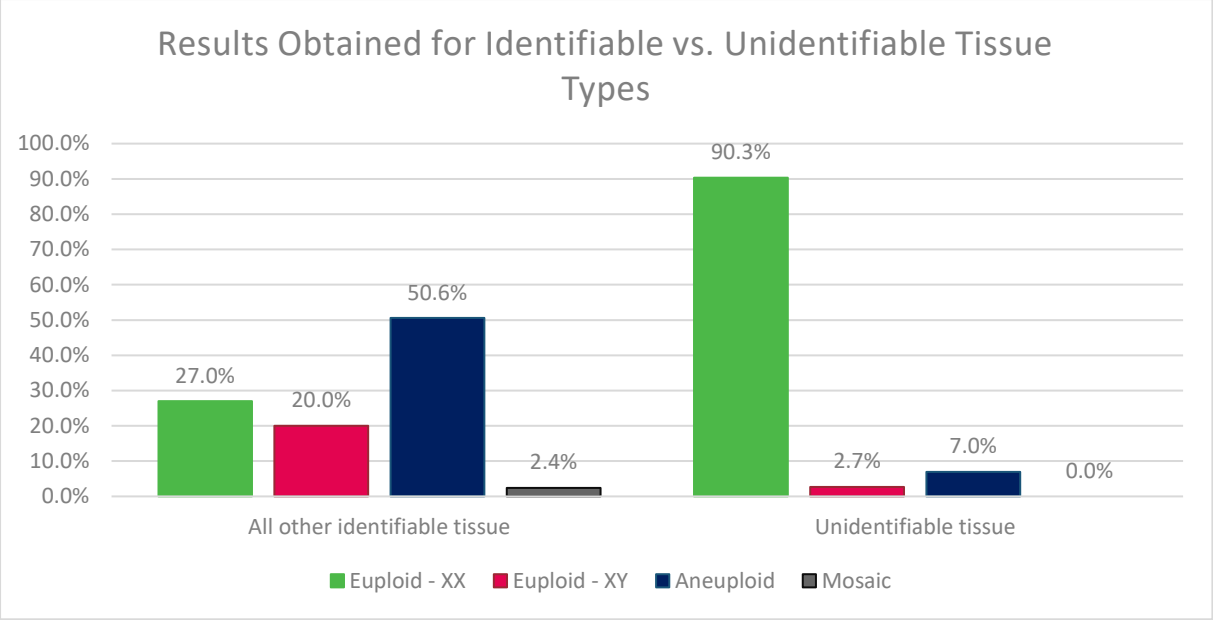


Sample Types Received and Tested

Samples received for POCScreen testing are examined and dissected to exclude maternal cell contamination (MCC) as far as possible. Ideally, products clearly identified to be of fetal or placental origin (e.g., fetal skin biopsy, chorionic villi, chorionic or amniotic membrane, umbilical cord, etc.) are isolated for testing. Chorionic villi is by far the most frequent tissue type submitted and isolated for testing, seconded only by products of unknown origin (unidentifiable tissue). The largest number of aneuploidies were detected in chorionic villi, of which 54.1% were aneuploid. The second largest number of aneuploidies were detected in fetal tissue, with 31.0% aneuploid.



Testing unidentifiable tissue significantly increases the risk of MCC. MCC is probably the most frequent laboratory factor leading to a decrease in the rate of detecting chromosomal abnormalities in POCs due to an over-reporting of a normal female profile. The graph below shows that euploid female results were obtained for 90.3% of unidentifiable POC samples which means that MCC cannot be excluded with absolute certainty. Submitting fetal or placental tissue therefore greatly improves the diagnostic yield for POC samples and the value of sampling these tissue types after a miscarriage cannot be overstated. Despite this limitation, a chromosomal anomaly was detected in 7% of POC samples with unidentifiable tissue, which is valuable information for those patients.


Results Obtained for Identifiable vs. Unidentifiable Tissue Types
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
27.0%
20.0%
50.6%
2.4%
90.3%
2.7%
7.0%
0.0%
All other identifiable tissue
Unidentifiable tissue
Euploid - XX
Euploid - XY
Aneuploid
Mosaic

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Nothing is inevitable. Anything is possible

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