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PREIMPLANTATION GENETIC DIAGNOSIS EVALUATION OF RESULTS AND EXPERIENCES

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PREIMPLANTATION GENETIC DIAGNOSIS – EVALUATION OF RESULTS AND EXPERIENCES

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To my parents who always

encouraged and supported me

ABSTRACT

Preimplantation genetic diagnosis (PGD) is an established alternative for couples at high risk of having an affected child, with the advantage that the genetic testing is performed at the embryo stage and the couple can thereby avoid a pregnancy termination of an affected foetus. The disadvantage is that an IVF treatment is required, which can be a stressful experience. The main indications for PGD are monogenic disorders and chromosome abnormalities and there is an increasing demand for PGD each year. The PGD process can be divided into three steps *1)* The IVF treatment, *2)* The biopsy and *3)* The genetic analysis. The aim with this thesis was to identify factors of importance for an optimal PGD and to learn more about patient's experience of PGD in order to improve the advisory procedure and care of these patients. Another aim was to gain more knowledge regarding the segregation of different reciprocal translocations and their influence on fertility.

Carriers of reciprocal translocations are usually healthy but have an increased risk of producing sperm or oocytes with an unbalanced chromosome content which gives them a high risk of repeated miscarriages, infertility and an increased risk to have an affected child. The unbalance arises during meiosis when the sperm and oocytes are formed and are present in every cell in the body in the offspring. However, some abnormalities arise after conception during the early embryo development resulting in mosaicism where some cells have the abnormality and some do not. This was the case in **Paper I** where germline mosaicism was demonstrated to be the cause of repeated pregnancies with the same unbalanced chromosome abnormality, although karyotypes from both parents initially were interpreted to be normal. Extended investigations with microsatellite markers and FISH analysis revealed the same abnormality in 4-6% of the mother's fibroblasts. The couple went through four PGD cycles and the abnormality was found in 35% of the embryos. The low level mosaicism in the fibroblasts gave no phenotypic symptoms but since the abnormality seemed to be present at a higher frequency in her gonads, there was a high" hidden" recurrence risk for affected offspring or repeated miscarriages.

In **Paper II** a linear regression analysis was performed of data from all 569 PGD cycles performed between 1996 and 2009. We found two factors of significant importance for the PGD outcome. Firstly, the age of the woman at stimulation start where women under 36 years were three times more likely to achieve a pregnancy $P = 0.003$ and odds ratio 3.1 [95% confidence interval (CI) 1.5-6.5]. Secondly, the number of biopsied cells from each embryo where PGD cycles with one cell removal were twice as likely to result in a pregnancy compared to those cycles were two cells had been removed $P = 0.0013$ and odds ratio 2.55 (95% CI 1.44 – 4.52). Accordingly, we have now introduced an age limit of 40 years at stimulation start for the woman and changed policy to one cell biopsy for almost all indications since 2009.

Paper III was based on statistical analyses of data from a survey study concerning the experience of PGD between June 2005 and 2011. A questionnaire was sent to 222 couples and 146 answered, of which 20% had the experience from a pregnancy termination of an affected foetus, one third had the experience of previous traditional prenatal diagnosis and 35% had given birth to an affected child. The results showed that couples with monogenic disorders choose PGD because of an objection to pregnancy termination for psychological reasons while carriers of chromosome abnormalities opted for PGD because of previous miscarriages. We could confirm that there is an extensive stress associated with PGD and that

the couples seemed to have been less prepared for the psychological stress than for the physical stress.

It has previously been suggested that a sperm FISH analysis could predict the PGD outcome and that there is a linear correlation between the proportion of abnormal sperm and the proportion of abnormal embryos. In **Paper IV** sperm FISH analyses from ten male carriers of different reciprocal translocations was performed in connection with their first PGD and the result from sperm and embryos were compared. We found a difference with an increase of unbalanced embryos compared to sperm with no linear correlation. We could confirm that the number of balanced embryos available for transfer correlates to the pregnancy rate.
In conclusion, PGD is a valuable and preferred reproductive alternative for couples at high risk of having a child with a severe genetic disorder. It is a rapidly developing field worldwide and new techniques as well as new indications are continuously announced. It is of great importance that medical and ethical aspects are considered and up to date before the introduction of new methods and that new techniques are constantly evaluated regarding accuracy and safety, in order to optimise the PGD program.

LIST OF SCIENTIFIC PAPERS

The Thesis is based on the following articles;

I. Haapaniemi Kouru K, Malmgren H, White I, Blennow E.
Hidden mosaicism for a structural chromosome rearrangement: a rare explanation for recurrent miscarriages and affected offspring?
Fertil Steril, 2011; 95: 806-808.

II. Haapaniemi Kouru K, Malmgren H, Nordenskjöld M, Fridström M, Csemiczky G, Blennow E.
One-cell biopsy significantly improves the outcome of preimplantation genetic diagnosis (PGD) treatment: retrospective analysis of 569 PGD cycles at the Stockholm PGD centre.
Hum Reprod, 2012; 27: 2843-2849.

III. Haapaniemi Kouru K, Syk Lundberg E, Malmgren H, Ingvoldstad C.
Preimplantation genetic diagnosis in Sweden: patient's experience and attitudes.
Manuscript

IV. Haapaniemi Kouru K, Malmgren H, White I, Rodriguez Sanchez A, Syk Lundberg E.
Meiotic segregation analyses of reciprocal translocations in sperm and embryos: no support for predictive value regarding PGD outcome.
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

AMA	Advanced maternal age
AMEL	Amelogenin
CGH	Comparative genomic hybridisation
CI	Confidence interval
COH	Controlled ovarian hyperstimulation
der	Derivative
DNA	Deoxyribonucleic acid
ET	Embryo transfer
ESHRE	European Society of Human Reproduction and Embryology
FISH	Fluorescence in situ hybridisation
HADS	Hospital anxiety and depression scale
HLA	Human leucocyte antigen
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilisation
NGS	Next generation sequencing
OR	Oocyte retrieval
PCR	Polymerase chain reaction
PGD	Preimplantation genetic diagnosis
PGS	Preimplantation genetic screening
PND	Prenatal diagnosis
RCT	Randomised control study
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
SRY	Sex determining region Y

INTRODUCTION

PREIMPLANTATION GENETIC DIAGNOSIS

Preimplantation genetic diagnosis (PGD) is a method developed to perform genetic testing at the embryo stage for couples with a high risk of having an affected child. It is an alternative to traditional prenatal diagnosis (chorionic villus sampling or amniocentesis) and termination of an affected pregnancy can thereby be avoided. The disadvantage is that an *in vitro* fertilisation (IVF*)* treatment is required, which could be a stressful experience and with limited chances of achieving a pregnancy. PGD requires a close collaboration between a fertility clinic with special expertise in the embryo laboratory, where the IVF treatment and the embryo biopsy are performed, and a genetic laboratory where the genetic analyses are developed and performed.

Approximately 50,000 PGD cycles have been performed worldwide and more than 10,000 children have been born after PGD (Moutou et al., 2014). The method was first described in 1990 (Handyside et al., 1990). It has since then been further developed and is today used for the detection of a large number of inherited genetic conditions as well as for screening of chromosome abnormalities, so called preimplantation genetic screening (PGS) and sex determination for social reasons. PGS and PGD for social sexing are presently not allowed in Sweden.

There are two centres that perform PGD in Sweden, Sahlgrenska University Hospital in Gothenburg and the Stockholm PGD centre at Karolinska University Hospital. The first PGD cycles in Stockholm were carried out in 1996, in the start mainly for chromosome abnormalities. After a change in the Swedish law in 2006, PGD could also be offered for an increasing number of monogenic disorders. There is a continuous increasing demand for PGD and several analyses for new indications are developed each year. Today, 60% of the PGD cycles at the Stockholm PGD centre are performed for monogenic disorders (Figure 1).

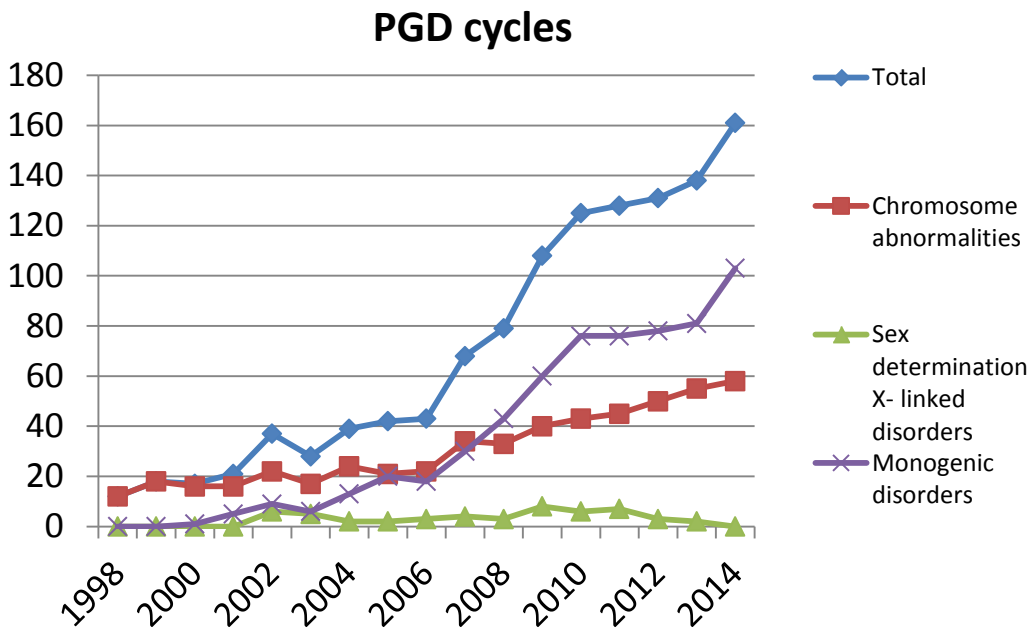

Figure 1. Indications and number of cycles over the years at the Stockholm PGD centre.

The PGD process

The PGD process can be divided into three steps:

1) The IVF treatment with hormone stimulation and the egg retrieval with fertilisation and cultivation in the laboratory.

2) The embryo biopsy on day 3 or day 5-6 after oocyte retrieval.

3) The genetic analysis.

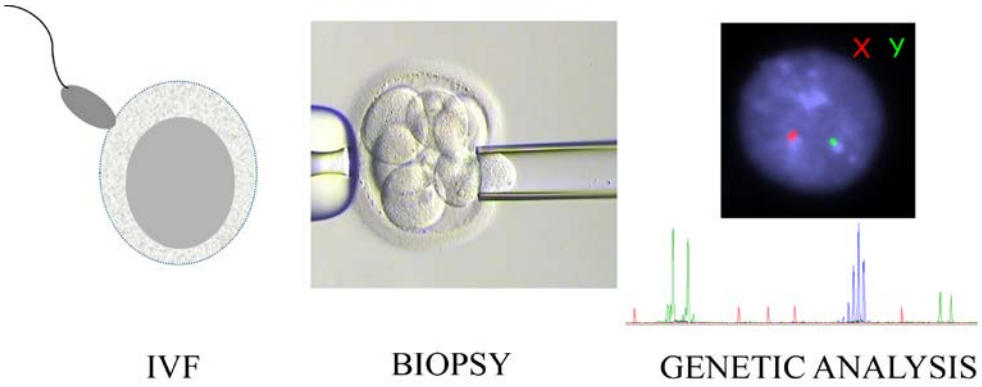

Figure 2. The PGD process.

There are several factors during the PGD process that affect the chances to achieve a pregnancy. Some are the same as for conventional IVF for fertility treatment, such as the number of collected and fertilised eggs and the age of the woman (Jansen, 2003), which both have a large impact on the success rate. Male factors of importance are sperm concentration and mobility. A PGD-related factor that significantly affects the pregnancy outcome is the number of biopsied cells from each embryo (Haapaniemi Kouru et al., 2012). In addition, the number of transferable or unaffected embryos suitable for embryo transfer during PGD will be of importance for success, and also depend on the genetic diagnosis (Ferraretti et al., 2004). Some genetic conditions will directly affect the oocyte reserve, e.g. Fragile X syndrome (Apessos et al., 2001).

Patients

Couples at high risk of having a child with a severe genetic disorder are in a difficult reproductive situation and may have a varying reproductive history. Some patients know from early years that they have a high risk through their mother's prenatal testing, their own personal experience of a disorder or through family history. Some couples have experienced repeated miscarriages, infertility problems, giving birth to an affected child or have been through a pregnancy termination of an affected foetus. They may also have lost an affected child in the neonatal period or later in childhood. It has been shown that the grief following a pregnancy termination in the second trimester due to foetal abnormality can be similar to that following a neonatal death (Kenyon et al., 1988). Couples with these experiences are often highly motivated to have prenatal testing. Other alternatives could be to opt for PGD or accept egg or sperm donation, but they also have the choice to take the chance without testing or to give up children. Moral and religious considerations could affect their decision as well as diverse regulations for funding of PGD in different countries, or different parts of a country. For example, there are counties in Sweden that do not offer PGD as an alternative. Some couples with a high risk of having affected children do not know that PGD exists and health care professionals that do not provide correct information may be seen as barriers in order to make a fully informed choice (Karatas et al., 2010).

Indications

Monogenic disorders

PGD can be used for the detection of monogenic disorders with autosomal recessive, autosomal dominant or X-linked inheritance as well as for chromosome abnormalities. Couples where both are carriers of an autosomal recessive disease-causing mutation are most often healthy, but have a 25% risk of giving birth to a child with the disorder. They often become aware of the high risk through the birth of an affected child. Autosomal dominant disorders are inherited from one parent who has clinical symptoms or will develop the disorder later in life. These couples often have a family history including affected family members and have a 50% risk that a child will be affected. X-linked recessive disorders mainly affect boys and are inherited from a carrier mother who is healthy, or very mildly affected. Fifty percent of her sons will be affected and 50% of the daughters will be carriers of the disorder.

We have presently performed PGD for over 100 different monogenic disorders. The ten most common indications are presented in Table 1.

Monogenic disorder	Couples
Myotonic dystrophy type 1	38
Huntington disease	31
Fragile X syndrome	19
Cystic fibrosis	11
Beta-thalassemia	10
Retinoblastoma	9
Neurofibromatosis type 1	9
Inherited breast cancer	8
Spinal muscular atrophy	5
Duchenne muscular dystrophy	5

Table 1. The 10 most common monogenic diseases as indication for PGD at Stockholm PGD centre, (2014).

PGD-HLA

PGD for a monogenic disorder may also be performed in combination with HLA (Human Leucocyte Antigen) typing, which enables the future child to be a donor of haematopoietic stem cells (Rechitsky et al., 2004). These stem cells are collected from the umbilical cord at birth and can be used for an affected sibling that is in need of stem cell transplantation in order to be cured from the inherited disorder.

Chromosome abnormalities

A chromosome abnormality is a change in the number or structure of the chromosomes. Numerical abnormalities are changes in the number of one or several chromosomes, e.g. missing in a pair (monosomy) or more than two chromosomes in a pair (trisomy). The most common at birth is trisomy 21, which will give rise to Down syndrome. Except for trisomy 13, 18 and 21 and numerical changes of the sex chromosomes, most numerical chromosomal abnormalities are not compatible with life. A structural chromosome abnormality is a change in the structure of a chromosome and there are several forms, e.g. deletions, duplications, translocations (reciprocal or Robertsonian), inversions and rings. Structural abnormalities can be inherited from a parent or occur for the first time in the individual (*de novo*). If the abnormality does not result in any loss or gain of chromosome material the rearrangement is balanced and will not affect the health of the individual. However, individuals with balanced abnormalities often have an increased risk of producing gametes (sperm or eggs) with an unbalanced chromosome content. This might cause reproductive problems such as infertility, repeated miscarriages or the birth of a child with intellectual disability, dysmorphic features and malformations.

Reciprocal translocations

Reciprocal translocations are the most common structural chromosome abnormalities with an incidence of 1/ 500-700 (Jacobs et al., 1992, Nielsen and Wohlert, 1991). A reciprocal translocation is an exchange of chromosomal material between two different chromosomes (Figure 3). The rearranged chromosome is called a derivative (der) chromosome and it is identified according to which centromere it possesses.

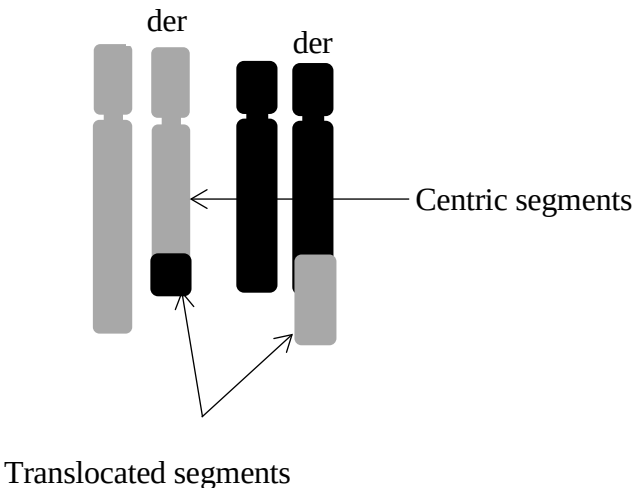

Figure 3. A balanced reciprocal translocation.

The chromosome unbalance arises during meiosis when the sperm and eggs are formed. During meiosis homologous chromosomes pair up and an exchange of genetic material between the two chromosomes takes place, so called crossing over or recombination. This contributes to the variation of genetic material in each gamete. In order to match homologous segments, a quadrivalent, including the four chromosomes involved in the translocation, is formed (Figure 4).

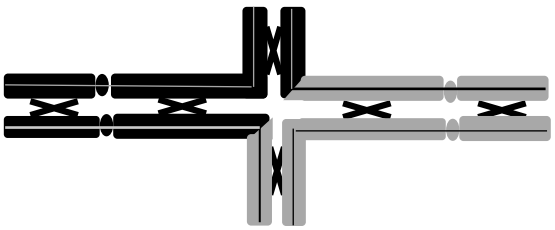

Figure 4. Quadrivalent in meiosis 1.

The chromosomes thereafter segregate in three principal ways; 2:2 segregation (alternate, adjacent-1 or adjacent-2), 3:1 segregation and 4:0 segregation. Gametes from alternate segregation are normal or balanced. All others will be unbalanced, usually very few that may proceed to the birth of a child. If crossing over occurs in the interstitial segments of the quadrivalent (between the centromere and the breakpoint), further unbalanced combinations

are generated. In theory, 36 different meiotic outcomes are possible for translocation carriers, of which only two are balanced (Figure 6, page 16).

The segregation pattern and number of balanced versus unbalanced gametes depend on the chromosomes involved in the translocation, as well as the breakpoints, and varies widely between 19 – 80% (Benet et al., 2005, Yakut et al., 2006). Results from sperm FISH analyses of 44 translocation carriers, reviewed by Benet et al. 2005, shows that alternate mode is the most common followed by adjacent-1. The 3:1 segregation is slightly more common than adjacent-2 and 4:0 is rarely observed. Several investigations have been performed in order to study the segregation pattern of different translocations. In male carriers, this can be done by fluorescence *in situ* hybridisation (FISH) analysis of sperm as well as studies of embryos after PGD. In female carriers, PGD with embryo analysis is the only possibility to study the segregation pattern. It has been suggested that there is a linear correlation between the proportion abnormal sperm and the proportion abnormal embryos in male carriers of reciprocal translocations (Escudero et al., 2003) and that a sperm FISH analysis could predict the PGD outcome. Men with levels of abnormal sperm over 60% are foreseen to have a decreased chance to achieve a pregnancy.

Male and female translocation carriers produce the same amount of balanced/unbalanced gametes (Lledo et al., 2010, Munne, 2005), but with a slightly different distribution of segregation modes. Male carriers produce more adjacent-2 segregation and female carriers produce more 3:1 segregation compared to males (Mackie Ogilvie and Scriven, 2002, Scriven et al., 2013).

Robersonian tanslocations

A Robertsonian translocation is a fusion of two acrocentric chromosomes (13, 14, 15, 21 or 22) with an incidence of 1/ 1000 (Blouin et al., 1994). Acrocentric chromosomes all have very small p-arms that contain genes coding for ribosomal RNA as well as repetitive genetic sequences. A Robertsonian translocation usually arise through the union and breaks of the short arms of two acrocentric chromosomes, most commonly chromosome 13 and 14, and results in one chromosome consisting of the two q-arms. The loss of p-arm material does not give rise to symptoms as there are multiple copies of genes coding for ribosomal RNA on the

other acrocentric chromosomes. As for reciprocal translocations, segregation problems may arise during meiosis. The chromosomes involved in the translocation will form a trivalent that theoretically may segregate in eight different ways, of which only two will result in a gamete with balanced chromosome content (one with normal chromosomes and one with a balanced translocation). There is an approximately seven fold excess of Robertsonian translocations among infertile couples and a 13-fold excess among oligospermic men (Van Assche et al., 1996).

Mosaicism

When chromosome abnormalities occur during meiosis the abnormality is present in every cell of the body in the offspring. However, some abnormalities arise after conception, during the early embryo development, resulting in mosaicism where some cells have the abnormality and some do not. Mosaicism can also occur after a mitotic correction in the zygote with an unbalanced chromosome content a so called “trisomy rescue” leading to one cell line with normal chromosome content and other cell lines with chromosome abnormalities. The effect of the mosaicism depends on the proportion of cells with a chromosome abnormality and what cell type that is affected. If the germ cells of the foetus are affected, so called germline mosaicism, the future individual may have a high risk of reproductive problems such as infertility, repeated miscarriages and affected offspring.

Misdiagnosis in PGD

Misdiagnosis in PGD may occur when a technical procedure has failed, is inaccurate or has been incorrectly interpreted and could be due to both technical and human errors, e.g. confusion of embryo and cell number, maternal or paternal contamination, allelic dropout, use of incorrect or inappropriate probes or primers, probe or primer failure and chromosome mosaicism. The misdiagnosis rate observed for PCR-based cycles has been estimated to 0.2% and for FISH-based cycles 0.06% (Moutou et al., 2014). In order to prevent misdiagnosis, General Guidelines has been developed by the ESHRE PGD consortium to promote that robust diagnostic methods are used in the laboratories, according to appropriate quality standards. Four different Guidelines have been published so far; 1) Guidelines for the organisation of a PGD centre (Harton et al., 2011a), 2) Guidelines for embryo biopsy (Harton

et al., 2011d), Guidelines for PCR based PGD (Harton et al., 2011b) and 4) Guidelines for FISH based PGD (Harton et al., 2011c).

Ethical considerations

When PGD was first introduced there were a lot of ethical discussions among the profession, decision-makers and the public. The fear of misuse of the technique was discussed (Wahlstrom et al., 2002). The method was developed in order to diagnose severe monogenic disorders and chromosome abnormalities, but could technically also be used to select for sex and qualities.

If an embryo is given less value than a foetus in the second trimester, PGD may be regarded as more ethical compared to prenatal testing (PND) with a possible pregnancy termination of an affected foetus. Others argue that it is unethical to create an overload of embryos in connection with the IVF treatment that later have to be discarded and that the selection per se is unethical. The PGD HLA method has raised further considerations. Is it acceptable to use a child, who cannot decide for itself, as a donor or that a child is born solely to be a donor for an affected sibling?

The PGD activity in Sweden is regulated through the law of Genetic Integrity and should only be used if the woman, the man or both are carriers of a genetic condition leading to a high risk of having an affected child. It is not allowed to use the method for selecting for qualities or sex for social reasons. PGD with HLA typing is only allowed after special permission from the National Board of Health and Welfare in each case.

AIMS

The overall aim with this thesis was to evaluate different factors of importance for the outcome during preimplantation genetic diagnosis (PGD) and evaluate the patient's experiences at Stockholm PGD centre in order to develop the programme and provide optimal care for the PGD patients. The specific aims were;

- To identify factors of importance for an optimal PGD outcome.

- To study the segregation of translocations in spermatozoas and embryos in order to gain more knowledge of the behavior of different translocations and the success of PGD-treatment for translocation carriers.

- To learn more about patients experience after PGD treatment in order to improve the advisory procedure and care of these patients.

MATERIALS AND METHODS

PATIENTS

All patients included in the thesis have experienced PGD at the Stockholm PGD centre. One third of the patients come from Norway and the rest from different parts of Sweden. The two main indications for PGD were: 1) Monogenic disorders (autosomal recessive, autosomal dominant or X-linked inheritance) and 2) Chromosome abnormalities.

IVF TREATMENT

IVF treatment is mainly used for couples with fertility problems but is also a prerequisite for PGD since the genetic analysis is made at the embryo stage. IVF is a process by which oocytes are retrieved from a woman's ovaries and fertilised with sperm from a man in a laboratory. The fertilised embryos are then cultivated in 37˚C in the laboratory and later transferred to the uterine cavity. In order to optimise the pregnancy results, the woman goes through controlled ovarian hyperstimulation (COH) to mature several oocytes at the same time. This enables a selection of an embryo with the best quality and highest chance to result in a live born child. During stimulation the development of the follicles in the ovaries is monitored and when there is at least three follicles ≥ 17 mm, maturation of the oocyte and luteinisation of the follicles is induced and oocyte retrieval (OR) is performed about 37 hours later. The oocytes are then fertilised in the laboratory, either by conventional IVF or in cases with male factor infertility by intracytoplasmic sperm injection (ICSI) where one sperm is injected into the cytoplasm of the oocyte. The fertilisation rate is controlled the day after OR. The blastomeres in the fertilised oocytes divide once a day and from the second day after OR, a quality assessment of the embryos can be made. The embryo transfer (ET) is made on day 2-5 after oocyte retrieval.

An IVF treatment is a necessary part of PGD in order to obtain a large number of embryos available for genetic analysis and transfer. The policy for fertilisation of the oocytes was that

IVF was performed in cases where FISH analysis was used and ICSI for all indications with PCR analysis and in cases of male factor infertility irrespective of genetic method.

BIOPSY

In order to perform genetic testing of the embryos, a biopsy has to be performed. This can be accomplished at different stages during the embryo development. Firstly, a polar body biopsy of the first and second polar body may be performed on the same day or day after OR. Secondly, a cleavage stage biopsy on the third day after OR, and thirdly, a trophectoderm biopsy may be done on day 5-6 after OR. For the PGD cycles included in this thesis, the strategy has been to perform cleavage stage biopsy. During the first years, acidified Tyrode's solution (pH 2.5) was used to drill a hole in the zona pellucida, followed by the removal of one or two cells (Inzunza J and . 1998) . Since March 2010, laser was used to drill the hole in the zona. Initially, the recommendation was a two-cell biopsy policy for almost all indications in order to obtain a robust genetic analysis. With two cells for analysis, each cell could serve as a control to the other. However, different studies have indicated that one-cell biopsy is less harmful to the developing embryo (Cohen et al., 2007, Pickering et al., 2003), and significantly improves the PGD outcome (Haapaniemi Kouru et al., 2012), which is the reason for our change to one-cell biopsy policy. At the Stockholm PGD centre, one-cell biopsy policy has been applied for almost all indications since 2009.

GENETIC ANALYSES

Fluorescent in situ hybridisation (FISH)

FISH is a molecular cytogenetic technique that is used to detect and locate the presence or absence of specific DNA sequences on metaphase chromosomes or interphase nuclei. A fluorescently labelled DNA probe is hybridised to the target cells that are fixed on a glass slide. The DNA strands of the probe and the target cells are separated by denaturation and the single stranded probe molecules can then hybridise to single stranded complementary molecules on the slide and be visualized in a fluorescence microscope. Metaphase-FISH makes it possible to characterise chromosome aberrations that are too complex or too small to

be detected by standard karyotyping. Interphase-FISH may be performed when metaphases are not available and has the advantage that many cells can be studied simultaneously. In this study, mainly interphase-FISH on blastomeres, sperm and fibroblasts was performed.

Blastomere FISH

Interphase-FISH on embryos from carriers of structural chromosome aberrations can be performed in order to separate balanced and unbalanced embryos. For Robertsonian translocations, usually a set of two or three DNA probes located on the chromosomes involved are used. For reciprocal translocations, a set of three or four DNA probes, on both sides of the breakpoints on the chromosomes involved, are needed in order to detect all theoretical variants of segregation. However it should be noted that, although derived from unbalanced segregation, adjacent-1 segregation in combination with crossing over in the interstitial segment is impossible to distinguish from alternate segregation and may therefore lead to an underestimation of adjacent-1 segregation (Armstrong and Hulten, 1998). The probe combination should be chosen with regard to the ability to reliably detect the translocation, especially important for segregation with possible viability in a future child. Different schemes that display the expected signal pattern simplify the interpretation, depending on the specific rearrangement and the chosen location of the probes (Figure 6). Before PGD, the probe efficiency and accuracy should be tested on peripheral blood lymphocyte metaphases and interphase nuclei from the couple. Interphase-FISH analysis may also be used for sex-determination for X-linked disorders, in order to do a selective transfer of female embryos.

Sperm FISH

Human sperm FISH was introduced to determine aneuploidy in the sperm from fertile and infertile men with altered semen samples (Calogero et al., 2001, Martin et al., 2003, Pang et al., 1999, Rives et al., 1999, Vegetti et al., 2000). It allows the simultaneous analysis of thousands of sperm, thereby increasing the statistical power. However, there are some technical problems that have to be considered. The highly compacted chromatin in the sperm head requires additional decondensation treatment to make the DNA sequences accessible to the FISH-probes. The expected normal number of signals in sperm is half of that seen in a blastomere nucleus (haploidy versus diploidy). However, overlapping signals may be more of a problem in sperm analysis.

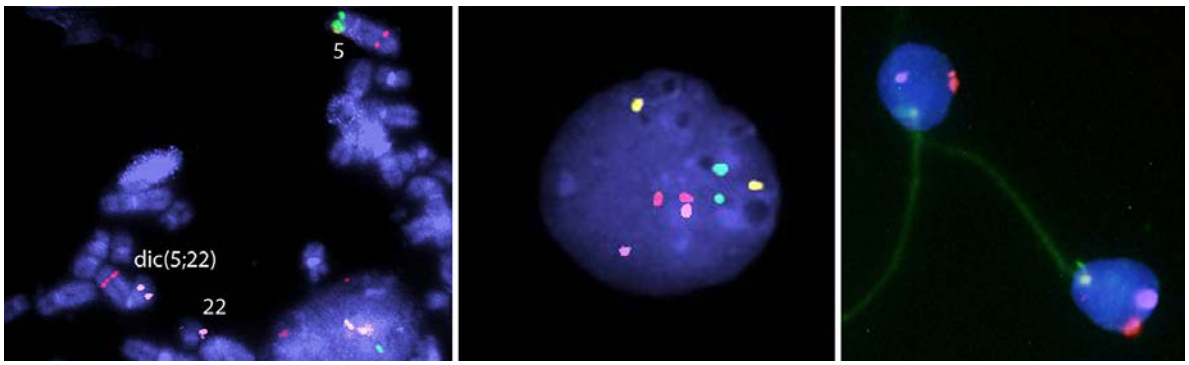

Figure 5. Metaphase-FISH (left), Interphase-FISH on a blastomere nucleous (middle) and sperm (right).

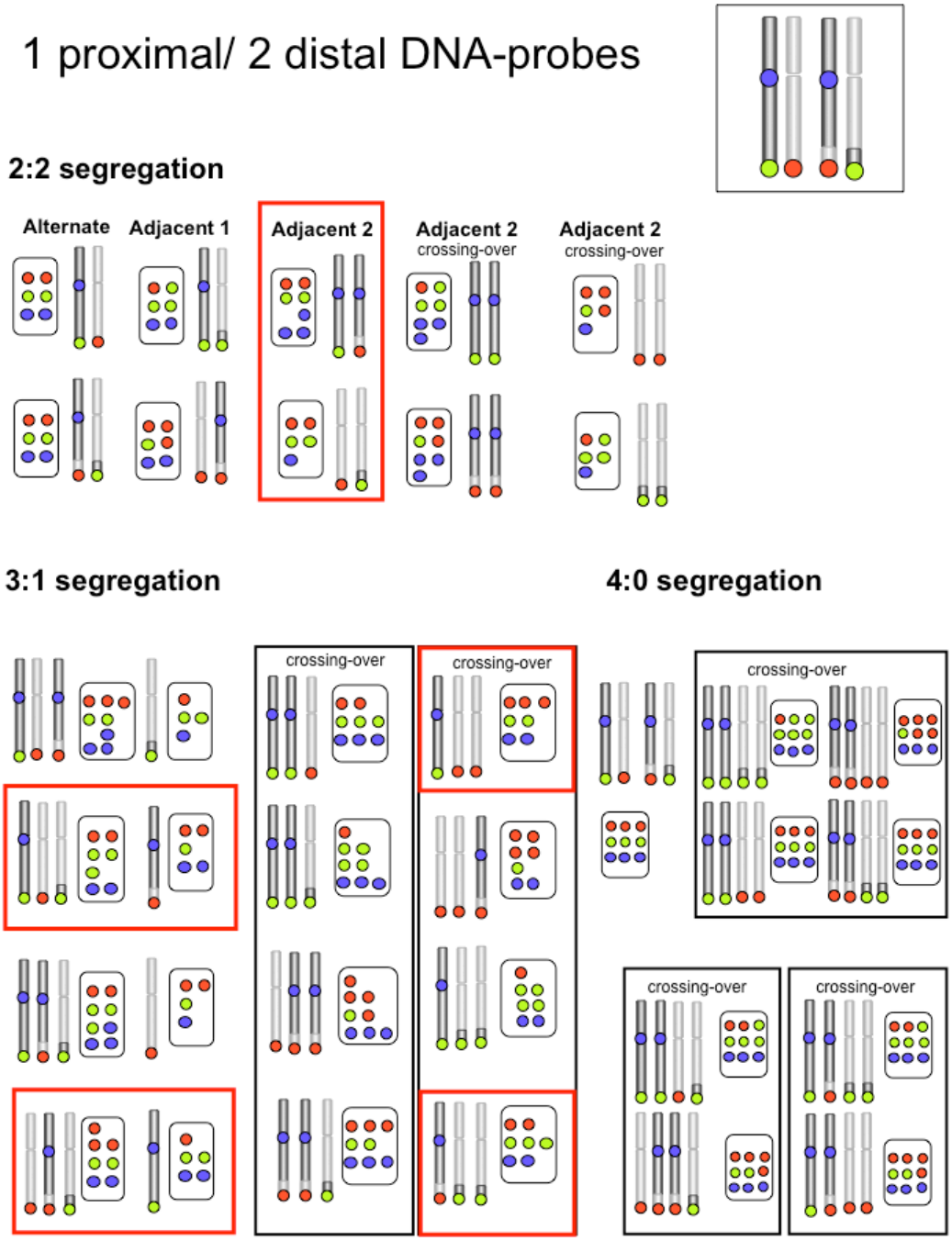

Figure 6. Probe-scheme showing the expected signal pattern in different segregations of a reciprocal translocation involving the long arms of two chromosomes. The haploid set is shown on the chromosomes to the left and the expected signals in the blastomere to the right. Segregation without "internal-check" in red boxes.

Polymerase chain reaction (PCR) analysis

For monogenic disorders, the strategy to distinguish affected and unaffected embryos is in most cases mutation detection in combination with linkage analysis using polymorphic microsatellite markers. Microsatellites are short tandem repeats of 2-5 base pairs of DNA and the normal variation in the number of repeats between different individuals may be used to follow the inheritance of different alleles within a family, i.e. linkage studies. These microsatellite markers are easily analysed by PCR amplification using primers specific for each marker, flanking the repeated sequence. The size of each generated PCR product can be determined by using fluorescently labelled primers, and fragment length analysis in a sequence analyser.

When establishing a PGD analysis for a family with a monogenic disorder, informative markers flanking the gene of interest, have to be identified in the family by investigating DNA from the couple as well as another relative with a known carrier status. At least two polymorphic microsatellite markers, intragenic or closely flanking the disease-gene has to be identified. If possible, mutation detection by PCR amplification of the target sequence, and subsequent cleavage with a restriction enzyme (RFLP) is included, and in most cases, three to six markers are used in the analysis. In addition, for X-linked diseases, markers for sex determination (*AMEL and SRY*) are included. In a second step, a multiplex PCR analysis is established and the accuracy evaluated on single cells.

QUESTIONNAIRE

A questionnaire study can be performed in order to retrieve structured information from a large number of individuals in a study-group. These data can often be used for generating statistics and for comparing subgroups regarding different issues, choices and results. In 2006, a pilot study was performed investigating couple's experience of PGD during the early years of PGD activity in Sweden from 1996 to May 2005 (Malmgren, H. 2014). A second questionnaire was sent out in May 2012 to patients that went through PGD at Karolinska University Hospital in Stockholm between June 2005 and December 2011, in total 222 couples. Both surveys included similar questions.

The questionnaires consisted of questions grouped into three parts. The first part included demographic information and information about previous reproductive history. The second part concerned the experience of PGD and the response alternatives were: 1) *easier than expected* 2) *as expected* 3) *more stressful than expected and* 4) *much more stressful than expected*. In the third part, information about the couple's choices regarding different reproductive alternatives after PGD closure was collected. The main issues where; What was the reason to opt for PGD? How did couples experience the procedure and which part was most stressful? Did factors like previous reproductive history or the experience of previous traditional prenatal diagnosis affect the experience of PGD? What reproductive options were considered after PGD closure? A validated self-assessment scale for detecting states of depression and anxiety was included in the questionnaire, the Hospital Anxiety and Depression Scale- HAD Scale (Zigmond and Snaith, 1983).

STATISTICS

Different statistical methods can be used to interpret data and use them to estimate associations. To identify factors of importance for the PGD outcome (*Paper II)* we included the following predictors in the analysis: indication, carrier status, woman's age at stimulation start, parity, number of oocytes retrieved at OR, type of fertilisation, number of oocytes fertilised and number of cells biopsied and analysed. The characteristics were defined using

absolute and relative frequencies for categorical variables (*Paper II and IV)*, and means and medians with measures of spread for continuous variables. Comparisons of continuous variables were made using Student *t*- test and the Mann-Whitney test. Comparisons of categorical data were made using X^2 and Fishers exact test when appropriate. Linear regression analyses were used to detect the significant factors for the outcome. Multivariate models were constructed to determine the potential confounding factors (*Paper II).*

Comparisons between unselected and selected spermatozoa were made using Wilcoxon matched pair test. The relative number of abnormal sperm versus abnormal embryos was analysed in a basic linear regression model (*Paper IV).* In the analysis regarding patient's experience (*Paper III),* Independent T-test was performed when comparing two subgroups of participants. When more than two subgroups were compared, the Anova test was used. Pearson correlation was used to see the correlation between parametrical variables. In all studies a *P*-value of ≤ 0.05 was considered significant

RESULTS AND DISCUSSION

FACTORS OF IMPORTANCE FOR PGD OUTCOME (PAPER II)

We performed an analysis of all PGD cycles at our centre between 1996 and 2009. During this period, 569 treatments for 256 couples were performed, thawing cycles excluded. The number of cycles per couple varied between one and five. The mean age of the women at stimulation start was 33.7 years (22-43). Embryo biopsy was possible in 83.7%, and embryo transfer was possible in 63.3% of all started cycles. The majority of the pregnancies were achieved during the first two cycles.

Logistic regression analysis of all data identified two factors of significant importance for the pregnancy outcome. It was the age of the woman at stimulation start and the number of biopsied cells from each embryo. Women under 36 years of age were three times more likely to achieve a pregnancy $P = 0.003$ and odds ratio 3.1 [95% confidence interval (CI) 1.5-6.5]. This confirms the results presented by other groups (Feyereisen et al., 2007, Verpoest et al., 2009) and we have therefore introduced an age limit of 40 years at stimulation start.

PGD cycles were one cell was removed from each embryo were twice as likely to result in a pregnancy compared to those cycles were two cells had been removed $P = 0.0013$ and odds ratio 2.55 (95% CI 1.44 – 4.52). The delivery rate per ET was 29.5% after biopsy of one cell and 14% after biopsy of two cells. Detailed information is presented in Table 2. We could thereby answer the previous raised question if the removal of two cells might have a negative effect on the pregnancy outcome (Cohen et al., 2007, Pickering et al., 2003). An explanation to the higher delivery rate in the one-cell biopsy group might be that these cycles were performed mainly during the later years, when the laboratory was more sophisticated and experienced. However, a comparison over time regarding the two groups showed that there were only four cycles with one-cell removal from 1996 to 2003. From 2004 to 2009 the results were the same as for the whole cohort comparing one- and two-cell removal. When comparing the two-cell biopsy group over the years, the delivery rate was 14% in both the early and the late years. This indicates that the general performance in the laboratory has not changed over the years.

Table 2. Outcome comparing one- and two-cell biopsy

Characteristics	One-cell biopsy	Two-cell biopsy
Mean age of woman[1]	33	33.7
Number OR[2]	117	359
Oocytes retrieved[3]	13.4	13.5
Oocytes fertilised[3]	8.4	8.3
Embryos biopsied[3]	5.9	5.6
Embryos analysed[3]	5.5	5.3
ET rate[4]	75.2 %	75.8 %
Delivery / stimulation	22.2 %	10.6 %
Delivery / ET[4]	29.5 %	14 %
Delivery/ ET in translocation group (Rec. and Rob.)	28.6% (56 ET)	14.7% (109 ET)
Delivery/ ET in autosomal dominant group	2 liveborn children (4 ET)	14.9% (74 ET)
Delivery/ ET in autosomal recessive group	No pregnancy (5 ET)	9.3% (43 ET)

1. At stimulation start, 2. Oocyte retrieval, 3. Mean number, 4. ET – embryo transfer

There has been a fear that the diagnostic efficiency (number of successfully diagnosed embryos) may be affected by analysing only one cell (Fiorentino et al., 2006, Goossens et al., 2008). In our material there were no differences in the success of the genetic analysis, 93% vs. 95% for the one-cell and the two-cell group, respectively, nor in the embryo transfer rate,

which was 75% versus 76%. In addition, to the best of our knowledge, there is no case of misdiagnosis in our material. Therefore, the advantages in delivery rate after one-cell biopsy seem to overweigh the possible disadvantages. However, one-cell biopsy sets a greater demand on the design and accuracy of the genetic test for each patient, as the possibility to use the two biopsied cells as controls of one another is lost. To compensate for this, four DNA-probes for interphase FISH analysis of reciprocal translocations may be used, or three DNA-probes with optimal localization, i.e. chromosome segregation likely to give rise to viable offspring should give unbalanced FISH-signal patterns with an "internal check". This means that failure of one signal or co-localization would still give an abnormal signal pattern (Scriven et al., 1998). The strategy to use linkage analysis with multiple markers for the PCR based analysis, and if possible in combination with mutation detection, also allows for a reliable test on one cell. In some situations, for certain couples where the diagnostic test is sub-optimal, the two-cell biopsy strategy may still be considered as the best choice. It is important to make an individual evaluation of each case regarding the calculated risk for possible misdiagnosis and chose the optimal strategy.

We did not find a significant correlation between the number of collected oocytes and the delivery rate in our logistic model, which is in contrast to previous publications (Grace et al., 2006, Verpoest et al., 2009). Nor did parity, carrier status or indication for PGD affect the outcome. There was a surprisingly low delivery rate in the autosomal recessive group, even if they had ET to a greater extent than the autosomal dominant group. The results may be explained by the fact that in the majority of cycles two cells were biopsied but also by the fact that the mean age of the woman at stimulation start was higher in this group than in the other groups; 35.5 years (27-40) compared to the autosomal dominant group were the mean age of the woman was 33.2 years (24-41) and the reciprocal translocation group 33.6 years (22-42). This in turn could be a consequence of the fact that most couples are not aware that they are carriers of an autosomal recessive disorder until they give birth to an affected child. This often means that they lose valuable time during their most fertile period.

As previously reported by Fridström et al., 2001, when comparing Robertsonian vs. reciprocal translocations, we experienced that couples performing PGD due to a Robertsonian translocation were more likely to have an embryo transfer. However, if a woman performing PGD due to a reciprocal translocation had an embryo transfer, the chance to establish a

pregnancy is even slightly higher than for a woman performing PGD due to a Robertsonian translocation. The couples with Robertsonian translocations were more likely to conceive if it was the woman who was the carrier of the translocation, which is in opposite to the couples with reciprocal translocations where the chance to conceive was higher if the man was the carrier of the translocation.

MOSAICISM (PAPER I)

We investigated a family with recurrent offspring with the same unbalanced structural chromosome aberration, although the parental karyotypes initially were interpreted as normal. Extended investigations using microsatellite markers showed a maternal origin of the aberration, and further metaphase and interphase FISH analyses on fibroblasts and lymphocytes from the mother were performed. This revealed a low level mosaicism in 4-6% of the fibroblasts. The couple went through 4 PGD cycles and 17 embryos were analysed by interphase FISH. Embryo analysis showed an unbalanced segregation in 6 out of 17 embryos (35%), of which one with a signal pattern corresponding to the previous abnormal pregnancies. The other five showed variable unbalanced segregation patterns. The only one found in more than one cell was loss of chromosome 22 (monosomy 22). Embryo transfer with one or two balanced embryos was performed during each cycle, but no pregnancy was established. Standard karyotype investigation includes the analysis of up to 10 or 11 metaphases. In addition, in most cases only a few of these metaphases will be fully karyotyped and the rest counted. With this approach, the ability to detect mosaicism includes 30% or higher grade mosaicism (Hook, 1977). If detection of low-level mosaicism for a structural aberration (<10% of the cells) is to be included, at least 100 metaphases have to be karyotyped. The cost and effort to perform such an analysis is in most cases too high compared to the chance to find an abnormality. Another problem is that there might be a variable frequency of the abnormal cell line in different tissues (Sciorra LJ, 1992). Unless the abnormality gives rise to fertility problems, abnormal children or an abnormal phenotype in the carrier, the aberrant cell line will in most cases remain undetected throughout life. However, it is important to have the possibility in mind following the detection of more than one pregnancy outcome with the same chromosome abnormality. In these cases greater efforts should be made to establish the carrier status and the level of mosaicism, in order to give a proper recurrence risk to the couple. In some cases, the option to perform PGD should

be considered as this may shed more light on the presence of germ line mosaicism and the segregation pattern for the specific abnormality.

SEGREGATION OF RECIPROCAL TRANSLOCATIONS (PAPER IV)

We analysed 17,500 sperm from 10 different translocation carriers and 160 embryos derived from in total 25 PGD cycles (1-4 per couple). Both unselected and selected sperm were analysed and no significant difference in segregation pattern was found. The most common segregation mode in the whole sperm count was alternate (51.5%) followed by adjacent-1 (18%), adjacent-2 (13%), 3:1 (13%) and 4:0 (0.5%). Four percent showed a segregation pattern that was not compatible with any of these, called “Other”. The number of embryos per couple varied from 3 to 29 and the segregation modes in the embryos were as follows; alternate 21%, adjacent-1 23%, adjacent-2 13%, 3:1 20%, 4:0 1% and other 22%. The distribution of the segregation modes in both sperm and embryos are presented in Figure 7 (Paper IV).

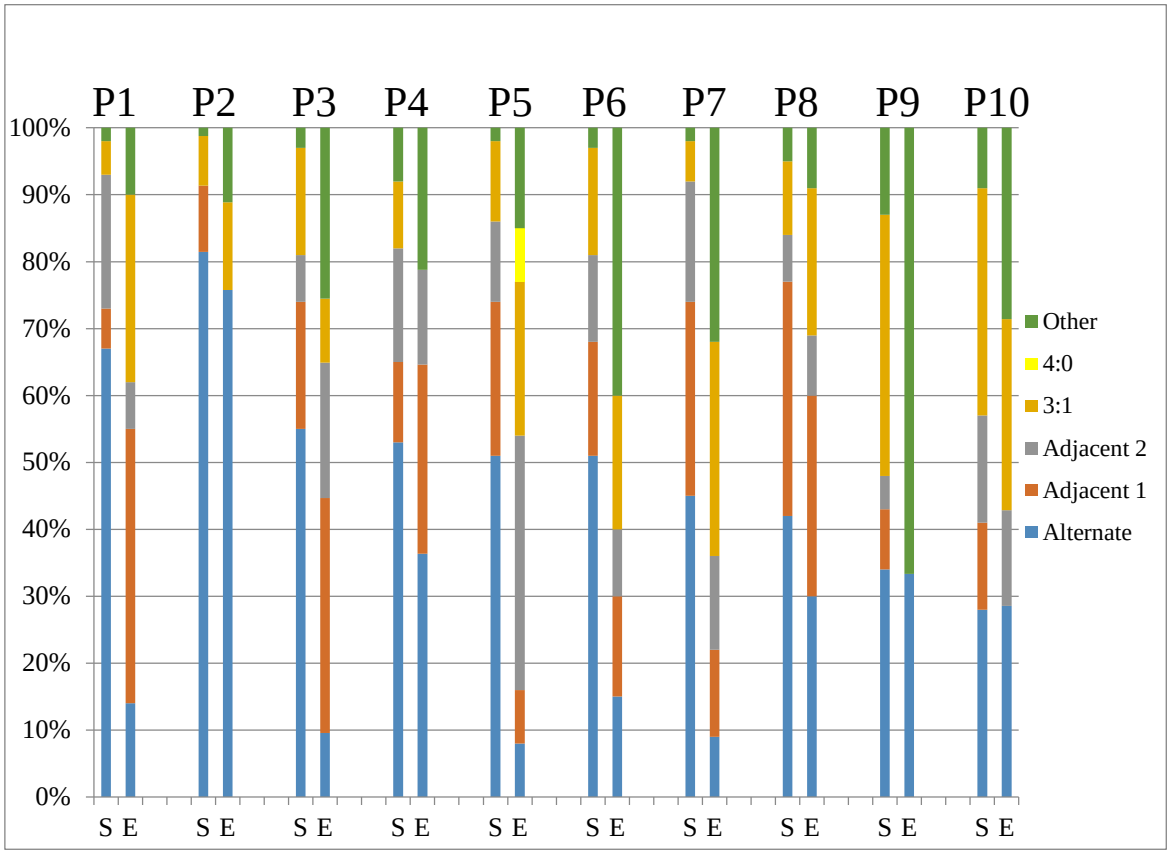

Figure 7. Comparison of the segregation in sperm (S) and embryos (E) for each patient.

A high level of unbalanced segregation was found in the embryos. The difference in the number of analysed cells may have affected the outcome but also the biological difference between sperm and blastomeres, where the sperm have a compact haploid nucleus compared to the bigger diploid blastomere nucleus, which could cause different hybridization and interpretation errors (Paper IV). Another explanation to this difference might be an on-going cell division in the blastomere where the chromosomes have replicated but not yet segregated. It has also been shown that there is a high frequency of mitotic errors in cleavage stage embryos leading to mosaicism (Iwarsson et al., 2000) which could also contribute to the difference in segregation pattern in analysed sperm and embryos.

PREDICTION OF EMBRYO AND PREGNANCY OUTCOME (PAPER IV)

It has been stated that the number of balanced embryos generally correlates with the chances to achieve a pregnancy (Munne et al., 2000) and the first PGD cycle has been proposed to have a predictive value, where the rate of balanced embryos during the first cycle is considered to be similar (±20%) to that in the following cycles (Munne, 2005). This was the case for only three out of ten patients in our cohort (Paper IV). In addition, in these three patients the number of balanced embryos per cycle was low (0-4, mean 1.24) and calculated percentages should be interpreted with caution. Three out of four patients that achieved a pregnancy had balanced embryos over the mean (4, 5 and 7). These findings support the conclusion that the pregnancy rate is correlated to the number of balanced embryos available (Munne et al., 2000). We found no strong correlation between the proportion of abnormal sperm and abnormal embryos when a linear regression analyses was performed.

PATIENT'S EXPERIENCE OF PGD (PAPER III)

The survey study among PGD patients had a response rate of 66%. The indication for PGD was a monogenic disorder in 54.8% and a chromosome abnormality in 45.2%. Twenty percent of the couples had experienced a previous pregnancy termination of an affected foetus, 35% had given birth to an affected child and 45% had experience of miscarriages.

It has been shown that PGD is mostly chosen for emotional reasons, since the couple cannot tolerate the emotional stress of repeated pregnancy termination (Franklin, S. and Roberts, C. 2006). The emotional stress may be illustrated with a citation from one of the patients "How can you kill a child when you have already lost one?" In our cohort there was a significant difference in the reason to choose PGD when the couples were divided in subgroups according to indication. Carriers of monogenic disorders claimed objection to pregnancy termination as their main reason ($p < 0.001$), while carriers of chromosome abnormalities said that the experience of previous miscarriages was their essential motive to choose PGD ($p < 0.001$), followed by the need of IVF. This is perhaps not surprising since carriers of chromosome abnormalities in general have a high risk of miscarriages and infertility. It has also been shown in previous studies that PGD decreases the miscarriage rate for these couples (Munne et al., 2006, Otani et al., 2006).

Previous studies from different parts of the world indicate that couples who have experienced a pregnancy termination of an affected foetus are more willing to opt for PGD (Chamayou et al., 1998, Palomba et al., 1994, Pergament, 1991, van Rij et al., 2011). However, the results from our study does not indicate that patients with this experience (one out of five patients in this cohort) are more likely to choose objection to pregnancy termination as their main reason to prefer PGD and couples that wanted to avoid a pregnancy termination did not always have the actual personal experience.

Figure 8. The experienced stress during PGD as compared to expectations

We could confirm that there is an extensive stress associated with PGD (Alsulaiman et al., 2010, Karatas et al., 2011, Lavery et al., 2002). The couples seemed to have been better prepared for the physical stress in connection with PGD than for the psychological stress (Figure 8). The information that they received prior to PGD most likely affected their expectations, which demonstrates the importance of accurate information before the procedure. A comment from one patient "It is important to be prepared that the PGD might not result in a child" furthermore underlines the importance of proper information. The most physically stressful event was the oocyte collection, while waiting for the pregnancy test was the most psychologically stressful part. In addition, some couples expressed that the most stressful moments could vary from one PGD cycle to another. Those who had the experience of both PGD and PND (32%), considered PND with a possible pregnancy termination as more psychologically stressful.

In this study there was no correlation between the experienced stress and previous reproductive history. This is in contrast to a qualitative interview study from Australia where they found that memories from previous reproductive trauma like death of an affected child, repeated termination of affected pregnancies or repeated miscarriages were activated during the PGD- procedure and increased the experienced stress (Karatas et al., 2010). This difference may be explained by the study design where surveys often are used to assess thoughts, opinions and feelings and can describe the attitudes of a population, including

changes over time. Qualitative interview studies on the other hand give a more In-depth understanding of the behaviour and decision making procedure in a smaller population.

When the couples had closed for further PGD cycles, the majority of couples with a monogenic disorder had chosen natural conception with or without PND as their reproductive alternative. However, couples with chromosome abnormalities chose adoption or donation to a higher extent. Since couples with chromosome abnormalities have a high risk for infertility and repeated miscarriages, which cannot be avoided with traditional PND, adoption or donation could be their best chance to have a child. We could also notice a change over time regarding the choice of reproductive alternative after PGD closure. In the early years, gamete donation was less attractive compared to adoption (Malmgren, H., 2014), while the opposite was seen later. There may be different reasons to this shift in preferences, e.g. a change in information provided by healthcare personal, the fact that oocyte donation was introduced in Sweden 2003 and an established alternative some years later, as well as changed conditions for adoption over the years.

The fact that 94% of all couples would recommend PGD to other couples in the same situation confirms that PGD is a preferred reproductive alternative for couples at high risk of having a child with a severe genetic disorder despite the experienced stress. It is important that accurate information regarding reproductive options is given to couples with a high risk of having an affected child, so that well informed and independent choices can be made.

CONCLUDING REMARKS – FUTURE PERSPECTIVES

PGD is a valuable reproductive alternative for couples at high risk of having an affected child. These couples are in a difficult situation and often have a complicated reproductive history with the birth and sometimes death of an affected child, termination of an affected pregnancy, repeated miscarriages or infertility. Each couple has its own experience and reason to opt for PGD. Since the regulations for reproductive alternatives vary in different counties in Sweden it is however not an actual alternative for all couples. An obvious suggestion is that the regulations should be equal regardless if you live in the north, south, east or west of Sweden.

We could confirm that there is an extensive stress associated with PGD and that the couples seemed to have been more prepared for the physical stress than for the psychological stress. This demonstrates the importance of accurate information prior to the procedure and that the multidisciplinary PGD-team also includes a social worker resource. The fact that 94% of all couples would recommend PGD to other couples in the same situation, in spite of the extensive experienced stress, supports that PGD is a preferred reproductive alternative.

The PGD procedure has undergone several progresses over the years and new techniques such as laser drilling, one-cell biopsy with optimised genetic tests and trophectoderm biopsy with array-CGH analysis have recently been introduced. It is a rapidly developing field worldwide and new techniques as well as new indications are continuously announced. It is of great importance that medical and ethical aspects are considered and up to date before the introduction of new methods, and that new techniques are constantly evaluated regarding accuracy and safety in order to optimize the PGD program (Harper et al., 2014). We recently introduced trophectoderm biopsy and array-CGH analysis in the clinical setting for translocation carriers. The advantage with trophectoderm biopsy is the increased amount of cells (5-10) for analysis, making the genetic test more robust. In addition the biopsy at this point (day 5 or 6) is also considered less harmful to the embryo. Only embryos that reach the blastocyst stage have a high potential of resulting in a pregnancy, and only these embryos will be biopsied and analysed. The disadvantage with trophectoderm biopsy is the short time frame for the genetic analysis. This problem has been overcome by an improved freezing

program using vitrification of blastocysts for later transfer of unaffected embryos. Trophectoderm biopsy will most likely also be used for other indications in the near future. Next generation sequencing (NGS) is a rapid high troughput parallel sequencing method that provides information of the whole genome and might in the future be applied in PGD. The method enables information of both mutations causing monogenic disorders, karyomapping (linkage studies) as well as structural chromosome aberrations and aneuploidy at the same time.

A disadvantage associated with PGD is the limited chance of achieving a pregnancy. This limitation is mainly due to the live birth rates after IVF treatment and research is therefore focusing on the selection of the embryo with the highest chance to result in a live born child. Preimplantation genetic screening (PGS) is a method that is used to screen for chromosome abnormalities, and may theoretically improve the pregnancy rates since it enables only euploid embryos for transfer. In other countries the technique is used for indications such as advanced maternal age (AMA), repeated IVF failure or recurrent miscarriages. However, previous studies with PGS using FISH analysis from cleavage stage embryos showed a decrease in pregnancy rates (Hardarson et al., 2008, Mastenbroek et al., 2007). Contributing factors to these disappointing results may be that only a limited number of chromosomes could be analysed with FISH and also that the cleavage stage embryos have been found to be highly mosaic (Iwarsson et al., 2000). The biopsied cell might therefore be euploid while the majority of the remaining blastomeres in the embryo are aneuploidy. Published RCT studies with trophectoderm biopsy and array-CGH analysis have shown positive results, but results from larger studies are needed to evaluate the utility and advantages of the method. PGS is presently not allowed in Sweden but if future PGS studies can provide evidence for a significantly improved pregnancy outcome, the law may be revised. Furthermore, if further research could identify genes that are crucial for implantation and early embryo development, PGS in conjunction with NGS might be used to select the embryo with the highest chances to implant and end up in a live born child.

POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Preimplantatorisk genetisk diagnostik (PGD) är ett alternativ till traditionell fosterdiagnostik med moderkaksprov eller fostervattenprov för par som har en hög risk att få ett barn med en svår ärftlig sjukdom eller kromosomavvikelse. Fördelen med PGD är att den genetiska diagnostiken sker på embryostadiet innan embryot återförs till kvinnan, vilket innebär att paret kan undvika att behöva göra ett graviditetsavbrytande av ett sjukt foster. Nackdelen med PGD är att paret måste genomgå en provrörsbehandling (IVF), vilket i sin tur kan vara en påfrestande upplevelse både fysiskt och psykiskt. PGD utvecklades i början av 1990 talet och debatterades livligt av patienter, vårdgivare, beslutsfattare och allmänheten. Det fanns en rädsla att tekniken kunde användas i andra syften, så som att välja ut embryon med önskade egenskaper. I Sverige regleras PGD-verksamheten idag genom lagen för Genetisk integritet och är bara tillåten vid svåra ärftliga sjukdomar och kromosomavvikelser. Det är inte tillåtet att screena för alla typer av icke ärftliga kromosomavvikelser (PGS) eller att selektera för kön av sociala skäl. PGD utförs vid två enheter i Sverige, på Sahlgrenska Universitets- sjukhuset i Göteborg och på Stockholms PGD center vid Karolinska Universitetssjukhuset.

Det har varit en kontinuerlig ökad efterfrågan av PGD vid Stockholms PGD-center för varje år sedan starten 1996 och idag görs runt 160 cykler per år. Varje år tillkommer nya indikationer och fram till idag har PGD utförts för över 100 olika ärftliga sjukdomar och olika kromosomavvikelser. PGD kräver ett nära samarbete mellan en reproduktionsmedicinsk enhet där IVF behandlingen samt embryoprovtagningen utförs och ett genetiskt laboratorium där den genetiska analysen och bedömningen utförs. PGD processen kan delas in i tre olika faser: 1) IVF behandlingen med hormonstimulering, ägguthämtning samt befruktning och odling i laboratoriet. 2) Provtagningen (biopsi) av embryot på dag 3 eller 5-6 efter ägguthämtning. 3) Den genetiska analysen.

Det övergripande syftet med detta projekt var att identifiera vilka faktorer som var av betydelse för graviditetsutfallet efter PGD samt att lära mer om patienternas upplevelse av PGD för att kunna utveckla verksamheten och förbättra omhändertagandet av patienterna. Ett annat syfte var att genom utvidgade genetiska analyser inhämta kunskap om hur olika kromosomavvikelser påverkar fertiliteten.

Delarbete I: En kromosomavvikelse kan finnas i endast en del av kroppens celler vilket kallas för mosaicism. Om andelen avvikande celler är liten är det inte säkert att det medför några synliga tecken eller symptom. Finns avvikelsen i könscellerna kan det visa sig genom att paret upprepade gånger får barn med samma kromosomavvikelse trots att ingen förändring kan återfinnas hos någon av föräldrarna. En låggradig mosaicism är svår att upptäcka med dagens rutinmetoder eftersom ett begränsat antal celler från blodet analyseras rutinmässigt. Ett par med ovanstående historia identifierades och undersöktes med utvidgade undersökningar för att finna ut vem som var bärare av förändringen. Våra resultat visade att avvikelsen var nedärvd från modern och även fanns i en låg andel av hennes hudceller. Paret genomgick även PGD, tyvärr utan att någon graviditet uppnåddes, då samma förändring kunde återfinnas i embryon, vilket bekräftade germinal mosaicism hos modern.

Delarbete II: Flera olika faktorer påverkar chansen att uppnå graviditet med PGD. En del är samma som vid vanlig IVF och några är specifika för PGD-metoden. I delarbete II gjordes en stor genomgång av resultaten på Stockholms PGD-center mellan 1996 och 2009. Totalt 569 PGD-cykler analyserades. Vi fann att kvinnor under 36 år hade tre gånger så stor chans att få barn efter PGD jämfört med kvinnor över 36 år. Vi fann också att provtagningsmetoden var av signifikant betydelse för utfallet. PGD cykler där endast en cell per embryo togs för analys hade dubbelt så stor chans att leda till en graviditet jämfört med de cykler då två celler per embryo togs för analys. Vi har sedan 2009 övergått till en-cells biopsi för i princip alla indikationer och infört en åldersgräns för kvinnan på 40 år vid stimuleringsstart.

Delarbete III: En enkät skickades till patienter som genomgått PGD på Stockholms PGD-center mellan juni 2005 och 2011, sammanlagt 222 par. Svarsfrekvensen var 66%. En femtedel av paren hade genomgått graviditets avbrytande av ett sjukt foster, 35% hade fött ett sjukt barn och en tredjedel hade tidigare genomgått traditionell fosterdiagnostik. Vi fann att par med en ärftlig monogen sjukdom valde PGD på grund av en ovilja att genomgå abort och par med kromosomavvikelser valde PGD på grund av tidigare missfall, vilket inte är så överaskande eftersom par med kromosomavvikelser har en ökad risk för missfall och infertilitet. Vi kunde inte se att tidigare erfarenhet av tillexempel graviditetsavbrytande av sjukt foster, födelse av sjukt barn, missfall eller tidigare fosterdiagnostik påverkade upplevelsen av PGD. Paren uttryckte att den psykiska påfrestningen i samband med PGD var större än förväntat och att PGD generellt var en påfrestande upplevelse, vilket även tidigare

studier visat. Vår slutsats att är att PGD är ett önskat alternativ då 94 % av paren skulle rekommendera PGD till andra par i samma situation.

Delarbete IV: Vi undersökte om utfallet vid PGD kan förutsägas för manliga bärare av ärftliga kromosomavvikelser och hur olika avvikelser påverkar fertiliteten. Det har tidigare framförts att män med en obalanserad kromosom uppsättning i över 60% av spermierna har en nedsatt chans att få egna biologiska barn och att de bör rekommenderas spermiedonation. Ett linjärt samband mellan andelen spermier med obalanserad kromosom uppsättning och andelen obalans i embryon vid PGD har också presenterats. Vi undersökte tio män med olika ärftliga kromosomavvikelser genom FISH-analys av spermier i samband med PGD och jämförde sedan med utfallet av embryoanalysen samt graviditetsutfallet. Vi fann en ökad förekomst av obalanserade embryon jämfört med spermier utan något linjärt samband. Fyra av paren uppnådde graviditet och i dessa fall hade männen en andel obalans i spermierna mellan 33-72%. Resultaten stödjer inte tidigare förslag att det går att förutsäga resultaten vid PGD. PGD har tidigare visats minska missfallsfrekvensen för par med ärftliga kromosomavvikelser och med det öka chansen till att få ett levande fött barn. PGD är därför ett värdefullt alternativ för denna patientgrupp som ofta har en historia av upprepade missfall och infertilitet.

Sammanfattningsvis så är vår slutsats att PGD är ett värdefullt och önskat alternativ för en liten grupp patienter med en hög risk att få sjuka barn av en ärftlig sjukdom eller kromosom-avvikelse. Våra fynd har lett till att kliniska rutiner ändrats så att utfallet och omhändertagandet ska förbättras. Det sker en snabb utveckling inom området och nya tekniker är på gång att införas. Syftet är att dels öka tillförlitligheten av den genetiska analysen samt även att öka chansen till graviditet efter PGD, genom att med nya metoder kunna välja det embryo med högst chans till att ge ett friskt levande fött barn. Det är av största betydelse att kontinuerliga utvärderingar av säkerheten och utfallet med nya metoder utförs så att PGD verksamheten kan optimeras.

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