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Genetics in Human Reproduction

Edited by
Elisabeth Hildt and Sigrid Graumann

GENETICS IN HUMAN REPRODUCTION

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Routledge
Taylor & Francis Group
LONDON AND NEW YORK

First published 1999 by Ashgate Publishing

Reissued 2018 by Routledge
2 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN
711 Third Avenue, New York, NY 10017

Routledge is an imprint of the Taylor & Francis Group, an informa business

A Library of Congress record exists under LC control number: 98074633

ISBN 13: 978-1-138-31497-9 (hbk)
ISBN 13: 978-1-138-31498-6 (pbk)
ISBN 13: 978-0-429-45661-9 (ebk)

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Preface

Since the birth of the first child conceived by in vitro fertilisation almost two decades ago the field of assisted reproduction is expanding continuously. Though, in the beginning of this development there has been an intensive discussion about the moral permittance of artificial intervention in human procreation and many aspects are still controversial in the public, today assisted reproduction is widely established as infertility treatment in medical practice.

In the 70s and 80s the ethical discussion was dominated by the problems related with artificial procreation as such, poor success rates of IVF, surrogate motherhood, split in social, biologic and genetic parenthood, cryoconservation and spare embryos, male domination of women's bodies, research with human embryos to improve the methods and similar topics. In spite of the fact that most of the stressed problems are still prevalent there is a change in the concentration on points of emphasis perceptible during the last years. The background for this alteration of the ethical discussion forms the experience of the establishment of the clinical practice of assisted reproduction and in vitro fertilisation as well as the presence of results of empirical follow-up studies on the one hand and the technological innovations in this field on the other hand. The new techniques pre-implantation diagnosis (PID), intracytoplasmic sperm injection (ICSI), in vitro ovum nuclear transplantation (IVONT), and in the future possibly germline gene therapy are bringing human genetics and assisted reproduction together.

Though, the theoretical possibility to check up the embryo in vitro for genetic "abnormalities" may have been from the beginning of in vitro

fertilisation an idea of great influence on the part of the involved scientists, the expected benefits and the feared dangerous consequences of pre-implantation genetic diagnosis are rather new topics of public interest. Although not being feasible in human beings at the moment, also germline gene therapy – for which IVF is the presupposition – is a matter of intensive medical and ethical discussion.

Medical, social and ethical issues relating to the latest developments in IVF are discussed in the first book of the *European Network for Biomedical Ethics* with the title "In Vitro Fertilisation in the 1990s – Towards a Medical, Social and Ethical Evaluation" which has been issued 1998 at Ashgate with Elisabeth Hildt and Dietmar Mieth being the editors.

The present volume concentrates on the issues related to the current as well as to the possibly future technological progress in genetic technologies linked to IVF, i.e. preimplantation diagnosis and germline gene therapy, from a scientific and medical as well as from a social, juridical and ethical point of view.

This book contents the contributions of the second symposium of the *European Network for Biomedical Ethics* 'Genetics in Human Reproduction' which took place from February 26th to March 1st, 1998 in Maastricht, Netherlands. It provides a multidimensional view on the moral questions raised by PID and related technologies by collecting contributions from researchers coming from various European countries, working in different disciplines and arguing on various theoretical backgrounds.

The basic scientific data concerning preimplantation diagnosis and other micromanipulative procedures, as well as considerations concerning the chances and risks going along with these technologies from a scientific and medical point of view are discussed in Part One of this volume. These contributors are all physicians and scientists which does not mean that they leave out the ethical questions. The individual interests playing a role in PID and other micromanipulative procedures and their moral implications, e.g. concerning the responsibilities of prospective parents, the scientists involved, and society as a whole, are further examined in Part Two. Part Three concentrates on moral rights and duties regarding the possibilities of the new techniques on the one hand and the moral status of the embryo on the other. Part Four collects contributions with controversial moral views on the social implications of PID and related technologies. The contributors to Part Five are stressing the moral significance of desires, moral implications of reproductive choices and the role of counselling in the decision making process in the context of PID and related technologies.

The book is completed by Part Six with questions of justice in health care systems and legal regulation of PID and other micromanipulative technologies in the European context.

Acknowledgements

This volume is a collection of the lectures given at the symposium "Genetics in Human Reproduction" (Maastricht, February 1998) which was organised by the *European Network for Biomedical Ethics* in co-operation with the Instituut voor Gezondheitsethiek, Maastricht.

We want to heartily thank Ruud ter Meulen for making this Maastricht conference possible. We are especially indebted to the local organisers Guido de Wert and Angelique Heijnen.

Without the co-operation of a great number of persons, the symposium could not have been held and this book could not have been prepared. In particular, we would like to thank Katja Ruppel and Christof Mandry for invaluable help with the organisation of the symposium. Katja Ruppel and Annika Thiem carried out a great deal of the editorial work on this book, Glenn Patten and Hille Haker gave us support with proof-reading. We also want to thank the Center for Ethics in the Sciences and Humanities, University of Tübingen, for technical support, and Michael von Doering for organisational help.

In particular, this volume owes its existence to the enormous co-operation of all contributors and to the great support of the members of the *European Network for Biomedical Ethics*. We want to take the opportunity to express our thanks for their personal engagement.

We are grateful to the European Commission, Dr. Christiane Bardoux, DG XII, Science, Research and Development, for the generous funding of the *European Network for Biomedical Ethics*, the symposium "Genetics in Human Reproduction", and the publication of its results.

Elisabeth Hildt
Sigrid Graumann

These lectures were also the contributions at the Second Symposium of ENBE in Maastricht/NL in April 1998. The first symposium resulted in the publication of *In Vitro Fertilisation in the 1990s* edited by Elisabeth Hildt and Dietmar Mieth, which concentrated on interdisciplinary approach and dialogue about IVF in a general meaning and in assisted procreation. This second volume focuses on PGD-techniques, scientific, social, legal and ethical aspects. It will be followed by a third volume (from the symposium in Sheffield in January 1999), the purpose of which is the social and ethical debate on Human Procreation, promoting the controversy but also common 'points to consider'.

As the Director of the Network I would like to thank the editors of this book but also say thank you for the teamwork in the co-ordination of the whole project, including management, newsletters and research activities.

Dietmar Mieth

List of Abbreviations

AC	Amniocentesis
ACGT	Advisory Committee on Genetic Testing
AID	Artificial insemination with donor sperm
ART	Assisted reproductive technology
bp	Base pairs
BRCA 1	(Breast cancer predisposition gene)
BRCA 2	(Breast cancer predisposition gene)
CA	Conjoint analysis
CBA	Cost-benefit analysis
CBAVD	Congenital bilateral absence of the vas deferens
CEA	Cost-effectiveness analysis
CF	Cystic fibrosis
CFTR	(Cystic fibrosis gene)
CHA	Catholic Health Assosiation of America
CUA	Cost-utility analysis
CVS	Chorionic villus sampling
DMD	Duchenne's muscular dystrophy
DNA	Deoxyribonucleic acid
EAGS	European Alliance of Genetic Support Groups
ECJ	European Court of Justice
ESHG	European Society of Human Genetics
ET	Embryo transfer
FAP	Familial adenomatose polyposis
FISH	Fluorescence in situ hybridisation
GLGT	Germline gene therapy

HBOC	Hereditary breast/ovarian cancer
HD	Huntington's disease
HEXAA	Beta-N-acetylhexoaminidase A
HFE (Act)	Human Fertilisation and Embryology (Act)
HFEA	Human Fertilisation and Embryology Authority
HIV	Human immunodeficiency virus
ICSI	Intracytoplasmic sperm injection
IRB	Institutional Review Board
IVF	In vitro fertilisation
IVM	In vitro maturation
IVONT	In vitro ovum nuclear transplantation
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MESA	Microsurgical sperm aspiration
mtDNA	Mitochondrial DNA
NABER	National Advisory Board on Ethics in Reproduction
nDNA	Nuclear DNA
NF 1	Neurofibromatosis type 1
NHS	National Health Service
PCR	Polymerase chain reaction
PEP	Primer extension preamplification
PGC	Principle of generic consistency
PGD	Preimplantation genetic diagnosis
PGS	Preimplantation genetic screening
PID	Preimplantation diagnosis
PKU	Phenylketonuria
PND	Prenatal diagnosis
PPA	Prospective purposive agents
TBHR	Take baby home rate
TESE	Testicular sperm extraction
TNT	Therapeutic nuclear transfer
WHO	World health organisation
WTP	Willingness-to-pay

Part One
MEDICAL AND SCIENTIFIC VIEW

1 Clinical experience with PID and ICSI*

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Abstract

Preimplantation genetic diagnosis (PID) is a novel procedure which may be considered as a very early prenatal diagnosis for couples at risk for transmitting genetic diseases. Using the polymerase chain reaction (PCR) or fluorescence in situ hybridisation (FISH) the genotype or the sex of biopsied cleavage stage embryos obtained after in vitro fertilisation can be determined and selected embryos are then transferred. In vitro fertilisation with intracytoplasmic sperminjection is the method of choice to obtain embryos analysed through PCR to reduce contamination by residual sperm DNA. In our series of 61 PID cycles for 29 couples at risk since a period of 4 years the ongoing pregnancy rate per cycle was 15 %, per transfer 19 % and per patient 31 %. Of the 6 morphologically normal children born, one who is still alive and doing well, weighed 850 gr. because it was born at 25 weeks after a complicated triplet pregnancy. More experience is needed to correctly evaluate the efficiency and safety of this novel technique as well as its place in the prevention of genetic disease.

Introduction

Preimplantation genetic diagnosis (PID) is a novel technique which permits determination of the genotype of an oocyte before fertilisation or of an embryo before implantation. On the one hand, this procedure became possible because of the almost simultaneous development of IVF, micromanipulation, PCR and fluorescence in-situ hybridisation (FISH). On the other hand, patients or couples with a recurrence risk for genetic diseases were asking such a procedure so as to avoid the need for pregnancy termination after conventional prenatal diagnosis. The first clinical PID was reported by Handyside and Winston in 1990 (Handyside et al. 1990). Preclinical studies had convinced them that their was apparently no harm in biopsying a cleavage-stage embryo at the 6- to 8-cell stage (Hardy et al. 1990). Since then several groups have performed PID successfully using either PCR or FISH to analyse blastomeres from 3-day-old embryos (Harper 1996, Lissens et al.1996) or to analyse polar bodies from oocytes before fertilisation (Verlinsky et al. 1996).

Intracytoplasmic sperm injection (ICSI) certainly has its use in PID. In the first place it has an advantage over conventional in vitro fertilisation (IVF) in that it avoids contamination by sperm in PID of a cleavage-stage embryo, using the polymerase chain reaction (PCR). Moreover, for some couples with a concurrent genetic risk such as cystic fibrosis (CF) in cases of congenital bilateral absence of the vas deferens (CBAVD), pregnancy will be obtained more often after ICSI with spermatozoa retrieved from the epidydimis than after regular IVF (Silber et al. 1994). Klinefelter patients who on rare occasions may produce a very low number of spermatozoa may try to father a child through IVF and ICSI with spermatozoa extracted from an ejaculate or more often from a testicular biopsy and in this experimental setting the number of sex chromosomes in the embryos will have to be evaluated before transfer. The outcome of these treatment cycles in Klinefelter patients have partially been published and will be updated separately (Staessen et al. 1996, Tournaye et al. 1996). In this article our clinical PID experience in case of a recurrence risk for monogenic diseases will be reported and the place of ICSI will briefly be discussed.

Materials and methods

Twenty-nine couples were counselled at the Centre for Medical Genetics between February 1993 and February 1997 prior to PID. Except for 3 of them with a lower risk (see appendix) they had a 25% or 50% risk of having children with CF (with or without CBAVD in the male) (n=8), with non-fragile X mental retardation (n=3), with hemophilia A (n=2), with Duchenne's muscular dystrophy (DMD) (n=4), with retinitis pigmentosa (n=1) or with

myotonic dystrophy (n=11). The reason why these couples chose PID rather than regular prenatal diagnosis were in general (A) infertility or subfertility necessitating IVF as well as the genetic risk (n=15), (B) one or several pregnancy terminations after chorionic villus sampling (CVS) or amniocentesis (AC) (n=8) and (C) moral, emotional or religious objections against abortion in itself (n=6) or in combination with another indication (n=4). Table 1 summarises the indications and the outcome of PID in Brussels over a period of 4 years. Couples were prepared for IVF (4 cycles) or for IVF with ICSI (57 cycles) according to standard protocols (Staessen et al. 1993, Van Steirteghem et al. 1993, 1995). A brief history of each couple is given in the appendix.

Blastomere biopsy

Embryos were biopsied in the morning of day 3 after insemination or microinjection. From the 7-cell stage on, two blastomeres per embryo were removed, while from 4-cell to 6-cell embryos only one blastomere was taken. A micropipette was used with an inner diameter of 40 to 45 μm while the embryos were immobilised by means of a holding pipette. These biopsies were performed in HEPES-buffered Earle's medium. First, a hole was made in the zona pellucida. This was done by blowing a stream of Acidic Tyrode's solution until the zona pellucida ruptured. Two different procedures to obtain blastomeres have been used. In the first cycles, the hole in the zona was turned to the 12 o'clock position. One or 2 blastomeres were pushed through the hole by pushing with a bevelled pipette with an inner diameter of 40 μm. Later, a blunt pipette with an inner diameter of 40 μm to 45 μm and a smoothened opening was passed through the hole. The hole was placed at the equatorial plane of a blastomere containing a nucleus before aspiration (Ao et al. 1996).

Diagnosis by the PCR method

Under continuous microscopical supervision, blastomeres were washed three times in Ca^{2+} and Mg^{2+} free M2 medium and placed in a 0.5 or 0.2 ml PCR tube. Lysis conditions and reaction conditions were worked out to detect the concerned mutations or DNA sequences in the most efficient and accurate way at the single-cell level. The resulting DNA fragments were further analysed on a polyacrylamide or Metaphor® agarose gel (Liu et al.1994a, 1994b, 1995, Sermon et al. 1997).

Diagnosis by the FISH method

The individual blastomeres were first rinsed in medium, then transferred to a 1-2 µl droplet of 0.01N HCl/0.1 % Tween 20 solution on a slide and the FISH procedure as described by Coonen et al. (1994) was used. Double target FISH was performed using directly labelled DNA-probes specific for chromosomes X and Y. The X (Vysis, ?-satellite DNA probe, Spectrum Green) and Y (Vysis, satellite III DNA probe, Spectrum Orange) were used for gender determination. To counterstain the nuclei 4'6-diamidino-2-phenyindole (DAPI) was used. The nuclei were then examined using a Zeiss Axioskop fluorescence microscope with the appropriate filter set (filter 10 for fluorescein isothiocyanate (FITC), filter 02 (DAPI) and Omega filter (FITC/Texas Red) (West et al. 1989, Griffin et al. 1991, Harper et al.1994). All nuclei were observed and FISH results (two green spots in case of the presence of female blastomere or one green and one orange spot in case of a male blastomere) were interpreted by two independent observers.

Embryo transfer, cryopreservation and follow-up

Whenever possible up to 3 unaffected embryos of grade A (no anucleated fragments), B (1 to 20% anucleated fragments) or C (21-50 % anucleated fragments), were transferred per cycle as indicated (female age, rank of trial, embryo quality). Spare unaffected embryos were cryopreserved and transferred in a subsequent cycle. The luteal phase was supplemented by micronised progresterone (600 mg daily) administered intravaginally or HCG (5000 units 5 days after ovum pick-up) administrated intramuscularly and serum HCG was determined from day 10 onwards. Where possible, a close pregnancy follow-up was organised, including regular ultrasound examinations, chorionic villus sampling or amniocentesis to confirm the result of the PID, registration of the pregnancy outcome and clinical evaluation of the child at birth and thereafter (Wisanto et al.1996, Bonduelle et al.1996). If no pregnancy ensued follow-up information on the couples was collected.

Results

So far 61 cycles for preimplantation diagnosis have been performed for 29 patients as reported in table 1. Of these cycles, 36 were performed during the last year. The mean age of the women at their first attempt was 30.4 years, with a range between 24 and 37. The number of attempts per couple ranged from 1 to 5 with an average of 2.1 cycles per couple (see appendix).

The number of cumulus-oocyte complexes recovered per cycle was between 2 and 43, providing a mean of 13.2 (805/61). Fertilisation, i.e., the presence of two pronuclei (2PN) was observed in 456 oocytes which corresponds to a mean of 7.5 per cycle. In 4 cycles there was no further development of the fertilised oocytes and therefore no further analysis. In 333 cleavage-stage embryos between the 4- and the 10-cell stage a biopsy was performed. The mean number of biopsied embryos per cycle was 5.8 (333/57). In 43 (12.9%) of the 333 embryos no diagnosis was possible because of no amplification, inconsistent results or contamination.

One hundred and twenty-nine unaffected embryos, a mean of 2.3 per cycle were available for transfer; except for 1 embryo of grade A, they were all grade B or C. In 12 cycles no embryos could be transferred; in 4 of these because no embryos developed and in the remaining 8 because no unaffected embryos were available. In 16 cycles only 1 embryo was transferred. Unaffected embryos were cryopreserved in 5 cycles and most of these were transferred in three additional cycles but without success.

So far 10 pregnancies have ensued from fresh transfers. One miscarriage has occurred, 4 singleton pregnancies are ongoing and 6 children have been born from the remaining 5 pregnancies. The children are between 3 months and more then 2 years of age. One of them is a boy, the others are girls.

Discussion

The success rate in terms of pregnancies is 10 out of 61 cycles or 16 %. Per transfer the pregnancy rate is 10/48 (21 %) and per couple it is 10/29 (34 %). Numbers are too small to calculate the take-home baby rate but if we subtract the one miscarriage and consider the ongoing pregnancies (n=4) plus the deliveries (n=5), the take-home baby rates are 15% per cycle, 19% per transfer and 31 % per couple. In our regular IVF or IVF/ICSI cycles the pregnancy rate per cycle is currently around 30 % and the take-home baby rate per cycle well over 20 %, and in the world figures for PID the pregnancy rate per cycle was 25 % and per transfer 29 % (Harper, 1996). The lower success rate in this small series cannot be explained by the age of our patients which is quite similar. One of the reasons for a lower pregnancy rate is most probably the higher number of cycles in which none or only 1 unaffected embryo of grade B or C were available for transfer. From our availabe data we therefore decided that cycles for PID with less then 9 cumulus-oocyte-complexes should be cancelled. Another reason for a lower success rate may be a "subfertility" of the myotonic-dystrophy patients due to their disease, since 25 out of the 61 cycles were performed in 11 couples at risk for this disease (Sermon et al. 1997).

None of the 4 couples at risk of CF has become pregnant so far. Two of these had a subfertility problem as well as the genetic risk but, nevertheless one of them has since had two spontaneous pregnancies followed by the birth of non-affected children after CVS. Prior to the pregnancies the couple was intending to have another PID cycle. One couple had 4 cycles so far without success. After CF had been diagnosed in 1 of their 2 children 8 years ago, they waited for the development of PID so as to be able to have at least 1 other healthy child, especially since the wife could not cope with the idea of prenatal diagnosis followed by a possible pregnancy termination. Although this couple was proven to be fertile, the oocytes and embryos produced during the 4 treatment cycles were always low in number and of extremely poor quality.

Pregnancies have ensued in 2 of the 4 couples at risk for CF because the wives of CBAVD-men were carriers. In 1 case the pregnancy occurred during the first treatment cycle after replacement of 3 embryos and a healthy boy now over 2 years of age was born (Liu et al. 1994a). Subsequent cycles were unsuccessful.

For the second couple, 5 cycles were needed to obtain a singleton pregnancy after transfer of 3 embryos.

In 6 out of 10 patients at risk for an X-linked disease, pregnancies have ensued. The mean age of these patients was 28 years. Four of the 10 patients were at risk of DMD. Two of them now have girls. In the first case the pregnancy occurred during the second cycle and the diagnosis was based on a PCR assay detecting the presence or absence of a dystrophin gene deletion. Two embryos were transferred (Liu et al.1995). The girl is now over 2 years of age and healthy. In the second case a triplet pregnancy occurred during the second cycle after transfer of 3 embryos. The triplet was one singleton and one twin (monochorionic, biammniotic) one of which was shown to be an acardiacus between 13 and 14 weeks of pregnancy. Five weeks later selective reduction of the malformed twin was performed extramuros and another 4 weeks later, the children were prematurely born at almost 25 weeks of pregnancy. The morphologically normal twin weighed 450 g and subsequently died. The singleton baby girl weighed 850g and is doing well according to the information we obtained so far. In 2 patients at risk for hemophilia A, 1 healthy girl was born after transfer of 2 embryos and 2 healthy twin girls have recently been born after the replacement of 3 embryos in the second patient respectively. Of the 3 patients at risk of non-fragile-X-linked mental retardation, 1 patient became pregnant after replacement of 3 embryos in the first cycle but a miscarriage occurred. Finally, 1 patient at risk of retinitis pigmentosa is currently pregnant after 1 treatment cycle with transfer of 2 embryos.

Two out of 11 couples at risk of myotonic dystrophy are currently pregnant with singletons, both after a 3rd cycle in which respectively 2 and 4 embryos were transferred (Sermon et al.1997).

The mean age of all the preceding pregnant women was 29.8 years (range 24-37); the mean number of embryos transferred per cycle was 2.5 (range 2 to 3 except in one case where 4 were transferred).

In our population of 29 couples who had requested PID, the indications, apart from the genetic risk, were infertility in the 4 cases with CBAVD, subfertility in 11 cases (most of which belong to the myotonic dystrophy group), a previous history of affected pregnancies which had to be terminated in 8 cases and moral problems with termination of pregnancy in 6 cases. The high pregnancy rate of 60% in the group of patients at risk of sex-linked diseases might be explained by the lack of subfertility problems (only 1 out of 10) and the younger mean age (28 years) of these patients. The one miscarriage occurred in the subfertile couple with a previous history of G4P1A3.

Only the first 4 cycles in couples without CBAVD involved classical IVF. Since then IVF with ICSI has been used for insemination. The aim was to reduce the risk for contamination in PCR reactions from residual sperm-DNA. We still consider this to be the insemination method of choice in PCR- based PID. In FISH-based PID for couples with no known subfertility or infertility, conventional IVF is probably equally valid as an option.

Before starting the treatment, PID patients were asked to agree to a prenatal diagnosis through CVS or amniocentesis to confirm the result of the PID should they become pregnant, since at least in PCR-based assays misdiagnoses have been reported (Harper 1996) and since diagnostic errors may occur as a result of contamination or allele-specific drop-out during the PCR reaction. Of the 10 pregnancies, 1 miscarried before prenatal diagnosis. Two patients pregnant after a FISH-based sex-determination and one patient pregnant after CF-diagnosis declined to have prenatal diagnosis. In 6 cases (1 CVS and 5 amniocenteses) the PID was confirmed or refined (CF carrier boy, non-carrier DMD girl).

The age of the 6 children born so far ranges from 3 months to over 2 years of age. Four of these are girls because female embryos were selected for transfer as a result of a risk of a sex-linked disease. The fifth girl was born to a carrier of Duchenne's muscular dystrophy but the PCR-based PID indicated affected boys (absence of fragment) versus unaffected male embryos and non-carrier as well as carrier female embryos (presence of fragment). This girl and the boy born at term in 1994 were morphologically normal (Liu et al.1994a, 1995). At birth and at 2 years of age their growth and developmental milestones were within the normal range. One of the 4 girls born in 1996 issued from the triplet pregnancy mentioned earlier; she was born at 25 weeks of pregnancy and weighed 850 g. At 4 months of age the girl weighed 3.2 kg and measured 49

cm. According to the parents, who plan to visit us, she was doing fine. The premature birth was probably the result of the selective reduction performed on 1 of the malformed twins at 18 to 19 weeks of pregnancy. The other twin weighed only 450 g at birth and did not survive. The cause of the acardiacus malformation is most probably linked to the twinning process and not to the biopsy procedure. The 3 other girls born in 1996 are doing well according to information obtained from the parents and their physicians. One girl was born at 36 weeks of pregnancy and had a birthweight of 2.4 kg, a length of 47 cm and a head circumference of 32 cm. She is now about 1 year old. The other twin girls were born at 35 weeks of gestation and weighed 2.6 and 2.1 kg respectively; they are now 3 months old. So far, the number of children born is too small to draw any firm conclusions concerning possible problems with morphology, growth or development. As in regular IVF and ICSI, multiple pregnancies should be avoided where possible so as to reduce the risk of complications (Bonduelle et al. 1996, Wisanto et al. 1996, Simpson and Liebaers 1996).

Our PID programme is now well structured and based on a close collaboration between the Centre for Medical Genetics and the Centre for Reproductive Medicine. Before starting, patients are counselled extensively by specialised physicians in both Centres. A nurse-coordinator schedules the cycles and informs the team members who will be involved and especially the laboratories dealing with cycle monitoring, IVF and ICSI, embryo biopsy and FISH or PCR analysis. Patients are asked to come to the clinic for pick-up and on day 3 post-insemination for a possible transfer. The outcome of the embryo diagnosis is discussed with the couple at the clinic. In any case a follow-up visit is scheduled with the geneticist as well as with the fertility specialist so as either to organise a pregnancy follow-up with prenatal diagnosis, ultrasound and finally a baby follow-up or to plan a subsequent cycle. Organising the follow-up of patients from abroad is more complex and the data obtained are less complete.

Possible reasons for the slow development of PID in our centres and elsewhere are probably linked to its experimental character and to the complexity of the procedure at the clinical as well as at the laboratory level. Moreover the take-home baby-rate is low as a result of this complexity and the cost is rather high. Finally, the availability of the procedure in general and of specific procedures for specific diseases is still limited. Nevertheless, the procedure does not appear to be too stressful for many of the patients, since several of them have had repeated PID (see appendix). Further development in diagnostic procedures as well as the evaluation of patient's experience are therefore to be expected. Moreover continuous data collection at the national and international levels will be of great value to correctly appreciate the value

of this new procedure (ESHRE Special Interest Group on Reproduction and Genetics, International Working Group on PID).

Table 1. PID in Brussels between February 1993 and February 1997 for monogenic diseases

Disease	Couples	PID indication		Cycles	Transfers	Pregnancies	Miscarriages	Ongoing pregnancies	Births	Children
Cf_a	4	Subfertility	2	9	7	-	–	–	–	–
		$History_e$	1							
		TOP_f	1							
CF_a / $CBAVD_b$	4	Infertile male needing $MESA_g$	4	12	8	2	–	1	1	1
MD_c	11	Subfertility	8	25	22	2	–	2	–	–
		$History_e$	1							
		TOP_f	6							
$X\text{-linked}_d$	10	Subfertility	1	15	11	6	1	1	4	5
		$History_e$	6							
		TOP_f	3							
	29			61	48	10	1	4	5	6

$_a$Cystic Fibrosis; $_b$Congenital bilateral absence of vas deferens; $_c$myotonic dystrophy; $_d$X-linked diseases such as Duchenne's muscular dystrophy, Hemophilia A, X-linked mental retardation and retinitis pigmentosa; $_e$previous history of prenatal diagnosis followed by termination of pregnancy; $_f$moral, emotional or religious objection to termination of pregnancy (TOP); $_g$microsurgical epididymal sperm aspiration.

Addendum

Since the publication of the above article, in total 170 PID cycles have been performed for 84 couples. Twenty-nine pregnancies were established. Five of these were multiple pregnancies.

One pregnancy was terminated because of a misdiagnosis detected at prenatal diagnosis. Seventeen healthy children were born, one acardiacs-twin died. Twelve pregnancies are ongoing.

Genetic indications for preimplantationdiagnosis were for monogenic conditions: several X-linked disorders such as Duchennes Muscular Dystrophy, hemophilia A, Wiskott-Aldrich disease, adrenoleucodystrophy, Charcot Marie Tooth disease, mental retardation, retinitis pigmentosa.

PID was also performed for autosomal recessive and dominant diseases such as myotonic dystrophy, cystic fibrosis with our without (CBAVD), Marfans disease, Charcot Marie Tooth disease, β-thalassemia, 21-β-hydroxylase deficiency, osteogenesis imperfecta and sickle cell anemia.

For chromosomal aberrations, PID has been performed for the velo-cardio-facial syndrome due to a 22q deletion, for a translocation (11;22), for a Yq deletion as well as for Klinefelter patients producing a few spermatozoa in their testes.

The demand for PID has increased over the years. New diagnostic tests are being developed and more centers are offering this new procedure. Evaluation of patients experience with this new procedure is necessary and ongoing.

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Acknowledgements

Research funds of the university and the F.W.O.-Vlaanderen have made the development of these new procedures possible. Besides the authors and all the other members of the Centres for Medical Genetics and Reproductive Medicine were helpful in taking care of the patients and their embryos. Special acknowledgements go to F. Winter for correcting the grammar and style and J. Heulaerts for typing the manuscript.

2 The various micromanipulative procedures: State of the art, chances, and risks

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Micromanipulation has added a new dimension to reproductive medicine. It entails the use of microtools that allow for a precise handling and manipulation of single cells or their subcompartments such as the cytoplasm or nucleus. With various types of micropipettes germ cells or parts of them as well as early embryos can be individually selected, held, drilled, cut, injected, or biopsied. The possible applications of this technology in research, diagnosis and therapy are manifold. Cloning of humans, considered to be on the horizon, would also have to rely on these micromanipulative technologies. But even some currently available techniques such as oocyte cytoplasm donation make conventional in vitro fertilisation (IVF) look like an old-fashioned and almost natural way of inducing pregnancies.

In quantitative terms, intracytoplasmic sperm injection (ICSI) is by far the most important procedure involving micromanipulation (Felberbaum and Dahnke 1997). Its main application is severe male factor infertility, an entity that in the pre-ICSI era had a dismal prognosis for a successful treatment outcome. Centers with ample experience now report clinical pregnancy rates exceeding 30 % per treatment cycle. The fact that this exceeds the natural conception rate in fertile couples illustrates what dramatic progress ICSI represents.

ICSI is supplemented by new techniques for surgical sperm retrieval in azoospermic or severely oligozoospermic men. Patients with obstructions of the seminal ducts may benefit from MESA (microsurgical epididymal sperm aspiration), individuals with non-obstructive azoospermia from TESE