ORIGINAL ARTICLE

Polar Body Biopsy in the Diagnosis of Monogenic Diseases

The Birth of Three Healthy Children

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SUMMARY

Background: For prospective parents at risk of transmitting a monogenic disease, polar body analysis is an option for pre-conception genetic diagnosis. In Germany, polar body analysis is currently performed in only two centers (Lübeck and Regensburg).

Methods: The authors present a clinical series of 9 couples at risk for the transmission of a monogenic disease who underwent in vitro fertilization with polar body analysis.

Results: Nine couples have undergone in vitro fertilization with polar body analysis at the center in Lübeck since 2004. Three healthy children were born after polar body analysis for mucopolysaccharidosis type I, incontinentia pigmenti, and cystic fibrosis. The decision to undergo in vitro fertilization with polar body analysis is not easy for prospective parents to take, even though it often follows years of emotional suffering. Treatment with the methods of reproductive medicine in general, and with polar body analysis in particular, can cause considerable physical and emotional stress.

Conclusions: For prospective parents in Germany at risk of transmitting a monogenic disease, polar body-based preimplantation diagnosis is an alternative to prenatal diagnosis and possible termination of pregnancy. The live birth rate per treatment cycle in this clinical series was 30%, which can be considered satisfactory. Nonetheless, most of the couples who did not achieve pregnancy after a first treatment cycle dropped out of treatment prematurely and did not go on to a second cycle.

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Polar body biopsy followed by molecular genetic analysis was first described in 1991 by Verlinsky et al. (1). Polar bodies, which are byproducts of oocyte meiosis, can be removed in vitro using a micromanipulator once the zona pellucida has been opened. Polar body analysis (PBA) allows a specific genetic defect to be studied in the first and second polar bodies and thus gives indirect information about whether the genetic defect is present in the oocyte. A precondition for PBA is that the genetic defect being sought in the polar bodies is known, and that a genetic linkage test can be carried out on the family in order to identify the haplotype. PBA is also suitable for indirect demonstration of numerical and structural chromosomal aberrations in the oocyte.

PBA is also described as a preconception diagnostic technique. Selecting oocytes for in vitro fertilization is permissible under German law (Embryonenschutzgesetz, Embryo Protection Law) so long as the selection is made before any of them has become an embryo (as defined in §8 of the Embryo Protection Law).

For couples in Germany who want to have children, but who are carriers of monogenic diseases, PBA represents an opportunity for preconceptional genetic diagnosis (2, 3). In the majority of European states, on the other hand, preimplantation diagnosis (PID)—genetic testing of the embryo before implantation—is the favored procedure. Unlike PID, PBA allows only indirect conclusions about the genetic make-up of the oocyte. Paternally transmitted (dominant) diseases cannot be diagnosed. In the case of autosomal recessive diseases, PBA leads more often than PID to the discarding of oocytes that would have become healthy heterozygous carriers (2, 4, 5). This must be regarded as a serious disadvantage of PBA.

PBA to detect numeric aneuploidies and chromosomal translocations is currently offered at several treatment centers in Germany. PBA for carriers of monogenic diseases requires far greater resources and in Germany is only offered at two centers, in Lübeck and in Regensburg (2, 5). The first healthy child to be born after PBA in Regensburg to a couple with a monogenic disease was born in August 2004 (5), and the second

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TABLE

PBA treatments carried out in Lübeck to date (November 2008)

	Cystic fibrosis	Hemophilia A	Mucopolysaccharidosis type 1	Myotonic dystrophy	Spinal muscular atrophy	Incontinentia pigmenti	X-linked hydrocephalus
Patients	3	1	1	1	1	1	1
Treatment cycles with oocyte harvesting	3	1	1	1	2	1	1
Treatment cycles in which at least one embryo was transferred	2	1	1	2	2	1	2
Pregnancy	1	0	1	0	0	1	0
Healthy babies	1	0	1	0	0	1	0

child followed shortly afterwards after PBA at the University of Lübeck (6). The Deutsches Ärzteblatt recently published data from Germany on aneuploidy screening of oocytes (2). The present article will describe the procedure for PBA for monogenic diseases and summarize experiences at the University of Lübeck so far (*Table 1*), with illustrative case reports.

Procedure for PBA in Lübeck

The University of Lübeck's program of counseling and treatment of couples with monogenic diseases who want children is run in close interdisciplinary collaboration between specialists in reproductive medicine and human geneticists. Before each new PBA treatment starts, agreement is sought from the relevant ethics commission. Every PBA requires in vitro fertilization and, in addition, intracellular sperm injection (ICSI) to be carried out. After ovarian stimulation and harvesting of oocytes, the zona pellucida is opened by laser and the polar bodies removed using a micropipette (7) (*Figure*). After lysing of the polar bodies, a cost- and labor-intensive series of molecular genetic pre-tests has to be carried out individually for each couple, and these then form the basis of the PBA itself. The "identification" of a mutation in the polar body DNA is by electrophoretic demonstration of several heterozygous genetic markers that are closely linked with the mutation. The markers are chosen such that they are as close as possible to the mutation, in order to minimize the chances of gene recombination between the marker and the mutation, and hence the likelihood of a false diagnosis. Oocytes in whose polar bodies the mutation being tested is present, and which therefore must carry wild-type alleles, are, after successful fertilization and development into the embryonic stage, transferred to the womb on the 2nd or 3rd day of preimplantational development. If a pregnancy ensues after PBA, the couple is advised to have a prenatal diagnosis carried out in order to confirm the PBA diagnosis.

Type 1 mucopolysaccharidosis

Type 1 mucopolysaccharidosis is an autosomal recessive lysosomal storage disease resulting from a mutation of the alpha-L-iduronidase gene (IDUA). Often a so-called Q70X mutation is present, which causes a severe form of the disease (Hurler syndrome). The enzyme defect leads to accumulation of dermatan sulfate and heparan sulfate in the body cells, leading to a progressive disease process of varying severity. The disease usually manifests itself during the first year of life with features including cardiomegaly, cataract, hydrocephalus, and marked mental retardation; in severe cases death usually occurs before the age of 10 years (6, 8).

Case report: A 34-year-old woman, Mrs. K., presented at the University of Lübeck in 2004 together with her 37-year-old husband, to whom she was related by blood. The couple reported having previously had a child who, after a long period of suffering, had died before the age of 4 from the consequences of type 1 mucopolysaccharidosis (Hurler syndrome). Two subsequent pregnancies (1999 and 2001) had been terminated on sociomedical grounds after prenatal diagnosis had shown the presence of a homozygous mutation (Q70X). The couple were both heterozygous for the Q70X mutation and phenotypically unremarkable. A PBA was set up for the family. Sixteen first polar bodies from 16 oocytes were analyzed. For two polar bodies the results of molecular testing were ambiguous and could not be clearly evaluated. Eight polar bodies proved to be heterozygous, in three the wild-type allele for the IDUA gene was found, and in three further polar bodies the mutated allele was found. Only one of the three oocytes that had a high probability of containing the wild-type allele was successfully fertilized in vitro. An embryo was transferred to Mrs. K. at the four-cell stage and a pregnancy ensued. Chorionic villus sampling showed a heterozygous Q70X mutation inherited from the father, thus confirming the PBA. When placental abruption occurred at the end of

Figure: Removal of the first polar body from a human oocyte

the 30th gestational week a cesarean section was performed with delivery of a healthy female baby. The child has continued to develop normally to the present day.

Incontinentia pigmenti

Incontinentia pigmenti (Bloch-Sulzberger syndrome) is a rare X-linked dominant monogenic disease that produces an ectodermal malformation syndrome. The incidence is reported at 1:40 000, with a male:female distribution of 1:37 (9). In males this genetic defect is usually fatal in utero (10). Affected females show great phenotypic variability. Clinically, pigmentation abnormalities are typical. In addition, in about 80% of cases multiple organs are affected, with dental defects, skeletal anomalies, and central nervous system malformations with mental retardation. The condition is caused by rearrangement of the gene coding for nuclear factor kappa B essential modulator (the NEMO gene) (11).

Case report: Mrs. L., 36 years old, presented with her 37-year-old husband in 2006. Apart from dental defects and skin changes, she showed no clinical signs of incontinentia pigmenti. The couple had had two male children who were stillborn in the 21st and 26th gestational weeks respectively (1994 and 1995). Both fetuses showed a mutation in the NEMO gene, which Mrs. L. carries. In 2004 she became pregnant with twins after stimulation with clomifene. Chorionic villus sampling showed mutation of the NEMO gene in both the male and the female fetus. Because of the prognosis of death for the male fetus and the uncertainty about the severity with which the female fetus would be affected, the patient underwent termination of pregnancy on sociomedical grounds in the 12th gestational week.

After oocyte harvesting, nine first polar bodies and five second polar bodies were analyzed. Two first polar bodies carried the mutation, and the second polar bodies contained wild-type alleles, so that it could be concluded that there was a high probability that both oocytes carried the wild-type allele. Both oocytes were successfully fertilized and transferred as embryos at the four-cell stage and a pregnancy ensued. Chorionic villus sampling ruled out the mutation in the fetuses. Mrs. L. had a healthy boy by primary cesarean section after 39 weeks and 2 days of gestation.

Cystic fibrosis

Also known as mucoviscidosis, this chronic metabolic disease has an autosomal recessive inheritance pattern. In Germany about 300 children a year are born with cystic fibrosis (12). It is caused by a mutation of the CFTR (cystic fibrosis transmembrane regulator) gene which leads to malfunction of the exocrine glands (13). In Germany, the delta-F508 mutation of the CFTR gene is by far the most frequent (67%) (12). Multiorgan involvement leads to progressive loss of lung function, exocrine pancreatic insufficiency, impairment of liver metabolism, and male infertility.

Improved treatment options have improved the average life expectancy of people with this hereditary disease, which used to be fatal before the end of childhood, to around 37 years (14).

Case report: Mrs. U. (29 years old) presented in 2006 together with her 38-year-old husband at the infertility clinic. Previous tests in connection with failure to conceive had shown azoospermia in the husband with congenital bilateral aplasia of the vas deferens, which is often due to mutations in the CFTR gene. Molecular genetic testing confirmed the suspicion that Mr. U. was heterozygous for cystic fibrosis with the delta-F508 mutation. Analysis of Mrs. U. showed that she too was heterozygous for the delta-F508 mutation. Cystic fibrosis did not appear in the couple's family history.

After ovarian stimulation and oocyte harvesting, 15 first and eight second polar bodies were available for diagnosis of the delta-F508 mutation. For one oocyte, the first polar body showed the delta-F508 mutation, the second the wild type. Two more first polar bodies were heterozygous for delta-F508; the second polar bodies showed the mutation. Thus, it was ascertained that the heterozygosity in the first polar bodies was the result of a crossover during the first cell division. It was deduced from this that the wild-type allele must be present in the three oocytes. After fertilization, two embryos at the four-cell stage were transferred to the womb. Mrs. U. became pregnant and developed severe ovarian overstimulation syndrome requiring hospitalization. The symptoms regressed rapidly with intravenous fluid therapy and anticoagulation treatment. Analysis of the fetal DNA by means of chorionic villus sampling ruled out the presence of the delta-F508 mutation in the fetus. Mrs. U. had a healthy male baby by cesarean section at 39 weeks and 3 days of gestation.

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease caused by a mutation of the telomeric region of the SMN1 gene (survival motor neuron gene 1) (15). The mutation leads to degeneration of the alpha motoneuron of the anterior horn of the spinal cord, with progressive symmetrical muscular weakness and atrophy (16). SMA has an incidence of around 1 in 6000 to 1 in 10 000 births (17). It is divided into four types depending on the age at manifestation and the severity of the neuromuscular symptoms. Type 1 SMA (Werdnig-Hoffmann disease, the infantile form of SMA) manifests itself during the first 6 months of life and leads to death within the first few years of life (16). Although promising therapeutic options exist, SMA is still regarded as incurable (18).

Case report: Mrs. B., 30 years old, attended with her 34-year-old husband because both of them were heterozygous for SMA. Mrs. B. had suffered a miscarriage in 2003 in the 9th week of gestation; human genetic analysis of the aborted tissue was not carried out. In 2004 she gave birth to a son. During the first

weeks of his life the parents noticed increasing muscular hypotension and restricted movements in their child. At the age of 6 weeks, type 1 SMA was diagnosed, and the child died at the age of 5 months. Mrs. B.'s family history showed that her maternal aunt had had two children in a consanguineous marriage who had died of SMA. Mrs. B.'s own mother had had one child stillborn. Mr. B.'s family history included two unexplained miscarriages by his sister.

After ovarian stimulation, 22 first and 14 second polar bodies from a total of 25 oocytes were available for PBA. Five first polar bodies showed heterozygosity for SMN1. The corresponding second polar bodies were hemizygous for SMN1, making it highly probable that the oocytes contained the wild-type allele. However, an "allele dropout" occurred during PCR of two of the second polar bodies, so that these cells could not be released for transfer. The remaining oocytes either had a high probability of containing the mutation on the basis of molecular genetic analysis or else were ruled out for transfer, since for these latter ones only the first polar bodies were available for analysis, and because of the heterozygosity no definite statement about the genetic status of the oocytes could be made. In the end, only 3 out of 25 oocytes could be released for fertilization with a high degree of diagnostic certainty. Two four-cell embryos were transferred into Mrs. B., but no pregnancy ensued.

The couple decided to embark on a further IVF cycle. After oocyte harvesting, 15 first and 13 second polar bodies were available for PBA. Four oocytes were diagnosed as carrying the wild-type allele. Three four-cell embryos were transferred to the patient, but no pregnancy ensued. Despite the couple's continued strong desire for children, they felt physically and psychologically unable to subject themselves to any more IVF cycles with PBA.

Discussion

For carriers of monogenic diseases in Germany who want preconceptional diagnosis, PBA is the only way to contemplate pregnancy without risking exposing themselves to the great physical and psychological trauma of a medically induced abortion or intrauterine fetal death. The case reports above show that affected couples often already have long experience of the disease and many years of suffering, and that the choice of PBA is not lightly made. They also show that many couples cannot stand the strain of the treatment for long and only make it through one treatment cycle (*Table 1*)—and this although the probability of one live birth per treatment cycle may be regarded as good, not least because most of the couples have normal fertility.

In contrast to prenatal diagnosis and medically induced abortion, the financial costs of PBA are not usually paid by the statutory health insurance companies. In the cases presented here, the PBA was financed out of research funds. The real costs of PBA would present a great financial hurdle for the couples involved,

so for most couples with a monogenic disease who for social or psychological reasons would decide against living with a child with a serious disease, a "trial pregnancy" is the only practicable route.

Paternally transmitted dominant diseases cannot be diagnosed by PBA. Diagnosis of autosomal recessive diseases leads to the discarding of oocytes that carry the relevant mutation, but which would have given rise to embryos not affected by the disease if fertilized by a sperm carrying the wild-type allele. These are serious limitations of the diagnosis of monogenic diseases by PBA.

PID does not have these disadvantages. This is why—given that the methodological, financial and temporal resources required and the health risks due to the IVF treatment are similar—PBA is hardly used at all for monogenic disease in other European countries (2, 5). The most recent report of the Preimplantation Genetic Diagnosis (PGD) Consortium of the European Society of Reproductive Medicine and Embryology documents only 500 treatment cycles of PID for monogenic diseases worldwide in the year 2005 (19). Even if there is no guarantee that all PID cycles worldwide have been recorded by the PGD Consortium, it does appear to be the case that one of the reservations originally expressed about PID, namely that it would be used too widely, is unfounded. We wish to make a plea for research efforts to make polar body biopsy easier, safer, and more efficient, and for financial support for affected couples, so as to make PBA more accessible.

Key messages

- There are only two centers for PBA for monogenic diseases in Germany.
- So far, only a few IVF treatments have been carried out in Germany using PBA.
- The probability of a live birth, according to the figures given in *Table 1*, is around 30% per treatment cycle.
- PBA is expensive in time and resources and is subject to methodological limitations, so couples must be given comprehensive information about the possible stresses and risks involved.

Conflict of interest statement

The authors declare that no conflict of interest exists according to the guidelines of the International Committee of Medical Journal Editors.

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REFERENCES

1. Verlinsky Y, Ginsberg N, Lifchez A, Valle J, Moise J, Strom CM: Analysis of the first polar body: preconception genetic diagnosis. *Hum Reprod* 1990; 5: 826–9.

2. Van der Ven K, Montag M, van der Ven H: Polar Body Diagnosis—A Step in the Right Direction? *Dtsch Arztebl Int* 2008; 105 (11): 190–6.
3. Griesinger G, Schultze-Mosgau A, Finas D, Felberbaum RE, Diedrich K: Präimplantationsdiagnostik: Methode und Anwendung aus reproduktionsmedizinischer Sicht. *Zeitschrift für medizinische Ethik* 2003; 49: 325–42.
4. Handyside AH, Pattinson JK, Penketh RJ et al.: Biopsy of human preimplantation embryos and sexing by DNA amplification. *Lancet* 1989; 1: 347–9.
5. Hehr A, Gross C, Bals-Pratsch M et al.: Polkörperdiagnostik für monogene Erkrankungen als deutsche Alternative zur Präimplantationsdiagnostik. In: Felberbaum RE, Bühler K, van der Ven H (eds.): Heidelberg: Springer 2007.
6. Tomi D, Schultze-Mosgau A, Eckhold J et al.: First pregnancy and life after preimplantation genetic diagnosis by polar body analysis for mucopolysaccharidosis type I. *Reprod Biomed Online* 2006; 12: 215–20.
7. Montag M, van der Ven K, Delacretaz G, Rink K, van der Ven H: Laser-assisted microdissection of the zona pellucida facilitates polar body biopsy. *Fertil Steril* 1998; 69: 539–42.
8. Scott HS, Litjens T, Nelson PV et al.: Identification of mutations in the alpha-L-iduronidase gene (IDUA) that cause Hurler and Scheie syndromes. *Am J Hum Genet* 1993; 53: 973–86.
9. Kaczala GW, Messer MA, Poskitt KJ, Prendiville JS et al.: Therapy resistant neonatal seizures, linear vesicular rash, and unusually early neuroradiological changes: incontinentia pigmenti: A case report, literature review and insight into pathogenesis. *Eur J Pediatr* 2008; 167: 979–83.
10. Scheuerle AE: Male cases of incontinentia pigmenti: case report and review. *Am J Med Genet* 1998; 77: 201–18.
11. Smahi A, Courtois G, Vabres P et al.: Genomic rearrangement in NEMO impairs NF-kappaB activation and is a cause of incontinentia pigmenti. The International Incontinentia Pigmenti (IP) Consortium. *Nature* 2000; 405: 466–72.
12. Sens B, Stern M, Wiedemann, BH: Qualitätssicherung Mukoviszidose – Überblick über den Gesundheitszustand der Patienten in Deutschland 2000.
13. Rowe SM, Miller S, Sorscher EJ: Cystic fibrosis. *N Engl J Med* 2005; 352: 1992–2001.
14. Yankaskas JR, Marshall BC, Sufian B, Simon RH, Rodman D: Cystic fibrosis adult care: consensus conference report. *Chest* 2004; 125: 1–39.
15. Sumner CJ: Molecular mechanisms of spinal muscular atrophy. *J Child Neurol* 2007; 22: 979–89.
16. Wirth B, Brichta L, Schrank B et al.: Mildly affected patients with spinal muscular atrophy are partially protected by an increased SMN2 copy number. *Hum Genet* 2006; 119: 422–8.
17. Pearn J: Incidence, prevalence, and gene frequency studies of chronic childhood spinal muscular atrophy. *J Med Genet* 1978; 15: 409–13.
18. Iannaccone ST: Modern management of spinal muscular atrophy. *J Child Neurol* 2007; 22: 974–8.
19. Goossens V, Harton G, Moutou C et al.: ESHRE PGD Consortium data collection VIII: cycles from January to December 2005 with pregnancy follow-up to October 2006. *Hum Reprod* 2008; 23: 2629–45.

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