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Conflicts of interest

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ABSTRACT

Introduction. Preimplantation genetic testing (PGT) is growing in importance and volume internationally. International societies such as ESHRE compile international results and these data are published in scientific journals. We present the first compilation of practices, quality measures and outcome data from Nordic clinics performing PGT. **Material and methods.** We conducted a structured online survey of PGT practices in the Nordic countries to compare clinical and laboratory techniques, outcomes and quality measures applied in Nordic clinics. The survey was designed by the authors and answered by the authors and members of the study group. The outcome data represents results from 2018. Results and details were clarified through iteration with responding clinics while maintaining anonymity. Response rate in the study was 80%, with eight of ten clinics performing PGT responding. **Results.** Most of the PGT cycles in the Nordic countries are funded through the public health care system with University Hospitals performing the majority of treatments, 716/848 or 84.4% of oocyte retrievals in this dataset. The genetic analyses are in five cases performed by the affiliated local genetic laboratory, while the remaining three consult with large international private enterprise laboratories. Genetic counselling is widely used. Results in the Nordic clinics compare well with international data. Systematic quality control procedures are in place and the larger clinics and laboratories utilize ISO certification or accreditation in the quality management. Automatic witnessing with detailed electronic documentation of laboratory processes is not utilized in the responding clinics although a majority uses manual witnessing procedures in the laboratory. The outcome after PGT in terms of clinical pregnancy per transfer is around 40% per embryo transfer and compares well with international data. **Conclusions.** PGT is organised in rather few clinics in the Nordic countries and most of them use local laboratories for genetic analyses of the biopsies. Laboratory procedures are largely in accordance with international guidelines and the outcome after PGT in terms of clinical pregnancy per transfer is comparable to results in international reports.

Keywords

Preimplantation genetic testing, aneuploidies, monogenic disorders, structural rearrangements , quality control, Nordic countries, assisted reproduction technologies

Abbreviations

PGT-A	preimplantation genetic testing for aneuploidies
PGT-M	preimplantation genetic testing for monogenic disorders
PGT-SR	preimplantation genetic testing for structural rearrangements
ART	assisted reproduction technologies
MPS	Massive Parallel Sequencing
IVF	in vitro fertilization
ESHRE	European Society for Human Reproduction and Embryology
ICSI	intracytoplasmic sperm injection

Key Message

Preimplantation genetic testing is performed in only a few of the IVF-clinics in the Nordic countries, most of them relying on local laboratories for genetic analyses. Laboratory procedures are largely in accordance with international guidelines and the clinical outcome is good.

INTRODUCTION

Preimplantation genetic testing (PGT) in combination with assisted reproduction technology (ART) treatment can be used as an alternative to traditional prenatal diagnosis in cases where there is a high risk of a genetically affected fetus due to known familial monogenetic mutations (PGT-M) or structural chromosomal rearrangements (PGT-SR). Being performed before implantation and by deselection of genetically affected embryos, PGT is a preferred option for couples who want to avoid termination of an affected pregnancy. For PGT-SR the procedure additionally elevates the chances of achieving a successful pregnancy per embryo transfer as all aneuploid embryos are most often deselected in addition to the embryos carrying the already known familial chromosomal aberration in an unbalanced form. Preimplantation genetic testing can also be used to establish a pregnancy with an embryo which is human leukocyte antigen (HLA) matched to a sibling having a hematological or immunological disease in need of a life-saving bone marrow transplantation.[1] Yet another PGT application is exclusion testing where individuals who may be at-risk for a late onset disease such as Huntington's disease, and who wish to prevent the birth of a carrier child without disclosure of their own carrier status may be eligible for PGT. This is achieved by avoiding the transfer of embryos carrying a *HTT* (huntingtin) gene allele from the affected family member, thus preserving the individual's right not to know.[2]

Finally, PGT can be used to screen embryos for chromosomal aberrations or aneuploidies (PGT-A) with the aim to optimize the in vitro fertilization (IVF)-treatment in couples lacking known familial genetic disease. Chromosomal aneuploidy is likely to be one of the main reasons why only 30-50% of human blastocysts result in a live birth after transfer. Thus, not only the decrease in implantation rate by female age, but also the increased risk of miscarriage can be explained by aneuploidy, increasing from 25%-30% for patients in their twenties to about 70-90% in patients above 40 years of age.[3] Screening of human embryos by PGT and selection of embryos with normal chromosome numbers is therefore expected to have the potential to increase the chance of pregnancy per transfer in ART, to reduce the risk of miscarriage and accordingly reduce the time to pregnancy.[4] Comprehensive chromosome screening can be highly predictive of the reproductive potential of human embryos.[5]

However the true impact on the cumulative pregnancy rate is not yet fully known as some discarded aneuploid embryos do have the potential to give rise to healthy babies.[6] Routine use of PGT-A in infertility treatment is therefore questioned and a subject of intense discussion.[7, 8]

The first PGT in humans leading to pregnancy was performed in 1990 by blastomere biopsy of cleavage stage embryos.[9] Since then PGT has moved from an experimental procedure to a specialized test which is currently performed on a large scale in many centers worldwide. Today PGT is in most cases performed by genetic analysis of biopsied trophectoderm cells from blastocyst stage embryos which may require whole genome amplification of the biopsied material before analysis (e.g. microarray, karyomapping, massive parallel sequencing (MPS)). This development has been supported by significant improvements in cryopreservation methods, now yielding embryo survival rates after vitrification exceeding 90% and which have a similar implantation rate as fresh embryos.[10] The genetic analysis can be time consuming and as a consequence requires cryopreservation and storage of all tested embryos until results are obtained.

Worldwide data on PGT are collected by the PGT consortium under the European Society for Human Reproduction and Embryology (ESHRE). The last report covered data from 71 centers performing 11 637 cycles resulting in 2 147 pregnancies (De Rycke personal communication).[11] PGT-A represented 52% of the 11 637 cycles reported. PGT-M requires a more thorough genetic work up of the couple, demanding highly specialized procedures, which may partly explain why PGT-M represent only around 30% of all PGT activity in the European register and 12% in the USA.[11, 12] While PGT-M and PGT-SR are performed to reduce the risk of a genetically affected child, the motivation to perform PGT-A is often related to ART efficacy, both during infertility treatment and as an add-on to PGT-M/SR.

With increasing utilization of PGT, the need for specialized genetic counselors has increased dramatically. In many countries special educational programs exist with the profession of genetic counselors being well established. An example of this is the US in which the first formalized training programs started in the late 1960's. In the Nordic countries, only Norway has a formal Masters (MSc) program for genetic counselors, but there are other formalized educational programs to achieve

genetic counselor status also in the other Nordic countries. Usually the counselors are clinical geneticists, specialized nurses or other trained health care professionals.[13]

Guidelines for best practice have been published both by ESHRE and The Preimplantation Genetic Diagnosis International Society (PGDIS).[14-17, 18] New versions of the ESHRE guidelines are in the final stages of review and the stakeholder versions have been accessed as background material for this study.[19-22]

Regulations for the use of PGT and the degree of governmental funding vary greatly across countries and continents.[23]

The aim of the present study was to describe the current status of activity, practices, quality, use and results for PGT in the Nordic countries.

MATERIAL AND METHODS

The study was performed as an on-line survey (SurveyMonkey) distributed to all Nordic clinics performing PGT. This included IVF clinics in Denmark, Finland and Sweden as clinics in Norway and Iceland do not currently perform PGT with patients utilizing centres in the neighbouring Nordic countries. At least one author acted as a contact person in each country and distributed access to the survey via a web link. The survey was open from September 9th to September 27th 2019.

To the authors' best knowledge, ten IVF clinics were performing PGT in the Nordic countries in 2018. In 2019 one additional clinic started performing PGT, however the data presented here only refer to the year 2018. We received responses from eight of the ten clinics performing PGT in 2018, four from Denmark, two from Sweden and two from Finland, giving a total response rate of 80% and 100% coverage for PGT in Denmark and Sweden. According to the Finnish ART data collection, 53 PGT-M/SR transfers and 66 PGT-A transfers were reported for 2018. We report data on 47 and 29 of these cycles, respectively. The clinics all cooperate with a Clinical Genetics department, where some have a strong affiliation with a local University Clinical Genetics department and others send the embryo biopsies to a commercial company for genetic testing.

Each clinic was assigned a number according to the time of response to the online survey, not according to country or any alphabetical arrangement.

All results presented are from the year 2018 in order to present the current status in the Nordic countries. A clinical pregnancy was defined as an implantation (the presence of a gestational sac) confirmed by ultrasound scan and the clinical pregnancy rate as the number of clinical pregnancies divided by the number of (frozen) embryo replacements. Ongoing pregnancy was defined as a viable pregnancy with confirmed foetal heart beat at >6 weeks of pregnancy. Pregnancy loss was defined as the difference between the number of clinical pregnancies and ongoing pregnancies.

Ethical approval

Ethical review board approval for the study was not relevant because of the nature of the study.

RESULTS

Demographics and availability

The responding clinics are large in terms of IVF+PGT cycle volumes in a Nordic context with the public funded clinics being largest. Figure 1 shows the annual number of oocyte retrievals and frozen/thawed embryo transfers.

Seven of the eight responding clinics offer PGT-M and PGT-SR while one exclusively offers PGT-A within a research protocol. Two clinics offer PGT with human leukocyte antigen (HLA) testing and one offers mitochondrial DNA (mtDNA) quantification. Three of the seven PGT-M/SR clinics also offer PGT-A as a stand-alone option, the others use aneuploidy data only as part of the analysis for PGT-SR. Figure 2 shows the cycle volumes for the main PGT techniques.

Organization

Table 1 shows highlights regarding the responding clinics from the text below. Six of the clinics are publicly funded and two are private. All of the PGT programs have access to a genetic counsellor. In

two cases a genetic counsellor is employed by the IVF clinic, five collaborate to varying degrees with the local University Hospital department of Clinical Genetics (three have allocated a dedicated specialist in the Clinical Genetics department) and one in collaboration with a genetics service provider.

None of the IVF-clinics runs an in-house genetic analysis. Four collaborate with the Clinical Genetics or Genomic Medicine departments of their own hospital and one is affiliated with the local hospital. Two clinics cooperate with Cooper Genomics and one with Igenomix UK, Invicta or BioArray, and in specific cases elsewhere, which are international private service providers, specialized in clinical molecular genetics.

Seven of the eight clinics have at least one dedicated clinician for the PGT-program in the IVF clinic and one clinic has a dedicated embryologist for this purpose. One clinic has two dedicated embryologists performing embryo biopsies and the other five have at least three dedicated embryologists performing the biopsies. Training of embryologists in performing embryo biopsy was achieved at courses or workshops (N=6), at other IVF laboratories (N=1) and all laboratories performed in house training (N=8). Training in embryo biopsy was validated in all laboratories, by internal controls (N=7) and/or by external evaluation (N=4).

Availability

For clinics performing PGT-M/SR the waiting lists are less than one year, four report 1-3 months, one reports 4-6 months and two centers report a 6-12 months waiting list. This includes the genetics work up of patients. For clinics performing PGT-A, waiting lists are short, less than three months or no waiting list at all.

For PGT-M/SR, four clinics treat international patients: two clinics report 10-25% international patients, one reports 25-50% international patients and one clinic has only a few international patients per year. For PGT-A, two centers report 25-50% international patients.

In the two private clinics, patients pay an additional fee for PGT-A on top of the costs for IVF, either per biopsied embryo or per cycle. This does not apply to the public funded clinics.

Patient selection

Ovarian reserve and response

Five of the responding clinics have no lower limits regarding ovarian reserve although one has a criterion of previous blastocyst transfer for inclusion in an ongoing PGT-A study. The remaining three all have a cut off level for anti Müllerian hormone, two of them also have a cut off regarding antral follicle count and one requires additionally a certain number of oocytes after FSH stimulation for inclusion in the PGT program.

Karyotyping

All centers perform karyotyping before PGT but for varying indications. Four perform karyotyping for PGT-SR carrier only, two for PGT-SR for both partners and one for PGT-M. Three perform karyotyping for PGT-A.

Indications

The most common indications for PGT-M are Huntington's disease (five clinics), Familial adenomatous polyposis (two clinics), Myotonic dystrophy (three clinics), Fragile X syndrome (three clinics), Familial breast-ovarian cancer (*BRCA 1* and *BRCA 2*, two clinics), Cystic Fibrosis (two clinics) and Marfan syndrome (two clinics).

Six of the reporting clinics allow PGT-M for Huntington's disease with exclusion testing (i.e. identifying alleles from a relative carrying the mutation for Huntington's disease allowing exclusion of embryos with risk of carrying a mutation for Huntington's disease without revealing the carrier status of the parent at risk); however the numbers are low for that specific activity. Three centres report 1-5 cycles per year, the other three did not report cycle numbers for this category.

The most common indications for PGT-A are advanced maternal age (two clinics), recurrent miscarriage and failed previous treatments (two clinics). Four report PGT-A in connection with PGT-SR/M.

Six centers use PGT-A during PGT-SR/M for embryo prioritzation for embryo transfer, one of them only when the aneuploidy information is generated by the analysis used for detecting the genetic disorder.

IVF Laboratory techniques

Six of the eight embryology laboratories have dedicated areas for tubing, five of eight have dedicated areas for embryo biopsy while the others use regular workstations for these procedures.

Protective gloves and gown are used during biopsy and tubing in seven of the embryology laboratories. In six laboratories a mask is used and in four of them as a minimum the biopsy pipette is changed between biopsies within the same patient. The holding pipette is not always changed between biopsies within the same patient. All of the eight laboratories change pipettes between tubing and six use negative control for each tubing.

DNA decontamination is applied for cleaning microscope and manipulators in five of the embryology laboratories and seven apply DNA decontamination for the tubing work-station.

Six laboratories perform intracytoplasmic sperm injection (ICSI) in all PGT cases whereas two perform ICSI only for PGT-M, otherwise IVF (unless the sperm sample motivates ICSI).

Biopsy technique

In one laboratory, zona breaching is exclusively performed at the cleavage stages to allow spontaneous hatching of the blastocyst before biopsy. The other laboratories perform zona breaching at the day of blastocyst biopsy. Three of the laboratories perform embryo biopsy both at the cleavage stage and the blastocyst stage depending on the case. In one laboratory this is operator dependent. Embryo biopsy is performed in all laboratories on days 5 and 6, additionally in one laboratory embryo biopsy is also performed on day 7. Details are shown in Table 2.

Two laboratories use exclusively laser cutting for biopsy dissection, the other six use either laser or manual cutting or a combination of both, depending on the embryo and/or the operator.

All laboratories use the Gardner's grading system with the minimum blastocyst grade for biopsy being expansion grade 3 in six laboratories and grade 4 in the remaining two. Three have a minimum of inner cell mass grade C and four laboratories apply a minimum inner cell mass grade B for biopsy. Three have a minimum trophectoderm grade C for biopsy and four have a minimum trophectoderm grade B for biopsy. One laboratory makes an individual judgment depending on the case.

Three laboratories perform embryo biopsy at the cleavage stage for embryos with a minimum of six blastomeres required for biopsy and one specifies a maximum of 20% fragmentation.

Quality

Six of the IVF-laboratories apply systematic quality control, such as International Organization for Standardization (ISO), and five of the genetics laboratories as well. Two centers report no systematic quality control.

None of the laboratories have automated witnessing procedures. Five of the embryology laboratories apply manual witnessing of procedures by a colleague in the procedure and in the laboratory in general. Three of the laboratories have no witnessing procedures in place.

Genetic analysis and interpretation

The analysis platform used for analysis of chromosomal aberrations is Massive Parallel Sequencing (MPS) (also called Next Generation Sequencing (NGS)) as reported by six centers.

The platform used in PGT-M is MPS in one center, PCR based methods in four centers, single nucleotide polymorphism (SNP) array or karyomapping in five centers. Some centers use multiple techniques depending on the case.

The platform used for the genetic analysis in PGT-SR is MPS in six centers and fluorescence in situ hybridization (FISH) in two of which one is now launching an MPS platform.

Interpretation of the genetic analysis

In all centers the PGT results are interpreted by specialists from the genetics lab performing the analysis and in one center it is the embryologist responsible for the PGT-program. Additionally, one clinic reports that the responsible clinician is included in interpreting the analysis and two report that the laboratory director is included in the interpretation of results. One clinic includes the genetic counsellor in cases where interpretation is not clear.

The maximum degree of mosaicism allowed for considering a blastocyst for transfer is 20% in one center, 30% in one center, 40% in three centers and 50% in one center. One center makes evaluations on a case by case basis focusing more on which chromosomes are involved rather than the level of mosaicism. One clinic does not transfer mosaic blastocysts at all. If mosaic blastocysts are transferred, patients receive separate genetic counselling in all clinics.

Three clinics recommend prenatal testing after PGT-A, six clinics after PGT-M/SR and two do not recommend prenatal testing after PGT. One of the latter clinics makes an exception if a mosaic blastocyst is transferred or if the genetic test has a lower accuracy compared to the applicable standard, e.g. 95% instead of 99%.

In one laboratory aneuploid embryos are automatically discarded after results are obtained whereas the other laboratories will store for research or future evaluation. Seven centers will not transfer aneuploid embryos by patient demand, one did not respond.

Figure 3 shows the PGT-A results in terms of the proportion of euploid embryos from the four clinics reporting stand-alone PGT-A, i.e. not including the combination of PGT-SR/A or PGT-M/A.

Clinical and laboratory results

The number of oocytes retrieved in PGT cycles ranges from 8-14 with no obvious correlations between clinics applying strict anti Müllerian hormone or antral follicle count cut off values and those which do not (data not shown). The proportion of cycles with no embryos to biopsy ranged from 0-30% and the average number of embryos biopsied per cycle was between two and five for the various clinics. The proportion of cycles with no embryos to transfer ranged from 22-60%, lowest in PGT-M and highest in PGT-SR.

Genetic analysis

The proportion of failed/inconclusive results in the genetic analysis ranged from 0-12%, highest in PGT-M. Summary results of the chromosomal analysis in PGT-A were provided by four clinics. The proportion of euploid blastocysts ranged from 25% to 45%, aneuploid from 42% to 70%, mosaic from 0% to 21% (Figure 3).

Clinical pregnancy rate

Clinical pregnancy rates for PGT-M/SR/A are presented in Table 2. Results range from 31% to 60% for PGT-M and from 27% to 75% for PGT-SR. Three clinics performed some Day 3 biopsy and fresh Day 4-5 transfers which are included in these results as numbers are low. Table 2 shows that the actual numbers of transfers in each group are in many cases low which means that the rates must be interpreted with caution. Data on clinical and ongoing pregnancy rates after PGT-A with single euploid blastocyst frozen-thawed embryo transfer were provided by three clinics with clinical pregnancy rates varying from 30% to 67%. The reported miscarriage rate was low with only one pregnancy loss reported for 2018 for PGT-A.

DISCUSSION

The present study presents unique data on the current status of PGT practices in the Nordic countries, representing the majority of PGT cycles performed in these countries. We estimate that the present data collection represents >95% of the PGT-M/SR cycles and at least 75% of the PGT-A cycles currently performed in the Nordic countries. In general, the results presented here in terms of pregnancy rates, rates of utilizable embryos and general quality aspects are well comparable or even superior to international data. The most recent ESHRE data from 2017 reported at the ESHRE consortium meeting in 2019 showed a clinical pregnancy rate of 20-25% per embryo transfer for PGT-M and PGT-SR (M De Rycke, personal communication). All of the clinics in the Nordic data presents results at equal or higher levels. It should be noted that embryo biopsy at the blastocyst stage is more prevalent in this dataset although varying between clinics, as compared to the most recent as well as previously published ESHRE data, which may partly explain this difference.[11] The results in

PGT-A are more varied, but in general the Nordic results compare well with the most recent ESHRE consortium data, see Table 2, which shows clinical pregnancy rates per transfer of around 40% per transfer in the majority of clinics for all treatment modalities and across nationalities. There is a striking difference between results from the three clinics reporting PGT-A in terms of proportions of mosaic and aneuploid embryos as well as clinical and ongoing pregnancies. This may be partly due to relatively low numbers of cycles, differences in patient populations including age (which were not accessible in this survey), but issues such as embryo culture, biopsy techniques, the diagnostic platforms and the interpretation of results (for example regarding the definition of mosaicism) may also be involved. However, many recent publications show very high clinical pregnancy rates per transfer when applying PGT-A, higher than the ESHRE average, although the difference might be explained by the difference in biopsy stage between newer studies and the ESHRE data.[24]

The clinical indications for PGT-M also compare well with the ESHRE consortium data in general with four out of five indications being identical to the top ones from the ESHRE dataset.[11]

Another interesting indicator of quality is the proportion of utilizable embryos, mostly regarding PGT-A but also for other indications. This has been discussed as an important quality indicator for both laboratory and clinical procedures.[25] The proportion of euploid embryos seems lower in the reporting Nordic clinics (Figure 3) in comparison with the ESHRE consortium data. Further, the rate of inconclusive results varied within the study. This can be related to the varied patient populations with parameters such as patient age possibly playing a role, but may also reflect technical aspects since in the published ESHRE data some clinics are still using older analysis methods and not MPS. Variance in the rate of inconclusive diagnoses between centers might be expected given differences in platforms used and interpreter skills, but the small sample sizes in the given dataset might also explain much of the variance.

Varying degrees of mosaicism in embryos is an issue which has become apparent in recent years. The International Preimplantation Genetic Diagnosis Society (PGDIS) has recently issued a statement regarding how to handle embryo mosaicism with recommendations on acceptable degrees of mosaicism, informed patient consent, prenatal testing and other issues.[26] The Nordic clinics allow different degrees of mosaicism in transferred embryos but all report extensive patient counselling in

these cases. The degree of mosaicism accepted has been changing over time and will continue to do so, as knowledge and safety issues influence opinions. Prenatal testing is not uniformly recommended by Nordic clinics after PGT. Nevertheless, misdiagnoses have been reported after PGT, and prenatal testing is recommended after PGT by the ESHRE PGTM working group.

Dedicated laboratory areas and strict quality measures are important to minimize contamination and maximize reliability of the analyses in PGT as specified in the most recent ESHRE recommendations. Most, but not all of the Nordic IVF laboratories are compliant in this respect. As noninvasive PGT by analyzing used culture media and/or blastocoel fluid is being introduced internationally, the importance of high laboratory standard becomes even more important and avoiding contamination of the samples by foreign DNA even more critical.[27, 28] Two clinics did not during the time period of the study perform biopsy in a special area dedicated for PGT, which is hardly in accordance with international guideline and represents a risk of contamination. One of the additional challenges for noninvasive PGT is avoiding potential maternal contamination in the culture medium which may be difficult to achieve despite all efforts to ensure a contamination free laboratory environment.[29]

The interest in PGT is increasing internationally. It is expected that non-invasive testing will increase the implementation of this technique, making the embryological work easier and potentially increasing accuracy of the methods,[27] although more research is warranted prior to clinical implementation. Additionally, the introduction of preconception carrier screening, where couples are proactively screened for recessive disorders and other conditions prior to even attempting pregnancy is becoming a realistic option.[30] This will likely increase the use of PGT in coming years and will place new and different demands on the clinics and genetic laboratories although many issues regarding this concept need to be addressed before large scale implementation.[31] Monitoring of the activities and continuous quality improvement are the key to offer patients high standards of care.

CONCLUSION

The present survey shows that PGT is organised in relatively few clinics in the Nordic countries. All of the PGT programs have access to a genetic counsellor. Majority of the clinics use local laboratories

for analysis of the biopsies. Laboratory procedures are largely in accordance with international guidelines. The platform for analysis of chromosomal aberrations is MPS as reported by six centers. The degree of mosaicism allowed for a transferable embryo varied from 0 to 50%. PCR is still used for PGT-M in half of the centers. The outcome after PGT in terms of clinical pregnancy per transfer is good when compared with international reports.

References

1. Ingerslev HJ, Hindkjaer J. Preimplantation genetic diagnosis with HLA matching - a way to save a child. *Acta Obstet Gynecol Scand*. 2012;91:765-768.

2. Sermon K, De Rijcke M, Lissens W, et al. Preimplantation genetic diagnosis for Huntington's disease with exclusion testing. *Eur J Hum Genet*. 2002;10:591-598.

3. Franasiak JM, Forman EJ, Hong KH, et al. The nature of aneuploidy with increasing age of the female partner: A review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril.* 2014;101: 656-663.

4. Rubio C, Bellver J, Rodrigo L, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. *Fertil Steril*. 2017;107:1122-1129.

5. Scott RT Jr1, Ferry K, Su J, Tao X, Scott K, Treff NR. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. *Fertil Steril*. 2012;97:870-875.

6. Patrizio P, Shoham G, Shoham Z, Leong M, Barad DH, Gleicher N. Worldwide live births following the transfer of chromosomally "Abnormal" embryos after PGT/A: results of a worldwide web-based survey. *J Assist Reprod Genet*. 2019;36:1599-1607.

7. Gleicher N, Orvieto R. Is the hypothesis of preimplantation genetic screening (PGS) still supportable? A review. *J Ovarian Res.* 2017;10: 21.

8. Penzias A, Bendikson K, Butts S, et al. The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. *Fertil Steril.* 2018;109:429–436.

9. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature*. 1990;344:768-770.

10. Rienzi L, Gracia C, Maggiulli R, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. *Hum Reprod Update*. 2017;23:139-155.

11. De Rycke M, Goossens V, Kokkali G, Meijer-Hoogeveen M, Coonen E, Moutou C. ESHRE PGD Consortium data collection XIV-XV: Cycles from January 2011 to December 2012 with pregnancy follow-up to October 2013. *Hum Reprod* .2017;32:1974–1994.

12. Stern, H.J. Preimplantation genetic diagnosis: prenatal testing for embryos finally achieving its potential. *J. Clin. Med*. 2014;3:280–309.

13. Abacan M, Alsubaie L, Barlow-Stewart K, et al. The Global State of the Genetic Counselling Profession. *Eur J Hum Genet*. 2019;27:183-197.

14. Harton G, Braude P, Lashwood A, Schmutzler A, Wilton L, Harper JC. ESHRE PGD Consortium-best practice guidelines for organization of a PGD center for preimplantation genetic diagnosis/screening (PGD/PGS). *Hum Reprod.* 2011a;26:14–24.

15. Harton G, Harper JC, Coonen E, Pehlivan T, Vesela K, Wilton L. ESHRE PGD Consortium best practice guidelines for FISH-based preimplantation genetic diagnosis (PGD). *Hum Reprod.* 2011b;26:25–32.

16. Harton G, DeRycke M, Fiorentino F, et al. ESHRE PGD Consortium best practice guidelines for DNA amplification-based preimplantation genetic diagnosis (PGD). *Hum Reprod.* 2011c;26:33–40.

17. Harton G, Magli C, Lundin K, Montag M, Lemmon J, Harper JC. ESHRE PGD Consortium/Embryology special interest group-best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). *Hum Reprod.* 2011d;26:41–46.

18. Preimplantation Genetic Diagnosis International Society (PGDIS). Guidelines for good practice in PGD: programme requirements and laboratory quality assurance. *Reprod Biomed Online*. 2008;16:134-147.

19. ESHRE PGT Consortium Steering Committee, et al. ESHRE PGT Consortium good practice recommendations for the organisation of preimplantation genetic testing. *HROpen, in press* OR Version for stakeholder review. www.eshre.eu/guidelines (last accessed July 2019).

20. ESHRE PGT Consortium and SIG-Embryology Biopsy working group, et al. ESHRE PGT Consortium and SIG-Embryology good practice recommendations for polar body and embryo biopsy

for preimplantation genetic testing. *HROpen, in press* OR Version for stakeholder review. www.eshre.eu/guidelines (last accessed July 2019).

21. ESHRE PGT-SR/PGT-A working group, et al. ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations. *HROpen, in press* OR Version for stakeholder review. www.eshre.eu/guidelines (last accessed July 2019).

22. ESHRE PGT-M working group, et al. ESHRE PGT Consortium good practice recommendations for the detection of monogenic disorders. *HROpen, in press* OR Version for stakeholder review. www.eshre.eu/guidelines (last accessed July 2019).

23. Ginoza MEC, Isasi R. Regulating Preimplantation Genetic Testing across the World: A Comparison of International Policy and Ethical Perspectives. *Cold Spring Harb Perspect Med*. 2019 Sep 10. pii: a036681. doi: 10.1101/cshperspect.a036681. [Epub ahead of print]

24. Munné S, Kaplan B, Frattarelli JL, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. *Fertil Steril*. 2019;112:1071-1079.e7.

25. Munné S, Alikani M, Ribustello L, Colls P, Martínez-Ortiz PA, McCulloh DH; Referring Physician Group. Euploidy rates in donor egg cycles significantly differ between fertility centers. *Hum Reprod*. 2017;32:743-749.

26. Cram DS, Leigh D, Handyside A, et al. PGDIS Position Statement on the Transfer of Mosaic Embryos 2019. *Reprod Biomed Online*. 2019;39 Suppl 1:e1-e4.

27. Huang L, Bogale B, Tang Y, et al. Noninvasive preimplantation genetic testing for aneuploidy in spent medium may be more reliable than trophectoderm biopsy. *Proc Natl Acad Sci U S A*. 2019;116:14105-14112.

28. Kuznyetsov V, Madjunkova S, Antes R. et al. Evaluation of a novel non-invasive preimplantation genetic screening approach. *PLoS One*. 2018;13:e0197262.

29. Capalbo A, Romanelli V, Patassini C, et al. Diagnostic efficacy of blastocoel fluid and spent media as sources of DNA for preimplantation genetic testing in standard clinical conditions. *Fertil Steril*. 2018;110:870-879.

30. Simpson JL, Rechitsky S, Kuliev A. Before the beginning: the genetic risk of a couple aiming to conceive. *Fertil Steril*. 2019;112:622-630.

31. Vaz-de-Macedo C, Harper J. A closer look at expanded carrier screening from a PGD perspective. *Hum Reprod*. 2017;32:1951-1956.

Figures and Table

Figure 1. Size of the reporting clinics in terms of the total number of oocyte retrievals (OPU) and frozen/thawed embryo transfer (FET) cycles per year, including preimplantation genetic testing. Y-axis indicates the number of cycles.

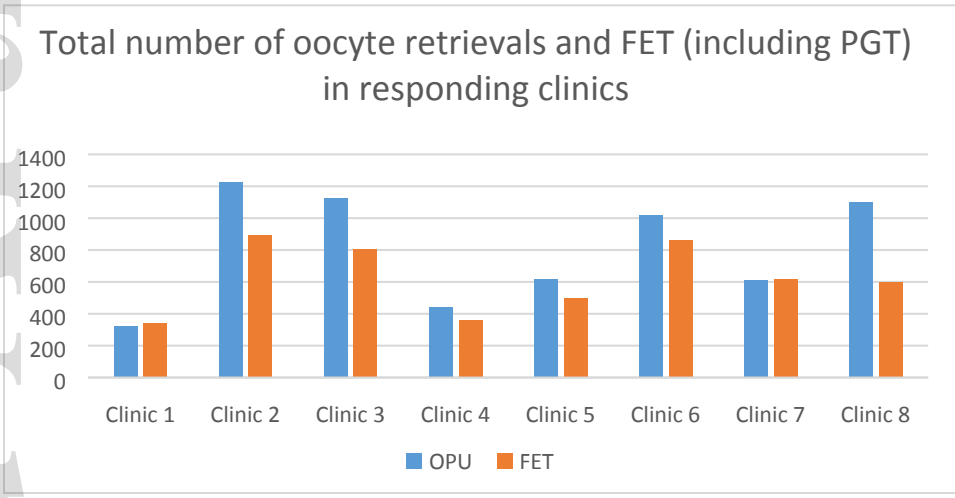

Figure 2. Cycle volume in terms of oocyte retrievals for the various preimplantation genetic testing (PGT) techniques. Y-axis indicates the number of oocyte retrievals. PGT-A, PGT for aneuploidies; PGT-M, PGT for monogenic disorders; PGT-SR, PGT for structural rearrangements.

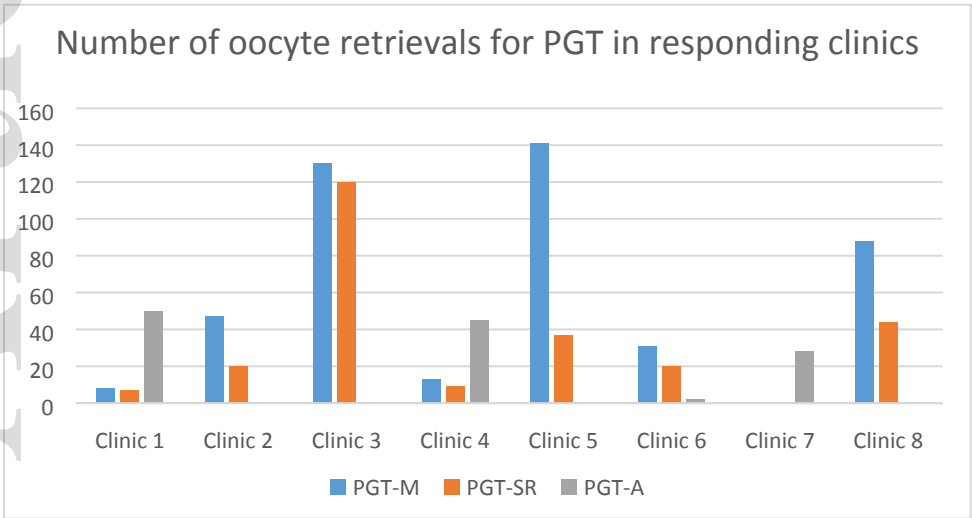

Figure 3. Chromosomal analysis and proportion of embryo euploidy from the four clinics reporting stand-alone preimplantation genetic testing for aneuploidies (PGT-A).

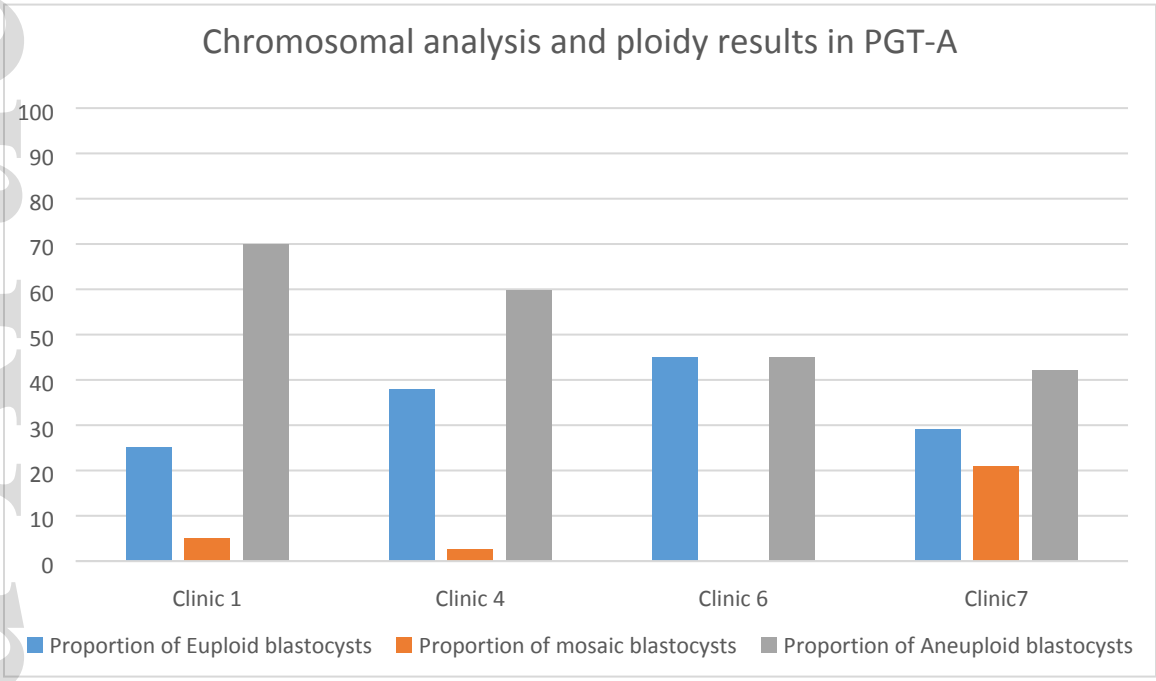

Table 1. Details on methods and status of reporting clinics.

	Nationality Organization	**Counselling and genetic analysis**	**Waiting list for PGT-M/SR**	**Day of biopsy, minimum embryos score for biopsy, ZP breach**	**DNA decon-tamination**	**ISO certification/ accreditation**	**Genetic analysis platform**
Clinic 1	Finland, Private	Own genetic counsellor. Private vendor for analysis	<3 months	Biopsy days 5-7, minimum grade 3. ZP breached at the day of blastocyst biopsy	70% EtOH and UV Light	IVF-clinic/lab	MPS/Karyomapping/ SNP array
Clinic 2	Sweden, Public	University clinic based counselling. University genetics laboratory	<3 months	Biopsy days 3, 5, 6. Grade 4BB min. or 6 blastomeres day3. ZP breached at the day of blastocyst biopsy	70% EtOH and UV light	Both IVF-clinic/lab and genetics dept.	PCR/FISH
Clinic 3	Sweden, Public	University clinic based counselling. University genetics laboratory	<12 months	Biopsy days 3, 5, 6. Grade 3BB min. or 6 blastomeres day3. ZP breached at the day of blastocyst biopsy	70% EtOH and detergents	Both IVF-clinic/lab and genetics dept	MPS/PCR/FISH/ Karoyomapping
Clinic 4	Denmark, Private	Own genetic counsellor. Private vendor for analysis	<3 months	Biopsy days 5, 6. Grade 4CC. ZP breach day 2 and 3	Quaternary compounds and UV light	No	MPS/Karyomapping/ SNP array
Clinic 5	Denmark, Public	University clinic based counselling. University genetics laboratory	<3 months	Biopsy days 5, 6. Grade 3BB min. ZP breach at the day of blastocyst biopsy	Detergents and UV light	Both IVF-clinic/lab and genetics dept	MPS/PCR
Clinic 6	Finland, Public	University clinic based counselling. Private vendor for analysis	<6 months	Biopsy days 5, 6. Grade 3BC min. ZP breach at the day of blastocyst biopsy	70% EtOH Regular detergents	Genetics dept	MPS

Clinic 7	Denmark, Public	University clinic based counselling. University genetics laboratory	n.a.	Biopsy days 5, 6. Grade 3CB min. ZP breach at the day of blastocyst biopsy	UV light	Genetics dept.	MPS
Clinic 8	Denmark, Public	University clinic based counselling. University genetics laboratory	<12 months	Biopsy days 3, 5, 6. Grade 3CC min. or 6 blastomeres day3. ZP breached at the day of blastocyst biopsy	70% EtOH and UV light	Both IVF-clinic/lab and genetics dept.	MPS/PCR

PGT, preimplantation genetic testing; M; for monogenic disorders; SR, for structural rearrangements; ZP, zona pellucida; ISO; International Organization for Standardization; EtOH, ethanol; UV, ultraviolet; IVF, in vitro fertilization; MPS, Massive Parallel Sequencing; SNP, single nucleotide polymorphism; polymerase chain reaction; FISH, fluorescence in situ hybridization; Gardner blastocyst grading system. Expansion: 1 to 6; Inner Cell Mass (ICM): A, B or C; Trophectoderm (TE): A, B or C. A fully formed blastocyst is graded 3 and above, A has the highest number of cells in ICM and TE and C has the lowest number.

Table 2. Treatments offered and the number of frozen/thawed embryo replacements (FET), ongoing pregnancies (OP) and pregnancy rates (OPR) with 95% confidence intervals for the participating clinics in 2018.

	Treatments offered	FET PGT-M	OP for PGT-M	OPRs (95%CI) for PGT-M	FET PGT-SR	OP for PGT-SR	OPRs (95%CI) for PGT-SR	FET PGT-A	OP for PGT-A	OPRs (95%CI) for PGT-A
Clinic 1	PGT-M/SR/A	5	3	60% (22-88)	4	3	75% (28-95)	25	12	48% (30-67)
Clinic 2	PGT-M/SR	32	10	31% (18-49)	8	4	50% (21-79)	0		
Clinic 3	PGT-M/SR	*	*	*	163	57	35% (28-43)	0		
Clinic 4	PGT-M/SR/A	5	2	40% (12-78)	5	2	40% (12-78)	27	8	30% (16-49)
Clinic 5	PGT-M/SR	107	41	38% (30-48)	19	8	42% (23-64)	0		
Clinic 6	PGT-M/SR/A	20	8	40% (22-62)	18	8	44% (24-67)	4	0	0
Clinic 7	PGT-A	0			0			18	12	67% (43-84)
Clinic 8	PGT-M/SR	56	18	32% (21-45)	52	14	27% (17-40)	0		

In PGT-SR chromosomal status is taken into account when selecting embryos for transfer even if the clinic otherwise does not offer PGT-A.

*Clinic 3 presents combined data for PGT-M and PGT-SR. In clinic 8, PGT-SR was performed by Day3 biopsy and fluorescence in situ hybridization (FISH) without PGT-A until 31 August 2018 and by TE biopsy and Next Generation Sequencing (NGS) from 1 September and onwards.
PGT-M, preimplantation genetic testing for monogenic disorders; PGT-SR, preimplantation genetic testing for structural rearrangements, PGT-A, preimplantation genetic testing for aneuploidies.