Faculty of Science and Technology

Is Non-Invasive Preimplantation Genetic Testing ready for Clinical Application?

A dissertation submitted as part of the requirement for the BSc Biological Sciences.

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Contents:

Table of Abbreviations.	
DNA	Deoxyribonucleic acid
ART	Assisted Reproductive Technology
IUI	Intra Uterine Insemination
IVF	In Vitro Fertilisation
ICSI	Intra Cytoplasmic Sperm Injection
PGT	Preimplantation Genetic Testing
PGT-M	Preimplantation Genetic Testing for Monogenic conditions
PGT-SR	Preimplantation Genetic Testing for Structural Rearrangements
PGT-A	Preimplantation Genetic Testing for Aneuploidy
TE	Trophectoderm
BF	Blastocoel Fluid
SCM	Spent Culture Medium
niPGT	Non-invasive Preimplantation Genetic Testing
PCR	Polymerase Chain Reaction
MALBAC	Multiple Annealing Loop Based Amplification Cycles
FISH	Fluorescence in situ Hybridisation
SNPs	Single Nucleotide Polymorphisms
CNV	Copy Number Variants
NGS	Next Generation Sequencing
cfDNA	Cell free DNA

Abstract:

Introduction: Many pregnancies end in miscarriage; the most common cause of miscarriage is chromosomal abnormalities. These abnormalities can be structural or numerical. Abnormalities occurring frequently can cause numerous pregnancies to end before they are clinically recognisable and the inability to become pregnant becomes known as infertility. Infertility affects 1 in 6 couples worldwide and the cause is often unknown, the only option for many couples is to seek in vitro fertilisation (IVF). When IVF still doesn't result in a pregnancy it is necessary to assess the chromosomes of the embryos before they are transferred back into the uterus. Preimplantation Genetic Testing (PGT) involves a biopsy of the embryo to evaluate its chromosomes to see if that is what is causing the IVF failure. This is a highly invasive technique that may also affect the implantation ability of the embryo so suitable non-invasive techniques are being sought. Cell free DNA found in the culture medium the embryo develops in could be the non-invasive answer but is it ready to replace PGT?

Research Question: Is non-invasive preimplantation genetic testing ready for clinical application?

Methods: Systematic review of published literature. Search topic "non-invasive preimplantation genetic testing", exclusion criteria applied until 18 appropriate papers were identified.

Results: Two studies were eliminated for not comparing invasive with non-invasive results. Ploidy concordance ranged from 36.4 to 97.3% and sex concordance ranged from 33.3 to 100%. No improvement was shown in the graph of averages over the years covered. Culture medium from vitrified and thawed day 5 embryos appeared to be optimal for highest concordance percentage.

Conclusion: Non-invasive preimplantation genetic testing is not ready for clinical application. Until many gaps in the research are filled, niPGT will not be suitable to replace PGT.

1. Introduction:

1.1 Chromosomal Abnormality and Achieving Pregnancy.

Up to one fifth of recognised pregnancies worldwide end in miscarriage, with 60% of these identified as having chromosomal abnormalities (Quenby et al. 2021). Chromosomal abnormalities can take many forms. Structural or segmental abnormalities occur when there is an aberration within a chromosome and include duplications, translocations (balanced or unbalanced), deletions, insertions, and inversions of parts of chromosomes. Numerical abnormalities occur when there are incorrect numbers of chromosomes and include monosomies, trisomies, triploidies, and tetraploidies, and are known under the umbrella term of aneuploidies (Ziotti 2020). Some abnormalities will cause fertilisation failure, many will cause zygote or early embryo arrest too early to be clinically recognisable pregnancies (McCoy et al. 2015.)

Other chromosomal abnormalities can progress to embryo stage and beyond. Magli et al. (2000) studied 143 day 3 embryos and found 51% to be aneuploid, of those 143, only 40 became blastocysts and only 16 of those were aneuploid suggesting a lot of chromosome abnormalities cease development at early stages. In blastocysts with chromosomal abnormalities, spontaneous abortion occurred most frequently in autosomal trisomies, then monosomy X (Turner's Syndrome), followed by triploidies and tetraploidies, further suggesting chromosome abnormality is highly detrimental to embryo development (Rubio et al. 2007). Errors can arise either in gamete meiosis or in zygote mitosis and can give rise to different types of abnormalities.

The majority of errors occur in meiosis and most often in oocytes rather than sperm, most likely due to the continuous nature of spermatogenesis compared to the stop-start meiosis of oocytes (Hunt and Hassold 2002). To create a euploid zygote once fertilisation has occurred, oocytes must meticulously go through the two divisions of meiosis over a protracted time period, stopping and starting (Mehlmann 2005). Meiosis I starts in the foetal ovary around week 7 of gestation, recombination of chromosomes occurs around weeks 10-11 and once S-phase is complete, meiosis I is arrested until puberty (Jones 2008). Recombination alone can be a treacherous stage with mispositioned chromosomes at cross over being

responsible for many trisomies, deletions, and duplications (Hassold and Hunt 2001).

Mis-segregation of sister chromatids is another meiotic error, one that increases with maternal age, sister chromatids prematurely segregate and this occurs more frequently when sufficient crossover hasn't been achieved (MacLennan et al. 2015). Mis-segregation should be caught at one of several check points in the meiotic process. Spindle Assembly Checkpoint (SAC) is one such point, its function is to hold progress of meiosis until all chromosomes are properly attached to the meiotic spindles and can segregate as expected without causing any chromosomal damage (Fragouli and Garrido 2020).

Most autosomal trisomies occur due to nondisjunction errors of meiosis in the maternal side, the most common of these occur in chromosomes 13, 15, 16, 18, 21 and 22 and have very different effects. Trisomies 13 and 18 both have >95% foetal loss rates but rarely, some affected foetuses survive pregnancy and birth. These infants usually have deformities so severe they are unlikely to survive to six months old. Trisomy 21, or Down's Syndrome, has a much higher foetal survival rate, and infants can survive into adulthood but will have some visible differences, learning difficulties and comorbidities (Witters et al. 2011). Trisomies 15 and 16 are common but always result in first trimester miscarriage, sometimes before clinically recognisable. Trisomy 15 causes structural defects of the skull, limbs, and umbilical cord whereas trisomy 16 causes over proliferation of trophectoderm cells at blastocyst stage and results in empty yolk sacs visible on early ultrasound (Shahbazi et al. 2020). Abnormalities that occur during meiosis causing an aneuploid oocyte will be present in every cell of any subsequent embryo and is often deleterious, in direct contrast to abnormalities that occur later in mitotic divisions of the zygote (Capalbo et al. 2021).

Abnormalities that occur in mitosis, after fertilisation, can include many of the same abnormalities that occur from meiosis, non-disjunction errors, mis-segregation of chromatids etc and often due to cell cycle checkpoint relaxation ahead of the embryo's own genome taking over control. The earlier the abnormality occurs, the more devastating its effects can be, however, it is possible that not all cells of the embryo will be affected (Fragouli et al. 2018). Errors in mitosis, which are unrelated to parental age, frequently occur within the first three cell divisions after fertilisation, later in development it is thought that in-

cell corrective mechanisms such as DNA repair, cell arrest and apoptosis reduce the rate of errors (Mantikou et al. 2012).

When a mitotic error occurs, it is common that any aneuploidy arising from the error does not affect all cells within the embryo, this is known as mosaicism. Embryonic mosaicism does not always end in spontaneous abortion, mosaic embryos can develop fully and go on to live birth and healthy human (Ariad et al. 2021). Although mosaic embryos can go on to live births, rates of mosaic preimplantation embryos greatly outnumber post implantation rates suggesting that mosaic aneuploidies are subject to selective elimination the same as uniform aneuploidies (Capalbo et al. 2021), or that mosaic embryos are able to self-correct and eliminate only the aneuploid cells (Orvieto et al. 2020).

There are different classifications of mosaicism, mosaic aneuploid is the most common and occurs in the very early embryonic cleavages, it is defined by more than one numerical chromosome abnormality cell lineage. It is understood that cell cycle checkpoint gene expression or lack thereof is responsible (Daphnis et al. 2005). Diploid-aneuploid mosaicism is characterised by 2 cell lineages within the embryo, one diploid and one aneuploid with usually only one chromosome involved, it often arises later at blastulation, and is frequently caused by non-disjunction and/or chromosome duplication (Bielanska et al. 2005). The final classification is chaotic mosaicism, this, like mosaic aneuploid arises during very early cleavages and causes most damage. Many cell lines contain numerous abnormalities across many chromosomes. Mechanisms leading to chaotic mosaicism is not understood and its lethality means embryos arrest before blastocyst stage when more research could be undertaken. Chaotic and aneuploid mosaicism are incompatible with implantation whereas diploid aneuploid can lead to live birth (Bielanska et al. 2002).

1.2 Infertility.

Infertility is defined as a condition of the male and/or female reproductive system that causes failure to achieve a pregnancy after twelve months of regular unprotected sexual intercourse. The World Health Organisation (WHO 2023) as well as providing this definition have stated that One in Six people worldwide will experience infertility in their lifetime. Aetiology of infertility is varied and widely unknown. In females, known causes include endometriosis, where overgrowth of endometrial tissues are found outside of the uterus, adhesions can cause

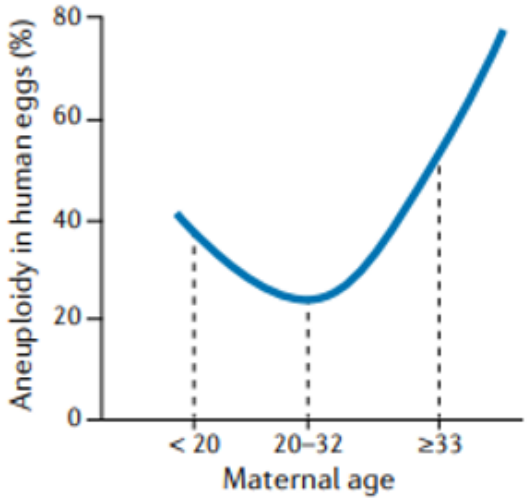

Figure 1. Increase in aneuploid oocyte % correlates with advancing maternal age. Graph adapted from Charalambous et al. 2023.

blockages of fallopian tubes and ovarian torsion which affect fertility (Vercellini et al. 2014). Hormonal or endocrine disorders can cause anovulatory cycles where no follicle is matured to be released, cycles can otherwise appear normal in length and menses initiation (McLaren 2012), polycystic ovarian syndrome (PCOS) is another very common endocrine condition that causes cysts to form on the ovaries and excesses of male androgen hormones to be produced disturbing menstrual cycles and ovulation as well as causing many physical effects to the sufferer (Siddiqui et al. 2022). Age is the biggest factor affecting fertility in women (See Fig. 1), the aneuploidies mentioned in part 1.1 increase exponentially with the advancing age of the woman due to decreasing oocyte quality and length of meiosis arrest where DNA damage and oxidative stress can develop (ESHRE 2005).

Many societal factors may also be influencing fertility. Women are choosing to establish themselves in a career before thinking about parenthood, often unaware of the risks of advanced age on their fertility (Schmidt 2012). Environmental factors also play their part. Atmospheric pollutants, smoking, alcohol consumption and drugs whether prescribed or recreational all have an effect on fertility (Brugo-Olmedo et al. 2001), whether directly in the case of pollutants, or indirectly in the case of recreational drugs and alcohol, by reducing inhibitions and causing risk taking behaviour that may result in sexually transmitted infections (Tsevat et al. 2017).

Male fertility problems contribute equally to infertility figures and similar environmental factors can apply to males as discussed for females. However, as spermatogenesis is a constant process, health and environmental factors can sometimes be reversed, and fertility restored (Agarwal et al 2021). Age is also a factor in male fertility, although new sperm are produced constantly, studies have

shown older men take longer to impregnate their fertile partners. Sperm analysis alone cannot explain this phenomena as, with exception of a slight reduction in motility, analyses are healthy (Hassan and Killick 2003).

Numerous tests and analyses will be conducted on infertile couples to assess the cause but in around 30% of cases no cause is found, and unexplained infertility is the diagnosis given (Deshpande and Gupta 2019).

In light of increasing infertility rates worldwide, assisted reproductive technology has had to keep up with demand. Assisted reproductive technology or ART is fertility treatment where natural conception is either aided or takes place out of the woman's body, in a laboratory (Graham et al. 2023). The first step in fertility treatment is often to try intrauterine insemination, IUI, this involves stimulating the ovaries with a drug such as Clomiphene citrate until a satisfactory follicle is produced then an ovulation trigger injection is given. Within 36 hours of the trigger shot the semen sample is introduced into the uterus via the cervix with a soft catheter. The patient will be asked to remain in a supine position for a short period of time after the procedure and supplementary progesterone treatment will be given for the duration of the luteal phase (Huniadi et al. 2023). As with natural conception, chances of achieving pregnancy with IUI decreases with maternal age (Wang et al. 2021).

If IUI does not work or is not likely to work for the type of infertility being treated, the next step is In Vitro Fertilisation, IVF. IVF is considerably more involved a process. A synthetic gonadotropin releasing hormone is given via injection to suppress pituitary function from cycle day 21 for 10 to 14 days to prevent spontaneous ovulation. After the 14 days, the first injections are replaced with follicle stimulating hormone injections for another 10 to 14 days. Once follicles reach an optimum size, approx. 18mm, assessed by transvaginal ultrasound, a trigger shot of human chorionic gonadotropin is given to induce the luteinising hormone surge that causes ovulation. Approximately 36 hours after the trigger shot, the patient is consciously sedated and using a transvaginal ultrasound guided needle aspiration, each of the follicles is drained. The drained follicular fluid is carefully examined by an embryologist and any oocytes collected and placed in a culture medium similar to the environment in the fallopian tubes and kept at body temperature. Around 100,000 sperm are added to the oocytes and fertilisation is assessed around 20 hours later. After 3- 5 days of development,

the highest quality embryos are chosen to be transferred back into the uterus via transcervical catheter or cryopreserved (Goldberg et al. 2007).

IVF treatment is still affected by advanced maternal age. Egg quality and ovarian reserve declines cannot always be overcome by fertility treatments (Ubaldi et al. 2019). The most popular technique and the one that can overcome most gamete problems is Intracytoplasmic sperm injection or ICSI. ICSI is IVF but instead of oocytes and sperm mixed in a dish, a single sperm and single oocyte are chosen according to ideal morphological attributes. The oocyte is held with a holding pipette and the sperm drawn up into the injecting pipette, the injecting pipette is then carefully pierced through the zona pellucida, effectively the shell of the oocyte and the sperm deposited inside (Haddad et al. 2020). Despite all these reproductive technological marvels, chromosome abnormalities can still undermine all that hard work. As previously mentioned, chromosomal abnormalities such as aneuploidies can cause embryonic arrest shortly after fertilisation, failure to implant or early spontaneous abortion before or after becoming clinically recognisable, these problems still apply if the pregnancy attempt involved ART (Zhang et al. 2010).

1.3 Preimplantation Genetic Testing.

All is not lost for the couples who continue to fail to achieve pregnancy using ART due to unknown factors. In some instances, embryos can be tested for abnormalities before they are transferred, this is known as Preimplantation genetic testing, PGT (Braude et al. 2002). PGT is performed to improve successful pregnancy and live birth rates by only transferring euploid embryos. It takes place on a day 5 embryo that has been fertilised by ICSI and incubated to the blastocyst stage (Greco et al. 2020). By day 5 after fertilisation, the dividing cells have differentiated into an inner cell mass which will become the foetus and the trophectoderm which will become the placenta (see Fig. 2). Morphologically good appearing embryos will be returned to the

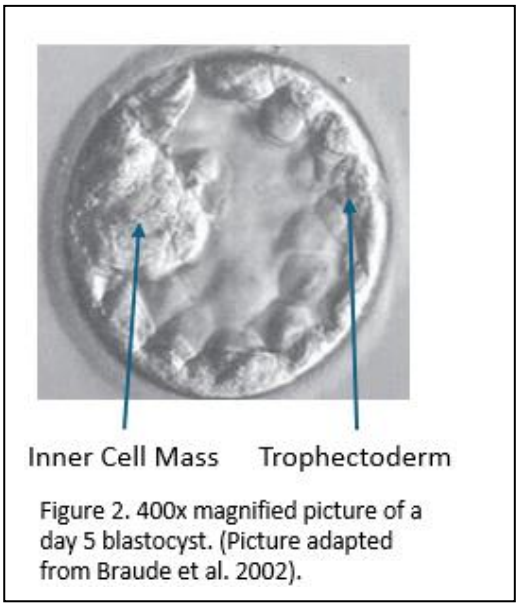

Figure 2. 400x magnified picture of a day 5 blastocyst. (Picture adapted from Braude et al. 2002).

micromanipulation equipment where ICSI was performed, held by the holding pipette and a laser is used to make a hole in the zona pellucida. A biopsy pipette then takes a small number of cells from the trophectoderm. Embryos are frozen until the tests are complete and then unaffected embryos can be thawed and transferred (Brezina et al. 2012).

There are several different types of preimplantation genetic testing according to what they are being used to test for. PGT-M stands for preimplantation genetic testing for monogenic disorders or single gene defects. It is used to identify numerous heritable conditions where the disease-causing loci have been identified, these will be either nuclear or mitochondrial. Nuclear loci include X-linked and dominant, or recessive autosomal conditions and mitochondrial loci include maternally inherited conditions (Besser et al. 2019). Indications for PGT-M would be parents or immediate family members who carry or have, cystic fibrosis, myotonic dystrophy, Huntington's, Duchenne muscular dystrophy, neurofibromatosis, some cancer syndromes, and other monogenic conditions. These specific genes would be identified and assessed in the embryonic cells and affected embryos not chosen for transfer (De Rycke and Berckmoes 2020).

PGT-SR is testing for structural rearrangements of chromosomes. Rearrangements or micro-duplication/deletions can cause repeated miscarriages or implantation failures. In order to maximise chances of a successful pregnancy, PGT-SR uses single nucleotide polymorphism (SNPs) arrays or sequencing based haplotyping to identify any unbalanced chromosomal rearrangements (Madjunkova et al. 2020). Indications for PGT-SR include patients aware of having a balanced Robertsonian translocation, however it is only advised if the applicable technique is capable of detecting all possible unbalanced rearrangements (van Montfoort et al. 2021).

Finally, there is PGT-A, testing for aneuploidies, this is the most commonly used in IVF facilities. Indications for PGT-A include advanced maternal age, recurrent miscarriage, repeated implantation failure and severe male factor infertility. It is recommended that in cases of recurrent miscarriage and implantation failures that parental karyotyping is carried out also (Carvalho et al. 2020). Genetic counselling is highly advisable alongside all preimplantation genetic testing (Carlson and Vora 2017).

1.4 Problems with Preimplantation Genetic Testing.

As impressive as these technological advances are, they are not without problems. In the absence of a random control trial, it is actually impossible to state for a fact that PGT of any kind increases chances of successful pregnancy. Mosaicism, as a cause for example, is difficult to assess or even confirm with any degree of accuracy as it is entirely dependent on the collected cells which may or may not be representative of the whole embryo (Franco Jr 2019).

The physical act of performing the PGT is fraught with potential problems, from pipetting or loading errors to errors in laser piercing of the zona pellucida. The embryologists performing the biopsies need to be highly trained and highly experienced, this in turn makes their time costly, further increasing the expense of PGT (Casper 2023). Embryologists undergo a lot of training to fine tune their skills and are the front line of IVF, from performing the fertilisation to monitoring progress of embryos and grading them in preparation for transfer, a lot of time is taken up performing PGT that takes away from these responsibilities and training of junior embryologists (Shapiro et al. 2023).

The lasers used in the processes are very expensive to purchase and require at least annual maintenance to keep them working to their full potential. Maintenance is also costly, and those costs are reflected in the costs of PGT overall (Go and Pool 2017). Costs of PGT on top of IVF are not at all negligible. According to CREATE Fertility website, for patients in the south of England, One cycle of private IVF with pre-treatment care, costs approx. £6000. PGT-A alone is £1300, PGT-M costs £2500, and both incur the additional costs of ICSI, vitrification of embryos, storage, thawing, and frozen egg transfer which come to a staggering additional £6130 for one embryo (CREATE Fertility 2023). £12,000 is not an amount that many couples could find easily and there are stringent rules restricting who can access ART on the NHS. Those who can receive ART on the NHS will find PGT is not an option for them unless there is a specific monogenic disorder (Yang et al. 2022). Despite all this cost, training and experience, embryo biopsy at any stage is far from without risk. Even at the more stable blastocyst stage, too many trophectoderm cells could be aspirated in the biopsy or the laser could cause DNA damage among other risks (Neal et al. 2017, Leaver and Wells 2020).

1.5 Non-Invasive Preimplantation Genetic Testing.

In an effort to reduce such risks, attempts have been made to reduce the invasive nature of PGT since 2013, when Palini and associates tried to isolate cell free DNA from the blastocoel fluid (BF) within the embryo which is usually removed via ICSI pipette prior to vitrification (Parikh et al. 2018, Palini et al. 2013). Some success has been had in comparing BF biopsies with trophectoderm biopsies in terms of ploidy concordance according to a study by Magli et al. (2016) but they struggled with getting enough cell free DNA to amplify for testing (Leaver and Wells 2020). Whilst it is less invasive, this method is clearly not non-invasive due to the piercing of the zona pellucida and the removal of fluid from within. Whilst looking for other sources of cell free DNA to test, it was discovered that some embryonic cell free DNA was left behind in the culture medium the embryos had been grown in and that that could be tested for chromosomal abnormalities (Sialakouma 2021). Collected DNA from both BF and culture medium is then subject to whole genome amplification and next generation sequencing to assess ploidy (Yin et al. 2021). The problem of not being able to collect enough cell free DNA from blastocoel fluid alone was solved by combining BF with DNA containing spent culture medium (SCM) and amplifying both samples together (Rogers et al. 2021). SCM testing is completely non-invasive so negates all of the potential problems that could be encountered with traditional PGT.

1.6 Aims and objectives.

Aim: To perform a systematic review of published studies and determine whether non-invasive preimplantation genetic testing is ready for clinical application.

Objectives:

1. To identify the most effective approach to perform non-invasive PGT from published studies/ To explore the different methodological approaches implemented for non-invasive PGT.
2. To systematically review the clinical implementation of non-invasive PGT/ To determine niPGT concordance in relation to PGT.
3. To evaluate the associated findings and determine whether non-invasive PGT is ready to replace standard PGT/ To explore whether niPGT is ready for clinical application.

2. Methodology:

A literature search was performed using EBSCO Host database using the search term “non-invasive preimplantation genetic testing” using the parenthesis to search the whole term as one. Limits were set to “Full Text” and sources were set to “Academic Journals”, the “results per page” slider was set to 50 results per page which removed any duplicate articles. The results by year option automatically set itself to 2018-2024 to represent the novel value of the search topic. Results were “saved to folder” and exported to csv file to be opened in Excel. The same search term was used on PubMed. Search term again in parenthesis to search as a whole term. Results by year again automatically set to 2018-2024, “full text” and “journal article” filters applied. The search result was saved as csv file and again opened in Excel. Excel sheets were merged into one sheet and article titles were rearranged into alphabetical order. This allowed duplicates of papers to be clearly identified and duplicates removed. Of the remaining papers, reviews were removed leaving only primary literature for this review. The final total was 18 original papers, see Fig 3 as to how this was achieved.

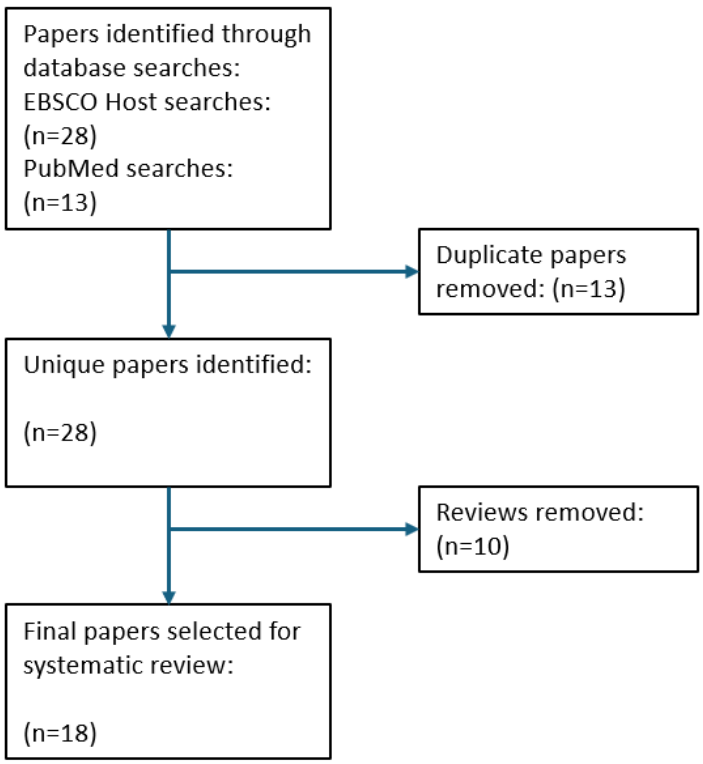

Figure 3. Pictorial representation of selection methodology.

3. Results:

Of the 18 studies included, two did not yield results. Olcha et al. 1&2 (2020) did not concurrently run trophectoderm biopsies to confirm their results. A further one, Orvieto et al. (2021) did not include sex concordance, see Fig 4. Ploidy concordance across the studies had an average of 64.1% with a range of 60.9%. Sex concordance had an average of 82% with a range of 66.7%.

Figure 4. Table showing studies reviewed by year, medium used for non-invasive PGT, either spent culture medium (SCM) or blastocoel fluid (BF). Also showing numbers of testable samples and concordance with trophectoderm or whole embryo biopsy regarding ploidy and sex.

Study	niPGT medium	No. of testable samples.	Ploidy Concordance %	Sex Concordance %
Vera-Rodriguez et al. 2018.	SCM	51	66.7	33.3
Kuznyetsov et al.2018	SCM & BF	28	71.4	100
Huang et al. 2019.	SCM	48	60.4	48
Yeung et al. 2019.	SCM	116	73.3	100
Olcha et al.(1) 2020.	SCM	3816	n/a	n/a
Olcha et al.(2) 2020.	SCM	146	n/a	n/a
Christopikou et al. 2021.	SCM	45	97.3	99.5
Jones et al. 2021.	SCM	108	52	100
Kulmann et al. 2021.	SCM	7	42.9	85.7
Li et al. 2021.	SCM	39	64.1	100
Orvieto et al. 2021.	SCM	1301	78.2	n/a
Shitara et al. 2021.	SCM	20	55	85
Sialakouma et al. 2021.	SCM	40	50	80
Sonehara et al. 2022.	SCM	22	36.4	81.8
Tsai et al. 2022.	SCM	40	52.5	42.5
Xie et al. 2022.	SCM	26	69.2	100
Yang et al. 2023.	SCM	218	78.2	100
Chow et al. 2023.	SCM	135	77.8	74.8

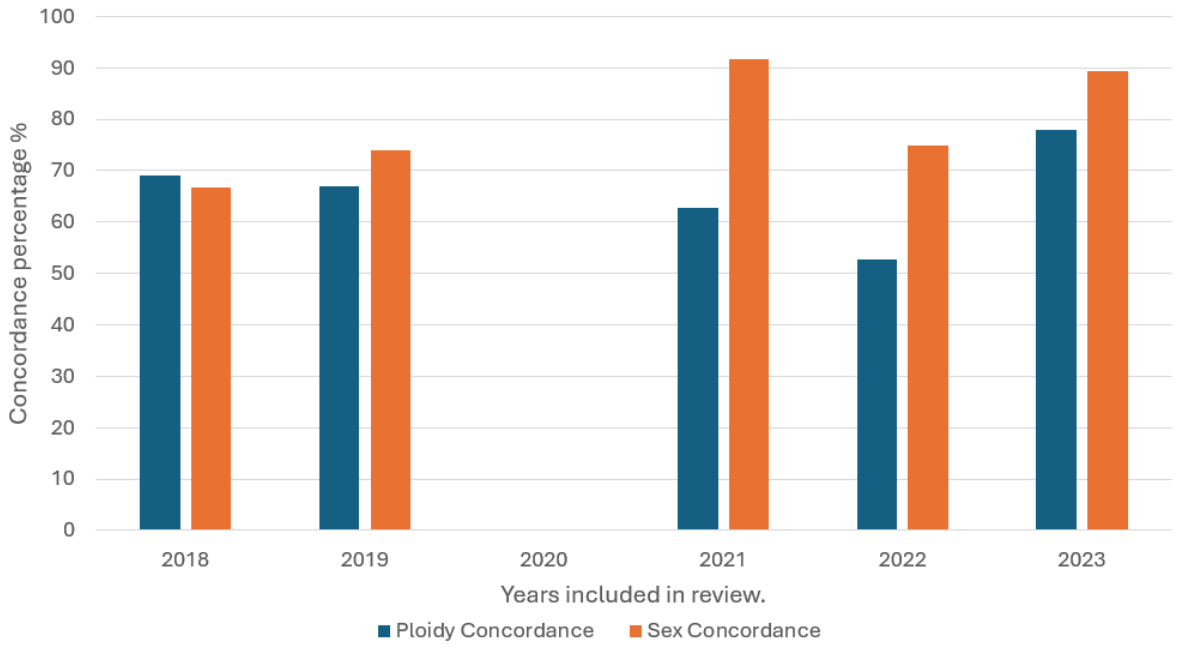

Figure 5. Graph showing mean concordance % over the 6 years included in the review.

The expectation was that as time went on and methods of testing became more advanced and specialised that concordance rates would show improvement. The mean averages as seen in Fig. 5 show no trend either up or down for ploidy concordance but a slight upward trend for sex concordance. 2020 is empty due to the two Olcha studies without trophectoderm control results. Sex concordance outperforms ploidy concordance in all years but 2018. Averages were presented per year to identify if changing methods of niPGT as more was learned, affected outcomes

Figure 6. Table showing testing protocols of each study for both PGT and niPGT. Whether blastocysts were fresh or frozen prior to culture medium collection, the day of sampling post fertilisation, method of DNA amplification, Polymerase chain reaction (PCR) or Multiple Annealing Loop-Based Amplification Cycles (MALBAC) and how ploidy of embryos is ascertained; either by Fluorescence in situ hybridisation (FISH), Copy number variants (CNV) or single nucleotide polymorphisms (SNPs), and ploidy concordance.

Study	Fresh or Frozen	Day of sampling	Amplification method	Method of ascertaining ploidy	Ploidy concordance %
Vera-Rodriguez et al. 2018.	Fresh	5	PCR	FISH	66.7
Kuznyetsov et al.2018	Fresh & Frozen	6	PCR	CNV	71.4
Huang et al. 2019.	Fresh	6	MALBAC	CNV	60.4
Yeung et al. 2019.	Fresh	6	PCR	CNV	73.3
Olcha et al.(1) 2020.	Fresh	6	MALBAC	CNV	n/a
Olcha et al.(2) 2020.	Fresh	6	MALBAC	CNV	n/a
Christopikou et al. 2021.	Frozen	5	PCR	CNV	97.3
Jones et al. 2021.	Fresh	6	PCR	CNV	52
Kulmann et al. 2021.	Frozen	6	PCR	CNV	42.9
Li et al. 2021.	Frozen	6	MALBAC	CNV	64.1
Orvieto et al. 2021.	Frozen	6	PCR	CNV	78.2
Shitara et al. 2021.	Frozen	6	PCR	CNV	55
Sialakouma et al. 2021.	Fresh	5	PCR	CNV	50
Sonehara et al. 2022.	Fresh	6	PCR	CNV	36.4
Tsai et al. 2022.	Fresh	6	MALBAC	CNV	52.5
Xie et al. 2022.	Fresh	6	PCR	CNV	69.2
Yang et al. 2023.	Fresh	6	MALBAC	CNV & SNPs	78.2
Chow et al. 2023.	Fresh	6	PCR	CNV	77.8

Within the time period covered by the papers in this review there has been little change in the PGT or niPGT testing protocols. FISH has been phased out as a method of testing due to its limitations which will be discussed further on. All of the other studies have used next generation sequencing (NGS) to then identify and count copy number variants or single nucleotide polymorphisms as markers of aneuploidy. DNA amplification has been undertaken using either polymerase chain reaction (PCR) or Multiple Annealing Loop Based Amplification Cycles (MALBAC) both are valid and current methods of amplification.

4. Discussion:

In order to answer the research question; Is non-invasive preimplantation genetic testing ready for clinical application, eighteen recent studies have been identified and systematically reviewed. The first objective of this review was to identify the most effective approach to performing niPGT.

The highest concordance rate from the studies included was from Christopikou et al. reporting a 97.3% ploidy and 99.5% sex concordance. The Christopikou et al. study used day 5 embryos for TE biopsy then after vitrification, thawed the embryos for 24 hours in fresh culture medium before amplifying the cell free DNA found in the medium with PCR. More failures to amplify were experienced with the culture medium than TE samples but concordance percentage was high, and the concordance was for both aneuploidy and structural rearrangements (Christopikou et al. 2021). Studies have suggested high DNA failure to amplify rates in SCM may be due to the length of time the embryo has spent in the culture medium, with indications that longer times may relate to better DNA amplification (Hanson et al. 2021). Sonehara et al. used the same protocol with regard to amplification method and ploidy determination but with fresh embryos and reported the lowest ploidy concordance in this review at 36.4%. The Sonehara et al. study used day 6 embryos, amplified DNA with PCR and after Next Generation Sequencing (NGS) were looking for Copy Number Variants (CNVs) (Sonehara et al. 2022). NGS is a powerful and cost-effective technology to determine ploidy of embryos whether PGT or niPGT, it does however take time. The majority of embryos undergoing PGT will have to be vitrified while amplification, sequencing and determination of ploidy takes place (Wilding et al. 2019).

Comparisons of fresh vs frozen embryos are currently undetermined, Maheshwari et al. compared fresh and frozen embryo transfers in 619 couples undergoing IVF and determined the live birth rate to be the same whether the embryo had been vitrified, thawed, and transferred or transferred freshly. Their only comment for one over the other is that fresh embryo transfer is less costly for the patients and the clinics (Maheshwari et al. 2022). However, Sanders et al. on reviewing the UK Human Fertilisation and Embryology Authority (HFEA) data collection between 2016 and 2018, reported a 2-3% increase in live birth rate

from frozen embryo transfers over fresh transfers (Sanders et al. 2021). The studies included in this review show a mean ploidy concordance of 67.5% from frozen embryos, while fresh embryos only show a 51.4% concordance, according to Chen et al. (2022) this may be due to a mixture of the aforementioned longer time the frozen embryo spends in the culture medium and that more cell free DNA is shed into the medium during thawing.

Multiple annealing and Looping-Based Amplification Cycles or MALBAC amplification was used in six of the eighteen studies, it is considered a more accurate genome coverage method of amplification as it can achieve 93% genome coverage and is free of the whole genome amplification bias which has been known to hinder other methods (Zong et al. 2012). The remaining twelve studies used PCR which has been the gold standard DNA amplification method since the 1980s (Zhu et al. 2020). Both methods amplify DNA satisfactorily to be able to provide readable sequences as is demonstrated by the reviewed study results.

The day of trophectoderm biopsy, either day 5 or day 6 is dependent on blastocyst development (Gordon et al. 2022). Traditionally, using morphological grading, blastocysts chosen for transfer would be the ones with the fully expanded blastocoel cavities, ideal shaped, tightly packed inner cell mass, and uniform trophectoderm cells (Gardner and Schoolcraft 1999). To achieve this ideal morphology, it can take the embryo until day 6 post fertilisation (Irani et al. 2018). Some studies infer that an embryo which does not fully expand until day 6, even with good morphological grading, is suggestive of aneuploidy and therefore lower implantation rates (Tong et al. 2022, Irani et al. 2018). Considerably more of the included studies used day 6 samples, the mean concordance of ploidy for day 6 samples is 62.4% whereas the few that used day 5 samples show a mean concordance rate of 71.3% which appears to support the literature saying day 5 sampling of the culture medium is optimal.

The methods for determining ploidy status did not change much at all across the six years covered by the studies. Copy number variant counting is the quickest, easiest way to assess all chromosomes have the correct or expected number of copies of segments of DNA or genes and it is ideal for PGT-A or PGT-SR and both invasive and non-invasive (Volozonoka et al. 2022). With NGS being the norm for genetic testing now, PGT-M, for monogenic conditions, is less

problematic. Previously allelic dropout, de novo mutations and DNA contamination made PGT-M particularly difficult, but SNP-based haplotyping has removed most of these difficulties (Zhao et al. 2024). For PGT-A or PGT-SR, SNP array is used less often. It can be very accurate for monogenic conditions, but when assessing for aneuploidy or structural rearrangements, studies have shown that SNP array accuracy in detecting embryonic mosaicism is woefully inadequate (Chen et al 2022). NGS for CNVs has a much higher accuracy rate for detecting mosaicism in embryos as well as aneuploidy overall (Xiao et al. 2021). The earliest study in this review used Fluorescence in Situ Hybridization (FISH) to assess ploidy of embryos (Vera-Rodriguez et al. 2018). FISH is a cytogenetic method of determining abnormalities in chromosome number or structure by using the complementary nature of DNA to attach probes to specific regions (Anyane-Yeboa 2011). FISH was phased out as a method of detecting aneuploidy in embryos as it cannot simultaneously assess all 24 chromosomes, autosomes 1-22 and X and Y sex chromosomes (Treff and Scott Jr 2012). Fluorochromes or coloured DNA complementary probes are fixed to specific chromosomes which can be counted under a fluorescence microscope, but only a certain number of fluorochromes are commercially available (Coonen et al. 2020). FISH can be used successfully to identify common trisomies 13, 18 and 21 and the sex chromosomes but is now vastly outperformed by processes such as NGS. FISH is also labour intensive, needing a skilled person to perform the fixing of metaphase cells and hybridization for it to work (Scriven et al. 2011). As FISH is only used in the earliest study from 2018, It is clear to see it has been phased out of use in niPGT in favour of more accurate methods like NGS.
While none of the studies explicitly say how sex and therefore sex concordance is determined, some extra research has made it clearer that NGS provides a clear read of all 22 autosomes and number of X and Y chromosomes. Five reviewed studies included these in their results section. Figures 7 and 8 show the NGS reads showing concordance between sample types as well as male and female examples (Kuznyetsov et al. 2018, Huang et al. 2019, Kulmann et al. 2021, Li et al. 2021, and Xie et al. 2022). Amelogenin is a commonly used highly conserved sex marker gene, the different number of short tandem repeats is clear between males and females when cytogenetically observed. Amelogenin and targeted gene sequencing, sequencing of one or a selection of specific

genes to identify known gene problems, are frequently used to determine sex in cases of X linked conditions that are passed from carrier mothers to sons (Liu et al. 2022).

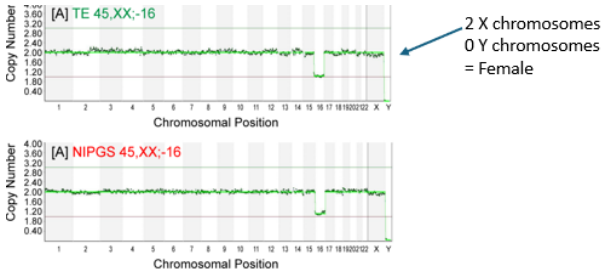

Figure 7. BlueFuse Multi software by Illumina representation of embryo chromosomal complement showing both concordance between trophectoderm biopsy and culture medium sample and how sex is determined to be female. (Picture adapted from Kuznyetsov et al. 2018)

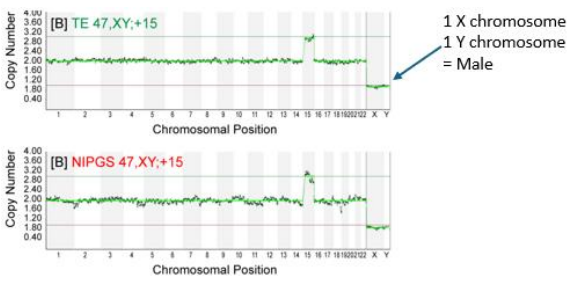

Figure 8. BlueFuse Multi software by Illumina representation of embryo chromosomal complement showing both concordance between trophectoderm biopsy and culture medium sample and how sex is determined to be male. (Picture adapted from Kuznyetsov 2018)

From the studies reviewed and published literature; the most effective approach to perform niPGT would appear to be to thaw a day 5 frozen embryo, which has fully expanded prior to vitrification, in a fresh drop of culture medium, use PCR to amplify the cell free DNA from the medium and NGS to analyse the content.

Of the eighteen studies in this review, only sixteen have given the comparable invasive and non-invasive results needed to address the research question. Two studies, both conducted by Olcha et al (2020), carried out non-invasive PGT experiments on spent culture medium as required by this review, however, they did not have control results from also testing whole embryo or trophectoderm biopsies. In both of these studies an apparent validated platform for performing niPGT was utilised, Non-Invasive Chromosome Screening or NICS™ by Yikon Genomics (Olcha2 et al. 2020). Due to the lack of TE biopsy control result for comparison, the Olcha et al. studies will be considered no further in this review, however, the niPGT platform will be further investigated.

On searching for literature relating to NICS™, a biotechnology company was discovered. Diagnostica Longwood is one company that provide laboratories and IVF centres with the NICS™ products by the brand Yikon Genomics. The webpage reports NICS™ is “simple and rapid, highly successful and highly representative” see Fig. 9 for a diagrammatic representation of the NICS workflow and timings (Diagnostica Longwood 2021). It is also important to note that on the webpage, NICS is referenced as Non-invasive Implantation Capability Screening not the aforementioned Non-Invasive Chromosome Screening.

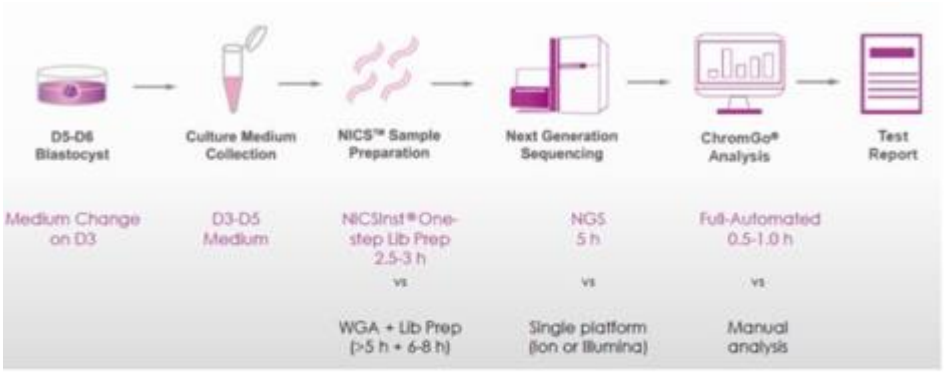

Figure 9. Diagram demonstrating the step-by-step workflow of the NICS process of completely non-invasive PGT-A including estimated times taken. (Picture adapted from Diagnostica Longwood.)

Further examination of the included studies found that three studies, Chow et al., Christopikou et al. and Sonehara et al. all used another specific non-invasive PGT platform, PG-Seq Rapid Non-Invasive PGT Kit by PerkinElmer. This kit uses a 2 step PCR to whole genome amplification of the DNA in the culture medium and attaches sequences specific adapters to result in sequence ready samples (Chow et al. 2023, Sonehara et al. 2022, Christopikou et al. 2021). Yang et al. developed a very fast SCM assay called NICSInst which can complete the whole amplification, sequencing, and results process in approx. 9 hours meaning embryos can be tested and transferred in the same day without needing to vitrify and thaw (Yang et al. 2023). All other studies used a modified PGT protocol for niPGT apart from Tsai et al. who used exactly the standard IVF workflow, with more IVF fertilisation than ICSI, with the aim of truly validating niPGT (Tsai et al. 2022).

As of April 2024, it is still unknown how or why cell free DNA is released into the culture media. Cell free DNA are fragments of DNA around 40-200base pairs long, released extra-cellularly from the nucleus and/or mitochondria by cell necrosis, apoptosis, or active releasing mechanisms (Qasemi et al. 2021). Shamonki et al. hypothesised that testing culture medium after any invasive piercing of the zona pellucida, either ICSI fertilisation or TE biopsy prior to SCM collection, increases the amount of cell free DNA released. Similarly, if the blastocyst hatches from the zona pellucida whilst still in the medium, a greater concentration of DNA is found (Shamonki et al. 2021). This does not illustrate how or why, but highlights what may encourage higher concentrations of cfDNA

in the media. Many included studies reference the origin of cfDNA in the media to be unknown but likely through cell lysis or entering the apoptotic pathway, meaning that it is waste products that are shed into the culture media (Sialakouma et al. 2021, Tsai et al. 2022). Conversely, while it is true that apoptosis is programmed cell death and those expelled products are waste products (Obeng 2020), All cells undergo apoptosis when it is their time. Within the embryo or more specifically the blastocyst, both the inner cell mass and the trophectoderm will go through apoptosis as it grows and develops, meaning the media will contain cfDNA from both of these structures and therefore potentially provide a better picture of the embryo as a whole than trophectoderm biopsy which purposefully does not take cells from the inner cell mass (Shitara et al. 2021).

Orvieto et al., included in this review, reported in their study that from 9 embryos confirmed euploid by TE biopsy, 5 of those embryos gave aneuploid results from SCM analysis. While this clearly does not show concordance, neither test result was wrong. In this case and possibly others, the cell free DNA in the culture medium came from the embryo expelling aneuploid blastomeres and DNA fragments with the end result being a healthy euploid embryo (Orvieto et al. 2021). Another study found that euploid embryos from women of advanced maternal age (AMA), which are known to have higher aneuploidy rates, shed notably more cfDNA into the culture medium. It does not specify any further on the content of the cfDNA but does later say euploid embryos from younger women have less cfDNA in the media inferring expulsion of more aneuploid material from the first group (Tsai et al. 2022). This raises many questions. Is it possible to tell which DNA fragments are representative of the embryo and which are discarded aneuploid fragments from a now euploid embryo? Will there ever be a way to be sure without using simultaneous trophectoderm biopsy (Tomic et al. 2022)? This review finds those questions repeated throughout the included studies with no conclusive answers, however, Huang et al. proposes improved amplification methods as a potential answer. Once contamination is ruled out, MALBAC amplification followed by NGS and a relatively high mosaicism threshold of ≥ 60% provided reportedly more accurate results (Huang et al. 2019).

Contamination is a big problem for niPGT using culture medium, prevention is essential! Treatment of the culture media and embryo should always be carried out under a laminar hood, with the practitioners wearing lab coats, nitrile gloves, masks, and theatre caps (Brouillet et al. 2020). These standard lab procedures reduce the chance of the testers DNA or any airborne contaminants accidentally ending up in the culture medium. The main source of contamination, however, is maternal DNA in the cumulus cells, the granulosa cells surrounding the oocyte (Turathum et al. 2021). To prevent, or at least reduce, maternal contamination the oocyte needs to be denuded prior to ICSI fertilisation or the zygote needs to be denuded after IVF before the culture medium is suitable for niPGT (Guerrero Sanchez et al. 2020). Denudation is performed with an enzyme called hyaluronidase and repeated pipetting through smaller and smaller diameter pipettes until all of the cumulus cells have been removed (Weng et al. 2019). Maternal contamination presents a challenge to niPGT because it means there is not only embryonic cell free DNA in the medium, the cumulus cells are full of maternal origin DNA also (Xie et al. 2022). Maternal contamination can cause false results for sex determination, showing XX when clinical determination is XY, and potentially false copy numbers of chromosomes where the embryonic and maternal profiles show as tetraploidy, four copies of each chromosome instead of two (Nakhuda et al. 2024) and other problems.

Embryonic mosaicism can be difficult to determine through PGT or niPGT, as discussed previously, only diploid aneuploid mosaic embryos can survive to blastocyst stage so is the only one of the three classifications likely to be encountered in PGT (Bielanska et al. 2002). Diploid aneuploid mosaicism consists of two distinct cell lines, one normal, diploid, one aneuploid with usually just one chromosome involved (McCoy 2017). The aforementioned three studies which used PG-Seq Rapid Non-Invasive PGT Kit by PerkinElmer do not mention mosaicism at all as this niPGT platform has not yet been validated to report mosaicism (Chow et al. 2023). Other studies that mentioned a mosaicism cut off level report setting their limit at 50-60% (Huang et al. 2019, Li et al. 2021). Mosaicism causes problems for IVF clinics because of the unknowns, even if the mosaicism is identified, the outcome if the embryo was to be transferred cannot be predicted (Fragouli et al. 2017). The main concern for niPGT with mosaic embryos is the ability of the embryo to self-correct. During the cell divisions up to

and beyond blastocyst stage the embryo has the capacity to expel mosaic or aneuploid cells and continue to proliferate healthy cells (Orvieto et al. 2020). This self-correction mechanism means that the expelled aneuploid cells are going to make up the majority of cell free DNA found in the culture medium to test for niPGT (Cai et al. 2022). An excess of aneuploid cells in the media are likely to result in a misdiagnosis of an aneuploid embryo without whole embryo analysis to compare to (Chen et al. 2022). Self-correction is without doubt an impressive trait of mammalian embryos but will require further research to overcome to the possibility of misdiagnosis through niPGT-A using culture medium (Leaver and Wells 2020).

The conclusions from the studies reviewed vary. One concludes that advances in non-invasive prenatal testing (NIPT), which identifies, isolates, and tests foetal DNA fragments in maternal blood, could be used to further develop niPGT platforms to isolate embryonic DNA from maternal DNA. They ultimately conclude that niPGT is not yet ready to be utilised without concurrent invasive testing (Vera-Rodriguez et al. 2018). Others conclude that there is a place for niPGT alongside morphology and morphokinetics in time lapse incubators to better predict the ideal embryos for transfer, but further trials and research are needed (Christopikou et al. 2021, Tsai et al. 2022). Some conclude that amplification methods of cell free DNA need to be greatly improved before any further advancement of niPGT in the clinical arena (Huang et al. 2019, Shitara et al. 2021). Two conclude that results are promising and niPGT is better than morphology assessment alone, but that it is not yet ready to replace standard PGT and many more randomised control trials are needed (Chow et al. 2018, Kulmann et al. 2021). Xie et al. conclude that results are promising for use with IVF and ICSI and can definitely be implemented to prioritise embryos for transfer which is similar to what Christopikou et al. and Tsai et al. said. They continue that niPGT is advantageous because of the huge reduction in cost compared to standard PGT and that it could be effective for patients where advanced maternal age or male factor infertility are NOT the case (Xie et al. 2019). Two further studies conclude that results are promising but further refinement of techniques need to be implemented and rigorously tested before implementation of niPGT and then further randomised control trials should be undertaken (Kuznyetsov et al. 2018, Sialakouma et al. 2021). Jones et al. conclude results are inconsistent

and niPGT is a long way from being standard practice, they propose further testing of prolonged exposure of the embryo in the medium to improve concordance rates then further trials (Jones et al. 2021). Sonehara et al. conclude that sex concordance is good and niPGT could be implemented for sex determination to assist in the diagnosis of X- linked mendelian conditions but no replacement of standard PGT yet (Sonehara et al. 2022). Finally, Orvieto et al. are quite firm in their conclusions. From their own experiments and reviewing of others, they conclude human embryos have numerous methods of self-correction and expel a high enough number of aneuploid cells that any testing of cell free DNA in the culture medium would be swayed considerably towards an incorrect aneuploid diagnosis. This will lead to disposal of perfectly healthy embryos with potential to implant and go on to a healthy live birth. They also state that these self-correction mechanisms can continue after the blastocyst stage so diagnosis of any kind at this stage is pointless. Finally, they conclude it is unethical that some IVF clinics are already offering unvalidated niPGT as an add on treatment, at a cost to the patient, and that these findings on niPGT should be heeded (Orvieto et al. 2021).

The findings of this review should be understood in light of some limitations. In the included years 2018-2023, the search results did not provide many papers to review. At the time of the literature search, non-invasive preimplantation genetic testing was still a topic in its infancy with not many published papers to include. Future reviews could include wider search terms.

5. Conclusion:

In conclusion, the systematic review of published literature finds that non-invasive preimplantation genetic testing is not ready for clinical application. In general agreement with the studies reviewed, niPGT needs many more trials and much more refinement before it is ready to replace PGT.

To date there is no confirmed method to ensure results that would be concordant with standard PGT. The highest concordance rate in this study came from the culture medium of a vitrified and thawed embryo, if using niPGT alone, there would be no need to have vitrified the embryo before testing. Vitrification, if necessary, would be undertaken whilst sampling the SCM and the results being processed. The literature suggesting vitrified and thawed embryos are optimal for sampling in a process where vitrification will not actually take place, leaves the fresh vs frozen debate deprived of practical significance.

15 of the 18 studies used day 6 blastocysts to test, regardless of fresh or frozen, literature has reported that blastocysts that do not reach full expansion until day 6 are often aneuploid and prone to implantation failure. This review confirmed day 5 to be optimal even from the small number of studies. Waiting to test SCM from day 6 blastocysts may reduce implantation potential even in euploid embryos.

The required time for the embryo to be in the culture medium for optimal cfDNA collection cannot yet be quantified, this alone requires further trials, perhaps comparing embryos from older and younger women and ones with known and unknown infertility causes. The origin of cell free DNA released out of the embryo needs to be investigated closely, also why it is released. While the origin of cfDNA is still unknown the risk of healthy embryos being declared aneuploid by niPGT is too high.

Non-invasive PGT may have a place for now, alongside morphology and morphokinetics time-lapse monitoring to suggest the best embryo to transfer in a standard cycle without PGT, perhaps under the name of chromosome screening. NiPGT could have a successful place in the diagnosis of monogenic conditions such as DMD or Cystic Fibrosis where only one specific gene is involved if trials can prove the genes necessary can be isolated from the cell free DNA. There is

also potentially a place for niPGT in the sex determination of embryos where it is important to know for reasons of X-linked mendelian conditions.

At this time, niPGT remains not appropriate to replace preimplantation genetic testing for structural rearrangements or aneuploidy.

Future work to improve the validity of niPGT is absolutely necessary. It is essential that a method of isolating only embryonic DNA from the culture medium is found to eradicate the risk of maternal contamination. It is of utmost importance to discover the exact source of cell free DNA to ensure the cfDNA in the medium is representative of the embryo and is not expelled aneuploid cells from an otherwise euploid embryo. A suitable platform for niPGT needs to be properly validated and rolled out for use in all settings to ensure that all testing is following the same protocols, the same equipment, and same methodologies so that results can be compared between settings and the platform adapted, adjusted, or improved as necessary. Ease of use or explicit instructions to account for user error will also be necessary. Christopikou et al. and Sonehara et al. reported the highest and lowest concordance respectively in this review, they both utilised a specific non-invasive PGT platform called PG-Seq Rapid Non-Invasive PGT Kit by PerkinElmer. One study reported concordance of 97.3% and the other only 36.4% with the same equipment. This needs to be further investigated to eliminate the possibility of human error.

Yang et al. reported development of a fast system for niPGT results called NICSInst, which can go from SCM sample to full results in 9 hours. Further trials of this method of testing and result reporting is essential. Validation of this platform would be a great leap forward. There is a potential for greater implantation success if a sample can be taken as soon as the blastocyst is fully expanded and dependent on results transferred into the uterus only 9 hours later.

With ever evolving scientific methods and expanding knowledge, it is possible that once the aforementioned problems and unknowns are addressed, non-invasive preimplantation genetic testing may be appropriate for clinical application in the future.

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Appendices:

Research Proposal Template

Student name: Louise Turner

Supervisor name: Elpida Fragkouli

Supervisor signature: 

Note to supervisors, by signing this form, I am agreeing that I will supervise the student research project in the academic year 2022/23. I have advised on equipment and methods, and I am able to explain necessary practical techniques and assess ethics and risk assessments.

Note to students:

Please complete the text boxes below. You should delete the guidance information (in blue) in the boxes as you go, and make sure you stick to all word counts. All word counts are maximum – there is no minimum length assuming you have all necessary information. The following online guide can be useful:

http://theprofessorisin.com/2011/07/05/dr-karens-foolproof-grant-template/

Summary (150 words max)

This should include the main background, references to key literature (1 or 2 references), the key gaps in current knowledge, the overall aim, research question or hypothesis and brief overview of the methods.

- Preimplantation genetic testing is used to improve IVF success.
- It is costly and invasive.
- Less invasive and non-invasive testing is possibly available.
- Can this non-invasive testing be as effective as the current PGT?
- This systematic review aims to assess whether non-invasive preimplantation genetic testing is ready for clinical application.

Background (400 words maximum)

Think of this like a short introduction to a scientific paper. Start broad, narrow to your focus, but make sure you cover the background to support your aim (you don't need to include the aim in here, as this will be in the next section)

- Preimplantation genetic testing (PGT) is used for both diagnostic and screening purposes to improve the chances of successful pregnancy following in vitro fertilization (IVF) (Brezina et al. 2012).
- PGT was first used in 1993 to identify euploid embryos which were optimal for implantation to increase live birth rates in IVF (Greco et al. 2020).
- PGT is considered ethically acceptable in diagnostic use but controversial in some screening situations (Brezina and Kutteh 2015).
- PGT is an invasive procedure which may cause damage to the embryo, and it requires skills that render it very costly (Leaver and Wells 2020).
- Noninvasive techniques are being investigated to provide less to no embryo damage whilst giving the same vital information. Cell free DNA has been found in the growth medium embryos are grown in, which can potentially be used to perform genetic testing (Navarro-Sanchez et al. 2022).

Aims and objectives (200 words max)

This should include a single overall aim, and 3-7 objectives. You can also phrase aims and objectives as hypotheses or research questions, as discussed in the Advanced Skills units.

The aim of this review is to assess if non-invasive preimplantation genetic testing is ready for clinical application.

Objectives:

- To summarise the current status of invasive PGT, focusing on methodological approaches and associated clinical outcomes.
- To explore the different methodological approaches implemented for non-invasive PGT.
- To determine niPGT concordance in relation to PGT.
- To explore whether niPGT is ready for clinical application.

Overview of the methods (300 words max)

These should be brief, but provide a clear overview of any techniques you will use and any equipment and consumables you will require. You should include an indication of how you will present and analyze your data (i.e. statistical tests to be used)

- A systematic review of published literature will be undertaken to assess the clinical readiness of noninvasive preimplantation genetic testing.
- An overview of existing PGT methods and outcomes will be analysed first.
- Relevance of literature will be clarified at several stages until final literature is decided.
- Data from final papers will be analysed, presented as graphs or tables with relevant statistical tests and results drawn from them.

Milestones and timelines (200 words maximum)

Milestones are key aspects of work you need to complete. These could include finishing reviewing the literature, finishing collecting data, submitting a draft to a supervisor and final submission of the project. You should list these with clear deadlines. You may wish to add a Gannt chart or written narrative of work you will be doing month by month.

- September 2023: begin literature searches.
- October 2023: conclude literature searches, begin writing introduction/methods.
- November 2023: Meeting to assess progress?
- December 2023: Results and data analysis.
- January 2024: Start discussion.
- February 2024: Meeting to discuss?? Finish discussion.
- March 2024: Submit draft, make recommended adjustments.
- April 2024: Submit final copy.

References

Please include all references you have cited in the proposal in alphabetical order, formatted in the BU Harvard Style

Brezina, P. R., Brezina, D. S. and Kearns, W. G., 2012. Preimplantation Genetic Testing. *British Medical Journal* [online]. 345 (0).

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LEARNING CONTRACT:

INDEPENDENT RESEARCH PROJECT

The learning contract is an agreement between student and supervisor: it should clearly indicate what is expected from both sides. The text in Sections 2 and 3 provides guidance and can be modified to give more details reflecting what has been agreed, such as deadlines for submission of drafts and provision of feedback, word count limits/exclusions and number/timing of meetings.

Importantly, the document checklist helps students to follow the required procedures (e.g. ethical approval and risk assessment) and communicate what has been done to the supervisor.

The student should submit a draft of the completed form to the supervisor and request a meeting to discuss and finalise the content. Both the student and the supervisor are responsible for keeping a signed copy of this document and following what has been mutually agreed.

1. YOUR DETAILS

Student name: Louise Turner

Degree Programme: BSc Biological Sciences

Proposed IRP Title or Set Project: Is non-invasive preimplantation genetic testing ready for clinical application?

Supervisor name: Elpida Fragouli

Excellent dissertations are made available for future students to refer to. Please tick this box to indicate that you agree to your dissertation being added to this collection, should it be selected.

☒ **YES**

2. As the student undertaking the above project I agree to:

- E-mail my supervisor on a fortnightly basis with a progress report.
- Meet with my supervisor at least once a month to discuss progress and I understand that it is my responsibility to organise these meetings.
- Comply with the terms of this learning contract and the guidance set out in the Guide to Independent Research Projects
- I understand that this is an *independent* project and that I am solely responsible for its completion.
- I agree to comply with all ethical, laboratory and fieldwork protocols established by the faculty.

3. As the supervisor of this project I agree to:

- Meet with the student undertaking this project on at least a monthly basis and to respond to the progress e-mails as appropriate.
- To meet formally with the student during the first week in November to undertake the interim interview.
- To provide guidance and support to the student undertaking this project bearing in mind that it is an *independent* research project. This is inclusive of commenting on drafts of the final report in a timely fashion.

3. DOCUMENT CHECKLIST

Research Proposal or Plan Attached? ☒ **YES** ☐ **NO**

☐ **YES**	☒ **NO**	**Risk Assessment for fieldwork and evidence of COSSH assessment for all laboratory procedures (online risk assessment completed)**
☐ **YES**	☒ **NO**	**Completed booking for all field equipment**
☐ **YES**	☒ **NO**	**Letters of permission where appropriate providing evidence of access to such things as field sites and/or museum archives**
☐ **YES**	☒ **NO**	**Completed Ethics Checklist**

4. INTERIM INTERVIEW – Progress evaluation

List what you have agreed to bring to your interim review (e.g. a spreadsheet showing your results and a plan for analysis).

Give the date of your Interim Review as agreed with your supervisor (ideally by early November).

Interim Review Date: Early to mid-November

5. Variance from the Independent Research Project Guide

The IRP assessment is normally governed by the guidance provided in the Independent Research Project Guide. Any variance in terms of format (e.g. technical report, scientific paper) and word limit should be agreed and specified here. Submission date cannot be changed unless evidence of mitigating circumstances is provided in accordance with the standard BU Guidelines.

Any changes? ☐ **YES** ☒ **NO** If YES please provide details below:

Both of the undersigned parties agree to be bound by this learning contract:	
Student Signature:	LMTurner
PRINT NAME:	Louise Turner
Date:	02/06/2023

Supervisor Signature:	
PRINT NAME:	Elpida Fragouli
Date:	02/06/2023

Independent Research Project
Interim Interview - Agreed Comments Form

Student Name: Lou Turner	Programme: BSc Biological Sciences
Date: 04/12/2023	IRP Title: Is non-invasive preimplantation genetic testing ready for clinical application?
Supervisor Name: Elpida Fragouli	

Agreed comments – to include progress and plans for completion:

Lou is making good progress in writing her Introduction Section.

We have planned for the Introduction section to be completed by the end of the 1st semester.

Once the Introduction section is complete, Lou will collect all the literature needed for her systematic review.

The plan is to start with the Methods and Results section at the beginning of the second semester.

Lou and I agreed on a plan for the Results and discussed about looking at other examples of systematic review IRPs.

Two copies of this form are needed – student to retain one copy and include in the appendices of the dissertation the other is to be emailed to the supervisor.

Student Signature: LMTurner.	Supervisor Signature: E. Fragouli