Infertility

Chromosome Testing Recommendations

PROVINCIAL GENETICS PROGRAM

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Ontario Health

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Introduction

Ontario Health is building a comprehensive and integrated system for clinical genetic services across the province. Through collaboration with health system partners, the Provincial Genetics Program (PGP) provides evidence-based guidance for genetic diagnostic testing and counselling services in key areas. The PGP and the Provincial Genetics Advisory Committee (PGAC) identified reproductive genetics as a priority domain for development in Ontario, resulting in the formation of the Reproductive Genetics Expert Group.

A priority-setting exercise was completed with the members of the Expert Group, and a topic-specific working group was formed to provide feedback on this document. The process for developing this guidance document included a scoping and literature review conducted by the Clinical Lead and the PGP. Upon completion, the draft was reviewed by the Expert Group as well as internal and external stakeholders.

Guidance Document Scope

This document is intended for clinicians who see individuals with fertility issues and laboratories who offer chromosomal genetic testing for fertility. It is acknowledged that there exists a broader selection of non-chromosome tests for the genetic work-up of infertility and hypogonadism, including Y microdeletion, *CFTR*, fragile X, infertility/hypogonadism panels but they are beyond the scope of this document.

Please note that data about prevalence, estimated proportions of affected populations, and other data presented represents the best and/or most useful data available in the literature, which can be limited depending on the condition(s) under investigation.

Equity Considerations

The Provincial Genetics Program recognizes that language is always evolving, and we aim to use inclusive language in our guidance documents. This includes considerations for person-centred and gender-inclusive language. The populations discussed in this document are people with ovaries, people with testes, and/or people who donate their eggs or sperm for the purposes of conception, who are recommended to undergo testing for fertility analysis. The use of the terms “female” or “male” are only used when referencing data or research that explicitly uses this terminology to maintain accuracy in how their guidance was developed. There are limitations in the research cited in how non-binary and trans people were engaged. In this guidance document, we aim to use inclusive language where possible unless referencing research data where the terminology may be different.

Background

In Canada, 8% of couples and/or individuals who contribute genetic materials, where the individual with ovaries is 15 to 45 years of age, experience infertility[1]. Infertility is a condition defined by the failure to achieve a pregnancy after 12 months with regular unprotected sexual intercourse[1]. Genetic variations can cause infertility in people assigned male or female at birth, and at times, karyotype analysis may be indicated to detect potential chromosomal abnormalities. Karyotype analysis can reveal important information on the structure (i.e., translocations, inversions) and number of chromosomes, including sex chromosomes. If sex chromosome aneuploidy is found, this could have health implications for the individual, such as cardiac risks with Turner syndrome or gynecomastia and increased risk for breast cancer with Klinefelter syndrome[2]. Furthermore, balanced translocation carriers are at increased risk for offspring with unbalanced chromosomal complements for the chromosomes involved in the translocation, and depending on the chromosome involved, could have a risk for uniparental disomy-related genetic conditions[3]. This in turn may influence the diagnostic testing offered during the pregnancy.

Chromosomal microarray testing detects extra or missing genetic material at a higher resolution than karyotype analysis but does not detect balanced chromosomal rearrangements. Currently in Ontario, microarray is publicly funded for several indications including developmental delay and multiple congenital anomalies. In contrast, testing criteria and access to karyotype and microarray analysis for infertility are inconsistent. Individuals or couples recommended to have karyotype analysis by health care professionals outside of the hospital setting routinely pay out-of-pocket, and the cost is about $600 CAD per test[4]. People planning a pregnancy and couples requiring a karyotype analysis to aid in fertility planning have significant difficulty accessing this test due to the lack of standardized testing criteria, variability in hospitals accepting specimens for testing from outside the hospital setting, and funding for testing. Identifying parental chromosomal abnormalities can support prospective parents with valuable information to make informed reproductive decisions and provide resolution to their infertility diagnostic odyssey.

Recommendations for Chromosome Testing for Infertility

To promote accessibility and consistency across clinics regardless of clinic or patient location, the indications below have been recommended for genetic testing related to infertility.

Indications for Karyotype

In people with testes: Sperm abnormalities including azoospermia or severe oligospermia; especially non-obstructive azoospermia or where the sperm concentration is less than 5 million/mL consistent with severe oligospermia.

In people with ovaries: Presence of primary amenorrhea or primary ovarian insufficiency with or without features of an abnormal sex chromosome.

In couples and/or individuals who contribute genetic material, any of:

- 3 or more unsuccessful in-vitro fertilization (IVF) treatment cycles and a diagnosis of unexplained infertility
- 3 or more recurrent clinical miscarriages or pregnancy losses
- 1-2 miscarriages with history of infertility

Clinical miscarriages are defined as any intrauterine pregnancy (gestational sac) detected on ultrasound and pathological confirmation of chorionic villi (even in the absence of fetal pole). Clinical miscarriages were considered rather than biochemical miscarriages as the diagnostic yield in the literature is based on clinical miscarriages was high enough to warrant testing and there is a chance of product of conception collection if it is a clinical miscarriage[5].

Indications for Rapid Aneuploidy Testing / Chromosome Microarray

In either partner: For individuals with primary amenorrhea or severe oligospermia (sperm concentration less than 5 million/mL) where there are features suggestive of sex chromosome anomalies such as Turner syndrome or Klinefelter syndrome, as recommended by the Canadian College of Medical Geneticists (CCMG), can consider microarray testing as first line testing[6]. If a more complex and/or multisystem genetic syndrome is suspected, such as developmental delay or congenital anomalies, a formal genetic assessment may be warranted to establish an appropriate testing strategy.

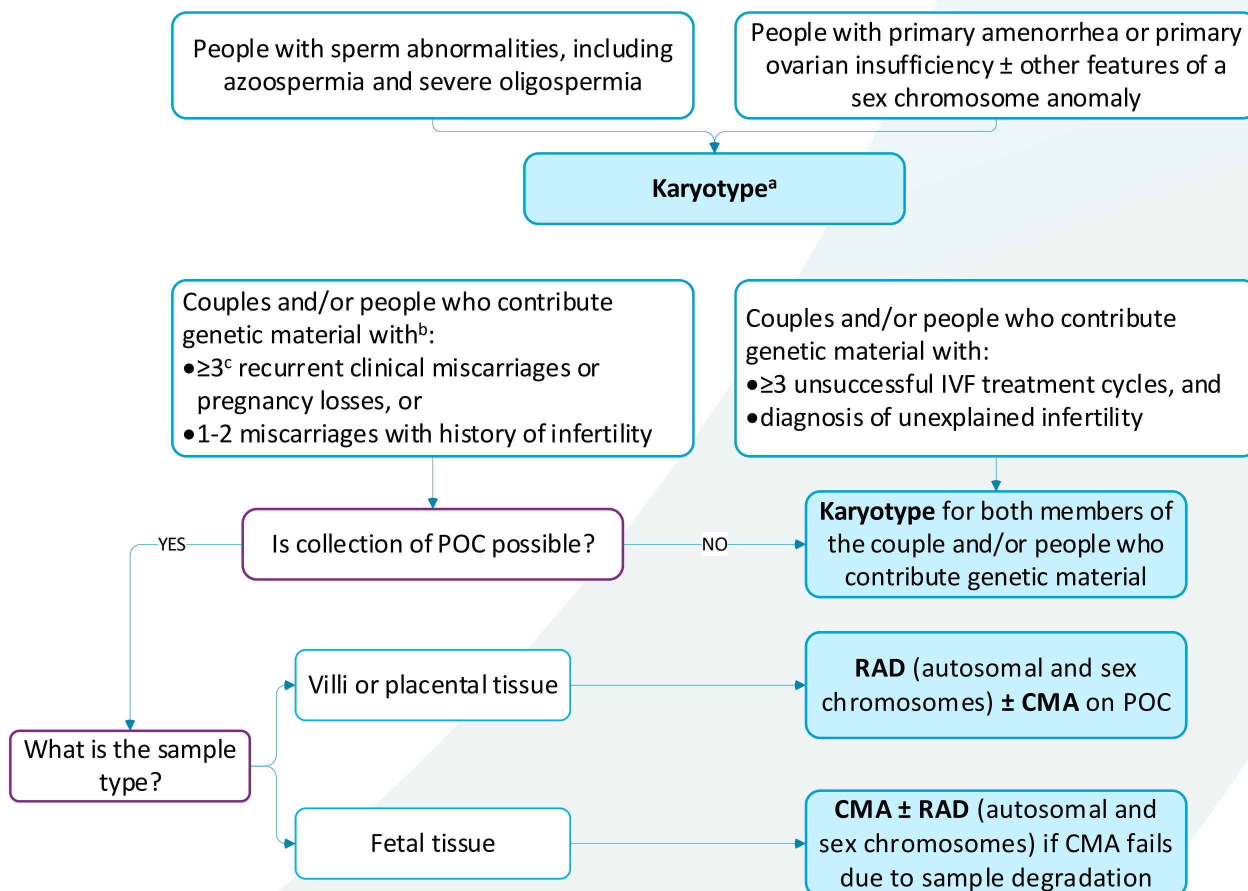
In couples and/or individuals who contribute genetic material: If there are 3 or more recurrent clinical miscarriages or pregnancy losses, or 1-2 miscarriages with history of infertility, testing of the product of conception (POC) through microarray can be considered as microarray have a higher diagnostic yield than routine parental karyotype[7]. On occasion, especially when there are concerns about the source of the sample (chorionic villus rather than fetal tissue requiring maternal cell

contamination testing) or the sample integrity (if the sample is degenerated and may yield an uninterpretable microarray), rapid aneuploidy testing may be considered to optimize the yield of a diagnosis.

As per international guidelines, consideration of testing can be made at 2 pregnancy losses if there are resources available in a different jurisdiction. For the purposes of developing an Ontario provincial guidance in the context of limited resources and turn-around times, the provincial recommendation is for testing to be initiated at 3 or more recurrent pregnancy losses. In the future, if resource limitations change and/or are removed, the recommendations will be revisited to determine feasibility to align with international guidelines.

In couples with infertility, consideration can be made for testing of the POC even after the first miscarriage as it may be informative[8].

Figure 1. Indications for Karyotype and Chromosome Microarray



CMA, chromosome microarray; **IVF**, in-vitro fertilization; **POC**, product(s) of conception; **RAD**, rapid aneuploidy detection

[a] If a sex chromosome aneuploidy is suspected, microarray can be considered as first-line testing.

[b] If products of conception (POCs) are available and/or appropriate sample type in these indications, rapid aneuploidy and/or microarray can be considered as first-line testing.

[c] *Clinical miscarriages* are defined as any intrauterine pregnancy (gestational sac) detected on ultrasound and pathological confirmation of chorionic villi (even in the absence of fetal pole).

Implementation Considerations

Technology

Karyotype analysis is time-intensive and requires specialized training to interpret the results. There are pressures related to the limited number of specialized individuals able to interpret these results. Therefore, considerations should be made when appropriate for less time-consuming tests including microarray and quantitative fluorescent polymerase chain reaction (qfPCR) for samples that may require maternal cell contamination or have decreased sample integrity. Microarray is a molecular test that can be batched, and the interpretation is aided by bioinformatic pipelines. qfPCR is a less expensive and more rapid method that can be used to detect common aneuploidies. Currently, karyotyping is considered the standard of care for cytogenetic studies for infertility diagnostics.

Feasibility

The increase in volumes projected for karyotype analysis may be challenging due to shortages in laboratory human health resources and competing clinical indications such as cancer testing.

Accessibility

To increase accessibility, ordering of karyotype and/or microarray analysis should not be restricted to genetics clinics or hospital-based clinics. There should be the ability and support for both primary care physicians and community-based specialists to organize the testing. Specimen collection should be available in local community laboratories. Transport of specimens to a cytogenetics laboratory is time sensitive as the sample should ideally be received in the laboratory within 72 hours. Physician and patient education will need to be provided for the process involved in products of conception (POC) collection. The possible implications of these factors for individuals in northern and/or remote communities should specifically be addressed in the implementation process.

Horizon Scanning

New technologies such as long-read sequencing and optical genome mapping may become important ancillary or front-line testing strategies in the future. Long-read sequencing has several advantages over short-read whole genome sequencing that may aid in the diagnosis of causes for infertility. Long-read sequencing generates reads that are two orders of magnitude greater than short-reads and have been able to map out areas of low complexity that are known as next-generation sequencing dead zones[9,10]. Enhanced mapping of structural chromosome rearrangements, which have an estimated prevalence of 0.96-1.10% in patients who have a history of recurrent miscarriages or undergo IVF, will support diagnosing infertility patients and this technology may be applied more frequently than karyotyping in the future[9]. Optical genome mapping can detect cryptic or sub-microscopic genetic causes of infertility. However, the current inability of optical genome mapping to detect Robertsonian translocations or balanced whole-arm translocations represents a known drawback of this technology[11] especially as it relates to telomeric and centromeric regions[12]. Further studies investigating the diagnostic yield of optical genome mapping in infertility will help guide future testing strategies. We recognize these technologies are forthcoming and the technology used in this algorithm may change at the discretion of the laboratory.

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Appendix

Appendix A: Glossary

Please note that some definitions may have been extracted verbatim from the references included.

Azoospermia: The complete absence of spermatozoa in two separate centrifuged semen specimens.

Chromosome microarray (CMA): A genetic test that compares the amount of chromosomal material from the individual to a standard reference to make sure that there are no missing or extra pieces of the chromosomes.

In-vitro fertilization (IVF): An assisted reproductive technology where the eggs are removed from the ovary and combined with sperm outside the body to form embryos. The embryo is then transferred into the uterus to promote implantation with the goal of achieving pregnancy[13].

Karyotype: A photographic representation of the chromosomes of a single cell, cut and arranged in pairs based on their size and banding pattern according to a standard classification[14].

Long-read sequencing: A DNA sequencing technique that enables the sequencing of much longer DNA fragments than traditional short-read sequencing methods.

Oligospermia: Low sperm count in the ejaculate.

Optical genome mapping: An imaging technology which evaluates the fluorescent labeling pattern of individual DNA molecules to perform an unbiased assessment of genome-wide structural variants at a resolution that far exceeds conventional cytogenetic approaches[15].

Primary amenorrhea: Amenorrhea is defined as the absence of menstruation in females of reproductive age. Primary amenorrhea is defined as the failure to reach menarche[16].

Primary ovarian insufficiency: Defined by loss of ovarian activity before the age of 40 years. POI is characterized by amenorrhea or irregular menstrual cycles with elevated gonadotropins and low estradiol[17]. Also known as premature ovarian insufficiency and premature ovarian failure.

Products of conception (POC): Any tissue derived from the union of the egg and sperm and may contain membranes, chorionic villi and fetal tissue. It may be collected after a miscarriage, planned termination or a delivery[18].

Rapid aneuploidy detection (RAD): Rapid testing for common aneuploidies in prenatal and postnatal samples and in the investigation of recurrent miscarriage through qfPCR.

Quantitative fluorescence-polymerase chain reaction (qfPCR): A form of PCR used to determine the relative amount of DNA or RNA in a sample; commonly used to detect heterozygous deletions and duplications[14]. By means of fluorescent primers, the amplified segments can be visualized and quantified as peak areas on automated DNA scanners.

Appendix B: Acknowledgements

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