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Preimplantation diagnosis in Switzerland – birth of a healthy child after polar body biopsy

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Summary

In Switzerland preimplantation genetic diagnosis is limited by law to polar body biopsy (PBB). The indications for PBB include unexplained recurrent miscarriage and improvement of the outcome of in vitro fertilisation (IVF) cycles in women at an advanced reproductive age. In this article we report the first birth of a healthy child after polar body biopsy in Switzerland in a case of unexplained miscarriage after IVF.

Key words: preimplantation diagnosis; polar body biopsy; recurrent miscarriage; advanced reproductive age; healthy child

Introduction

Preimplantation genetic diagnosis (PGD) has become a routine clinical tool in many countries outside Switzerland, especially Italy and the USA. The three leading centres in these countries have already reported more than 3000 cycles of PGD, either using blastomere biopsy of an embryo or polar body biopsy (PBB), and more than 500 babies have been born so far [1]. The breakthrough of PGD in Switzerland, however, is still awaited, mainly due to restrictive Swiss federal law on reproductive medicine (*Fortpflanzungsmedizingesetz*, Art. 5), which only allows PBB and prohibits embryo biopsy.

The polar body contains a chromosomal complement of the oocyte. Consequently, genetic investigation of the polar body allows conclusions to be drawn on the chromosomal status of a specific oocyte.

Common indications for PGD of aneuploidies are treatment of unexplained recurrent miscarriage and improvement of outcome after in vitro fertilisation (IVF) in women aged 35 years or over [2, 3]. The rationale behind these indications is that chromosome abnormalities are the major cause of miscarriage [4] and more than half of the oocytes in women at an advanced reproductive age are aneuploid [5]. Hence testing for aneuploidy may enhance the clinical outcome by avoiding the transfer of chromosomally abnormal, i.e. non-viable, embryos.

Material and methods

According to Swiss law on reproductive medicine (*Fortpflanzungsmedizingesetz*, Art. 5), polar body removal for chromosome testing remains the only PGD method in Switzerland, and biopsy of blastomeres from a cleaved embryo is prohibited.

A 38-year-old patient was referred to our clinic after four preclinical miscarriages. All pregnancies were induced in a highly reputed reproductive centre by in vitro fertilisation treatments, i.e. one egg retrieval followed by one transfer of fresh embryos and four replacements of frozen-thawed pronuclear stages. The indication for IVF was primary infertility for 5 years and the presence of mild endometriosis. Peritoneal endometriotic lesions had previously been removed by laser surgery. Ovarian stimulation cycles with gonadotrophins followed by intrauterine insemination were unsuccessful.

Before the patient's arrival in our unit extensive exploration of known miscarriage factors had been carried

Abbreviations

ESHRE	European Society for Human Reproduction and Embryology
FISH	Fluorescent in-situ hybridisation
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilisation
PBB	Polar body biopsy
PGD	Preimplantation genetic diagnosis

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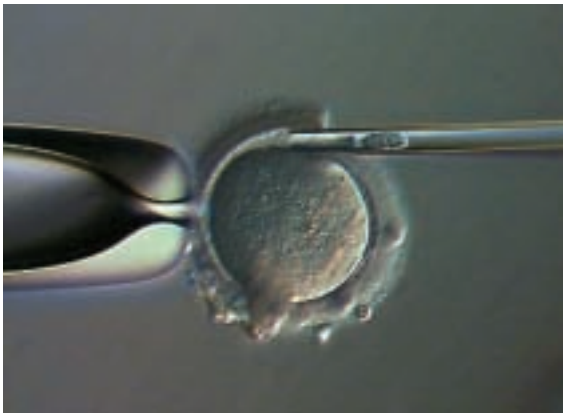

Figure 1

Removal of the first polar body.

out in a dedicated miscarriage clinic. Genital infection was ruled out and all types of potentially detrimental antibody (including anti-cardiolipin) and clotting factors were screened for. Karyotyping of lymphocytes showed normal results in both partners. Irrespective of these findings, the patient was treated in her IVF cycles with low dose aspirin. She sometimes also received low dose heparin.

Tender loving care was discussed as a first option, as this procedure is known to have a significantly beneficial effect on pregnancy outcome, especially in cases of unexplained recurrent miscarriage [6]. Since more than half of all miscarriages have a genetic background [7] and more than half of the oocytes in women aged 35 or over are aneuploid [5], oocyte chromosome testing by polar body biopsy was offered as a second option. The couple were informed extensively about the novelty of this treatment with its inherent potential advantages and limitations. It was explained that this investigation would not exclude any chromosomal abnormalities from the sperm, nor would it guarantee the birth of a healthy child. The couple's agreement was confirmed by written informed consent.

Stimulation and intracytoplasmic sperm injection (ICSI) were carried out as previously described [8]. ICSI was chosen instead of conventional insemination to prevent polyspermia after the creation of a hole in the zona pellucida for extraction of the polar body.

Polar body extraction from oocytes in metaphase II followed the method reported by Montag et al. [9]. In brief, after laser dissection of the zona pellucida (Octax; MTG Medical Technology, Altdorf, Germany) the first polar body was removed. A micropipette with an inner diameter of 20 µm was used to penetrate the opening in the zona and draw out the polar body by slight negative suction (Figure 1).

After removal of the polar body the oocytes were cultured in G-1™ medium (version 3; Vitrolife, Gothenborg, Sweden) under mineral oil at 37 °C and 6% CO_2 in air. Embryo transfer was performed on the morning of the third day after egg retrieval.

Polar bodies were washed in droplets of isotonic HEPES-buffered saline (G-Mops™, Vitrolife) for 5 minutes. The polar bodies were transferred into 2–3 µL of hypotonic solution of 0.56% KCl and 0.193% natrium citrate (3:1), and then placed on a microscope slide (Superfrost, Menzel, Germany) into 0.2 µL of a solution composed of 9 parts distilled water and 1 part of the solution described above. After drying, the polar body was fixed with methanol and glacial acetic acid (3:1) and immediately transported to the cytogenetic institute (Genetica AG, Zurich) for fluorescent in-situ hybridisation (FISH) analysis.

FISH was performed with probes for chromosomes 13,16,18, 21 and 22 in accordance with the manufacturer's instructions accompanying the hybridisation kit (Multi-Vysion Kit, Vysis, Downers Grove, USA). In brief, 3 µl of probes was applied to the polar bodies. The slides were coverslipped and sealed with rubber cement. Codenaturation was performed in HyBrite (Vysis) for 8 minutes at 69 °C followed by overnight hybridisation at 37 °C. Post-hybridization washings were made in 0.7% SSC/0.3% NP-40 (Vysis) for 7 minutes at 73 °C followed by 2×SSC/0.1% NP-40 for 1 minute at room temperature. The slides were air dried and mounted in Antifade II (Vysis). The polar bodies were analysed with a fluorescence microscope (Zeiss Axioplan, Germany) equipped with single bandpass filters for red (chromosome 13), aqua (chromosome 16), bue (chromosome 18), green (chromosome 21) and gold (chromosome 22). The images were analyzed with Quips software (Vysis).

Results and discussion

Follicular aspiration revealed 12 mature oocytes in metaphase II. Removal of the first polar body was successful in 8 cases. One polar body showed a euploid pattern for chromosomes 13, 16, 18, 21 and 22 (Figure 2). In the remaining three polar bodies the situation was aneuploid or unclear. Four polar bodies could not be analysed due to damage to the microscopic slide during transport. The inseminated euploid oocyte was cultivated until day 3 after egg retrieval when the 8-cell embryo was transferred into the uterine cavity. A pregnancy test two weeks later was positive. Ultrasound examination demonstrated an intact intrauterine pregnancy with positive foetal heart action a further two weeks later. The mother was delivered of a healthy boy by caesarean section in the 38th week of gestation.

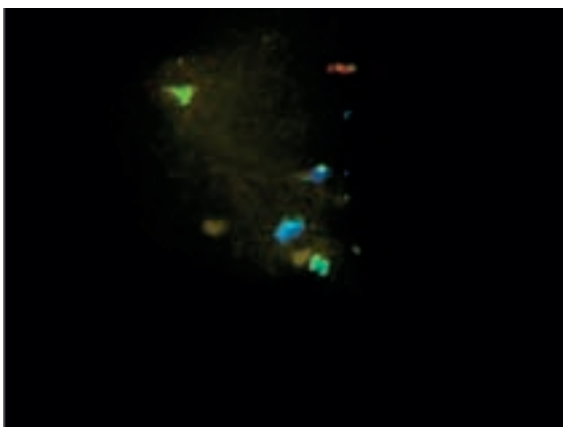

Figure 2

Fluorescent in-situ hybridisation of the polar body with the euploid pattern for chromosomes 13, 16, 18, 21 and 22.

In our unit polar body biopsy was introduced in late 2002. Out of three attempts, one patient became pregnant but this pregnancy ended with a missed abortion in the 8th week of gestation. Seven PBB procedures followed in 2003 with 3 pregnancies. The first was the reported case.

Polar body diagnosis is extremely demanding.

First, the reproductive biologist who must remove the polar body needs a very high degree of micromanipulative skill in order to take the tiny polar body out of the perivitelline space. Second, the geneticist is challenged by having only one chromosomal set for analysis. In addition, as the abovementioned transport problem clearly demonstrates, logistical difficulties must not be underestimated. Due to the possibility of misdiagnosis and the fact that the investigation of only 5 chromosomes does not rule out further chromosomal problems, the couple were informed of the possibility of confirming embryonic euploidy by chorion villus sampling or amniocentesis. However, the couple rejected this option in view of fears that the procedures could induce miscarriage. Counselling of the patient with regard to these limitations of PGD is mandatory.

PGD is applicable to couples at high genetic risk due either to single-gene problems or to chromosomal aberrations. Thus, PGD can prevent the transmission of incurable genetic disorders to offspring, avoiding diseases such as thalassaemia major or cystic fibrosis, and improve the clinical outcome in reproductive medicine (advanced maternal age, repeated IVF failures) as well as in cases of otherwise unexplained recurrent abortions [2, 3].

To monitor the safety of PGD the European Society for Human Reproduction and Embryology (ESHRE) formed a consortium in 1997 to undertake a long-term study of the efficacy and clinical outcome of preimplantation genetic diagnosis. In their third report, the consortium stated that the obstetric and foetal outcomes observed after PGD were comparable to an equivalent cohort of ICSI pregnancies and children without PGD [10]. This observation was confirmed specifically for PBB by the work of Strom et al. [11].

In Switzerland, PGD is limited by law to PBB. The advantages of polar body diagnosis over genetic investigation of removed embryonic blastomeres are principally ethical in nature. There is widespread fear that (mis)use of blastomere biopsy could serve to create so-called designer babies or induce germ line therapy. However, in view of these serious reservations, the legal rules surrounding these techniques offer far better long-term solutions than mere prohibition. In addition, there is growing pressure from the patients' side for review of the restrictive legal situation in Switzerland [12], since blastomere biopsy of an embryo does not confine genetic information to the oocyte but also provides conclusive information on a sperm's contribution to the embryonic genome. This would allow investigation of the genetic problems of an embryo originating not only from the female but also from the male side. Finally, affected couples can easily circumvent prohibition in Switzerland by travelling to countries where blastomere biopsy is available, e.g. Belgium.

Two approaches are currently under study with a view to improving the diagnostic reliability of PGD. The first method is to increase the number of available chromosome sets by removing not only the first but also the second polar body and, if legally permitted, one or two embryonic blastomeres in addition [13]. The second technique aims to enlarge the number of chromosomes tested [13]. Although plausible in theory and already widely used, the clinical value of PGD still needs to be confirmed by prospective randomised trials.

In conclusion, PGD offers new treatment options in difficult situations such as unexplained recurrent miscarriage. However, the restrictive legal situation in Switzerland limits PGD to polar body biopsy, thus restricting the availability of genetic information to the female side only.

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References

1 International Working Group on Preimplantation Genetics. Preimplantation genetic diagnosis: experience of 3000 clinical cycles. Reprod Biomed Online 2001;3:49–53.
2 Munné S. Preimplantation genetic diagnosis and human implantation – A review. Placenta 2003;24:70–6.
3 Gianaroli L, Magli MC, Fiorentino F, Baldi M, Ferraretti AP. Clinical value of preimplantation genetic diagnosis. Placenta 2003;24:77–83.
4 McFadyen IR. Early fetal loss. In Rodeck C (Ed.) Fetal medicine 1989:26–43. Blackwell Scientific Publishers Oxford, UK.
5 Kuliev A, Cieslak J, Ilkevitch Y, Verlinsky Y. Chromosomal abnormalities in a series of 6733 human oocytes in preimplantation diagnosis for age-related aneuploidies. Reprod Biomed Online 2002;6:54–9.
6 Clifford K, Rai R, Regan L. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. Hum Reprod 1997;12:387–9.
7 Hassold T, Chiu D. Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. Hum Genet 1985;70:11–7.
8 Imthurn B, Macas E, Rosselli M, Keller PJ. Nuclear maturity and oocyte morphology after stimulation with highly purified follicle stimulating hormone compared to human menopausal gonadotrophin. Hum Reprod 1996;11:2387–91.
9 Montag M, van der Ven K, van der Ven H. Erste klinische Erfahrungen mit der Polkörperdiagnostik in Deutschland. J Fertil Reprod 2002;12:7–12.
10 ESHRE PGD Consortium Steering Committee. ESHRE Preimplantation Genetic Diagnosis Consortium: data collection III (May 2001). Hum Reprod 2002;17:233–46.
11 Strom CM, Levin R, Strom S, Masciangelo C, Kuliev A, Verlinsky Y. Neonatal outcome of preimplantation genetic diagnosis by polar body removal: the first 109 infants. Pediatrics 2000; 106:650–3.
12 Moser H, Tamburlini U. Einmal erlaubt – ein andermal verboten. Für eine einheitliche Regelung der Pränataldiagnostik. Neue Zürcher Zeitung 2003;280:15.
13 Wells D, Escudero T, Levy B, Hirschhorn K, Delhanty JD, Munne S. First clinical application of comparative genomic hybridization and polar body testing for preimplantation genetic diagnosis of aneuploidy. Fertil Steril 2002;78:543–9.

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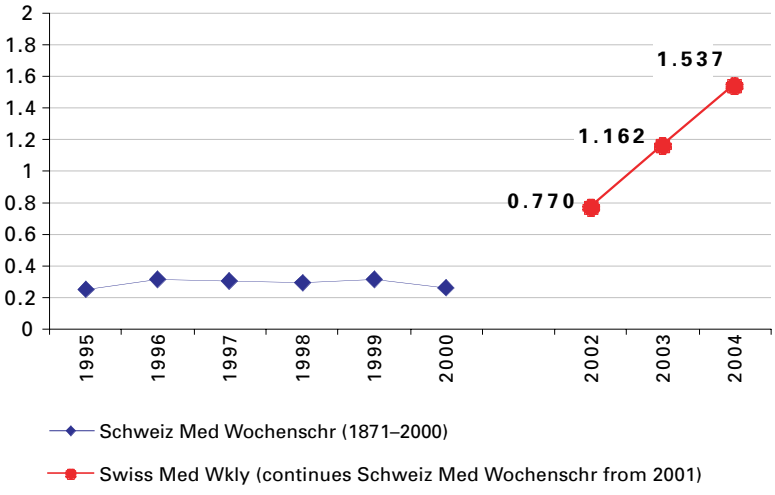

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