

Journal für

Reproduktionsmedizin und Endokrinologie

– Journal of Reproductive Medicine and Endocrinology –

Andrologie • Embryologie & Biologie • Endokrinologie • Ethik & Recht • Genetik
Gynäkologie • Kontrazeption • Psychosomatik • Reproduktionsmedizin • Urologie



Polar Body Diagnosis for Monogenic Disorders in Regensburg

Hehr A, Seifert B, Hehr U, Bals-Pratsch M

J. Reproduktionsmed. Endokrinol 2009; 6 (1), 27-31

www.kup.at/repromedizin

Online-Datenbank mit Autoren- und Stichwortsuche

Offizielles Organ: AGRBM, BRZ, DVR, DGA, DGGEF, DGRM, D-I-R, EFA, OEGRM, SRBM/DGE

Indexed in EMBASE/Excerpta Medica/Scopus

Krause & Pachernegg GmbH, Verlag für Medizin und Wirtschaft, A-3003 Gablitz



ENDO FERTI FORUM

ENDOKRINOLOGIE & FERTILITÄT
FÜR KLINIK & PRAXIS

20.-21. März 2026

Universitätsmedizin Mainz

Einladung zu unserer wissenschaftlichen Veranstaltung Endo-Ferti-Forum

Brücke(n) zwischen Unikliniken und Praxen an Rhein und Main(z)

– die aus dem bisherigen Format „Ferti Forum“ ab 2026 hervorgeht –



Freuen Sie sich auf spannende Vorträge und den lebendigen Austausch mit Kolleg:innen und Expert:innen aus Klinik und Praxis. Freitagabend laden wir Sie herzlich zu einem entspannten Empfang ein – eine perfekte Gelegenheit, Kontakte zu knüpfen und den Tag genussvoll ausklingen zu lassen.

Wissenschaftliche Leitung: Univ.-Professorin Annette Hasenburg, Dr. Susanne Theis, Universitätsmedizin Mainz, Sanitätsrat Dr. Werner Harlfinger, BVF Rheinland-Pfalz Dr. Rüdiger Gaase, BVF Hessen Dr. Klaus J. Doubek

Schirmherrschaften: Prof. Nicole Sänger, Uniklinik Bonn, Prof. Jan-Steffen Krüssel, Uniklinik Düsseldorf, Dr. Annette Bachmann, Uniklinik Frankfurt am Main, Prof. Christine Skala, Uniklinik Köln

Weitere Informationen
& Anmeldung unter



Polar Body Diagnosis for Monogenic Disorders in Regensburg

A. Hehr¹, B. Seifert², U. Hehr^{1,3}, M. Bals-Pratsch²

Polar body diagnosis (PBD) is currently the only legal option to perform a preimplantation genetic diagnosis (PGD) in Germany. The results of PBD for monogenic disorders performed at our center in Regensburg since 2001 are reported. Our data show that PBD can be safely performed on first and second polar bodies within the tight timeframe provided by the German Embryo Protection Act. It requires extensive interdisciplinary counseling of the couple, good and close cooperation between the IVF center and the genetics laboratory as well as meticulous development, validation, and performance of the individual genetic assay. Provided that these prerequisites are met, PBD can today be an acceptable option for German couples at high risk for a particular monogenic disorder in their offspring. Main arguments pro PBD usually include a decline of both conventional prenatal diagnosis and subsequently induced abortion of an affected offspring as well as the birth of an affected child. Major disadvantages of PBD in this situation include the requirement of assisted reproduction for couples in the absence of any obvious fertility problems with their immanent obstacles like pregnancy rate, remaining recurrence risk for the particular monogenic disorder, costs etc. Furthermore, PBD can only be offered for mutations, which are passed on by the female partner with her nuclear DNA (autosomal dominant, X-chromosomal as well as autosomal recessive traits). For heterozygous female mutation carriers of autosomal recessive or X-chromosomal inherited disorders PBD requires discarding all oocytes carrying the mutation, although they may result in healthy offspring if the sperm does not carry the mutation or the Y chromosome, respectively. Finally, both PBD as well as PGD can substantially reduce the recurrence risk for a particular monogenic disorder but not diminish it entirely. Therefore, conventional prenatal diagnosis (PND) should still be offered and in fact has been applied in three of seven pregnancies achieved at our center. **J Reproduktionsmed Endokrinol 2009; 6 (1): 27–31.**

Key words: polar body diagnosis, PBD, preimplantation genetic diagnosis, PGD, prenatal diagnosis, PND, monogenic disorder, PCR, polymorphic markers, ICSI

■ Introduction

The first embryo transfer after polar body diagnosis (PBD) for a monogenic disorder as a very early form of preimplantation genetic diagnosis (PGD) was reported by Verlinsky and his colleagues in 1990 [1]. In several countries PGD for both, monogenic as well as chromosomal conditions, is today an accepted tool for a restricted patient cohort to realize their family planning. More than 15,000 prospective PGD cycles have been registered by the ESHRE PGD consortium so far (data collections I–VIII up to December 2005). However, PBD cycles still account in the ESHRE data collection for less than 1 % [2]. In Germany PBD is currently the only legal option to perform PGD. We summarize the current status on PBD for monogenic disorders in Germany, the results at our center in Regensburg and the suggested practical approach for German couples interested in PBD for monogenic disorders.

■ Prerequisites

Legal Regulations in Germany

The handling of gametes is strictly regulated within the framework of the Ger-

man Embryo protection act [3] and recently in addition has been extensively reviewed [4]. Although PGD is not directly addressed in the act, prohibition of any form of genetic testing of blastomeres (cells of the blastocyst, e.g. 8 cell stage embryo) can be assumed based on the immanent exclusion from further development and subsequent disposal of blastocysts identified to carry the disease causing mutation(s). Likewise, PBD is not a direct issue of the Embryo protection act. However, in contrast to PGD, genetic diagnosis by PBD is carried out on polar bodies of oocytes and not on embryos according to the legal text (prior to fusion of the pronuclei) hence allowing disposal of mutant pronuclear stage (PN stage) oocytes. Furthermore, PBD was on the agenda of the National Ethics Council in 2004, which confirmed general compliance with both ethical as well as German legal regulations at least for the application for monogenic disorders (see <http://www.nationalerethikrat.de>) [5]. However, PBD still requires the handling of impregnated oocytes immediately before the fusion of the nuclei (emergence of a human embryo), and therefore any PBD application should be performed with greatest care, consider-

ing not only the clinical indication for each individual case, but also potential alternatives for the couple, accuracy of the testing procedure as well as the general ethical context. The development of national guidelines is desirable.

Medical Requirements

Considering these ethical as well as genetic aspects, each PBD should be accompanied by extensive interdisciplinary counseling of the couple prior to, during as well as after performed PBD:

- Genetic counseling should address age at the onset, clinical course, phenotypic spectrum of the particular monogenic disorder as well as the recurrence risk according to the Mendelian trait and potential alternatives including regular PND to realize family planning.
- Reproductive medicine should assess potential fertility problems of both partners (ultrasound, hormone and semen analyses) and explain the procedure of assisted reproductive techniques (ART) by intracytoplasmatic sperm injection (ICSI) as well as its risks and success rates considering the individual couple according to the current guidelines of the German Medical Association [6].

Received: December 30, 2008; accepted after revision: January 19, 2009.

From the ¹Center for Human Genetics, the ²Fertility Center Regensburg and the ³Department of Human Genetics, University of Regensburg, Regensburg, Germany

Correspondence: PD Dr. med. Ute Hehr, Center for and Department of Human Genetics, University of Regensburg, D-93040 Regensburg, Universitätsstraße 31; e-mail: ute.hehr@humangenetik-regensburg.de

Genetic requirements for PBD for monogenic disorders are:

- Identified disease causing mutation in the women planning to undergo PBD, which frequently is preceded by mutation detection in an index case of the family affected by the particular disorder. PBD only allows exclusion of mutation-positive impregnated oocytes from further development. Therefore, PBD in general is applicable for any Mendelian disorder, for which the mother is a heterozygous mutation carrier: e.g. any autosomal dominant, autosomal recessive or X-chromosomal inherited disorder. PBD cannot be offered for mitochondrial diseases, homozygous or compound heterozygous female mutation carriers of an autosomal recessive disease or heterozygous male mutation carriers of an autosomal dominantly inherited disorder.
- Mutation testing using only closely linked informative markers (indirect PBD) in addition requires blood or DNA sample(s) of at least one further close relative with known mutation status.
- Genes localized in complex genomic regions, e.g. with high genomic instability and/or large duplicated genomic regions may not be suitable for PBD.

Financial Requirements

Molecular genetic testing of polar bodies for monogenic disorders is not part of the regular list of services covered by the public health insurance funds. Couples can apply for individual cost coverage but have no legal claim. The cost situation for privately insured couples needs to be explored by each individual case.

■ How To Proceed

For couples interested in PBD who meet the requirements, we recommend the following steps:

1. Personal contact to a human genetics center offering PBD for monogenic disorders to discuss if testing for the particular disorder and mutation for this family will be available. In addition, further detailed information can be obtained upfront e.g. through the homepage of our center (<http://www.humangenetik-regensburg.de> and <http://www.kinderwunsch-regensburg.de>).
2. As a next step we recommend personal contact of the couple either at an ART center close to their hometown or as a combined personal contact both at our human genetics center as well as the ART center in Regensburg to assess in detail the genetic as well as medical situation prior to a decision of the couple for PBD. At our center the couple will also receive detailed information on genetic testing and on assisted reproduction both verbally as well as with a written consent form. In addition, further information regarding the individual situation of the couple including potential cost coverage may be passed on.
3. If the couple decides to proceed with PBD, we require at our center submission of the signed consent form for testing, assisted reproduction and cost coverage and then will start to develop the individual family-specific genetic testing assay within a timeframe of 3–6 months.
4. The couple and the ART center in Regensburg will be informed as soon as the family-specific genetic assay has been established to arrange parallel personal contact at both the genetic as well as the ART centers to obtain a fresh blood sample of the woman for determination of the allele dropout rate and to plan the first ICSI cycle. If stimulation is considered at an ART center close to her hometown, all necessary information will be passed on to the respective colleagues by phone and treatment will be coordinated by the ART center in Regensburg.
5. In the genetic laboratory we will perform a validation of the established test system using Fluorescence-Activated Cell Sorting (FACS) for single cells from a fresh blood sample of the patient. If necessary we will further optimize the multiplex Polymerase Chain Reaction (PCR) for single-cell application and finally calculate the individual allele dropout rate. As soon as the system is ready for use the couple as well as the participating ART center(s) will be informed in order to plan the first ART cycle in close contact between the couple, the human genetics laboratory and the ART center(s).
6. In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) as a prerequisite for PBD are not different from any regular IVF-ICSI

cycle performed for couples with fertility problems (for further details see <http://www.kinderwunsch-regensburg.de>).

All necessary additional genetic testing is done after oocyte retrieval in the ART and/or genetics laboratory. However, due to the additional rejection of PN-stage oocytes based on their genetic status and/or failed genetic diagnosis, the number of transferable PN-stages will potentially be lower when compared to a regular IVF-ICSI cycle without PBD. Hence, particularly in case of a low number of initially available oocytes the number of cycles without transfer will be higher.

7. Further treatment of the patients after oocyte retrieval and transfer can again take place in the collaborating ART center close to their hometown. In case of a pregnancy, again extensive genetic counseling of the couple should be offered regarding the PBD-immunant remaining risk of about 3 % for the recurrence of the particular monogenic disorder and the available options for prenatal testing.

■ Establishing the Family-Specific Assay System

Main reasons for the low availability of PBD for monogenic disorders in Germany are the tight timeframe and the very time- and cost-intensive development and implementation of specific assays (including 24-h work and night shifts).

If a couple contacts our center for PBD the procedure first starts with a check-up of the principal feasibility in this specific setting based on the medical findings (already known mutation in the family) and sequence information of the respective gene. Especially a high degree of homology to other genes of the same gene family, pseudogenes and repetitive sequences will render a polar body diagnosis impossible.

Whenever possible a direct diagnostic assay is preferred (Fig. 1), consisting of 2 closely linked polymorphic (variable number tandem repeats = VNTR) markers and direct detection of the mutation with a suitable method (e.g. length polymorphism in case of a repeat disorder or mini-sequencing). If direct detection of the mutation is impossible (e.g. for large

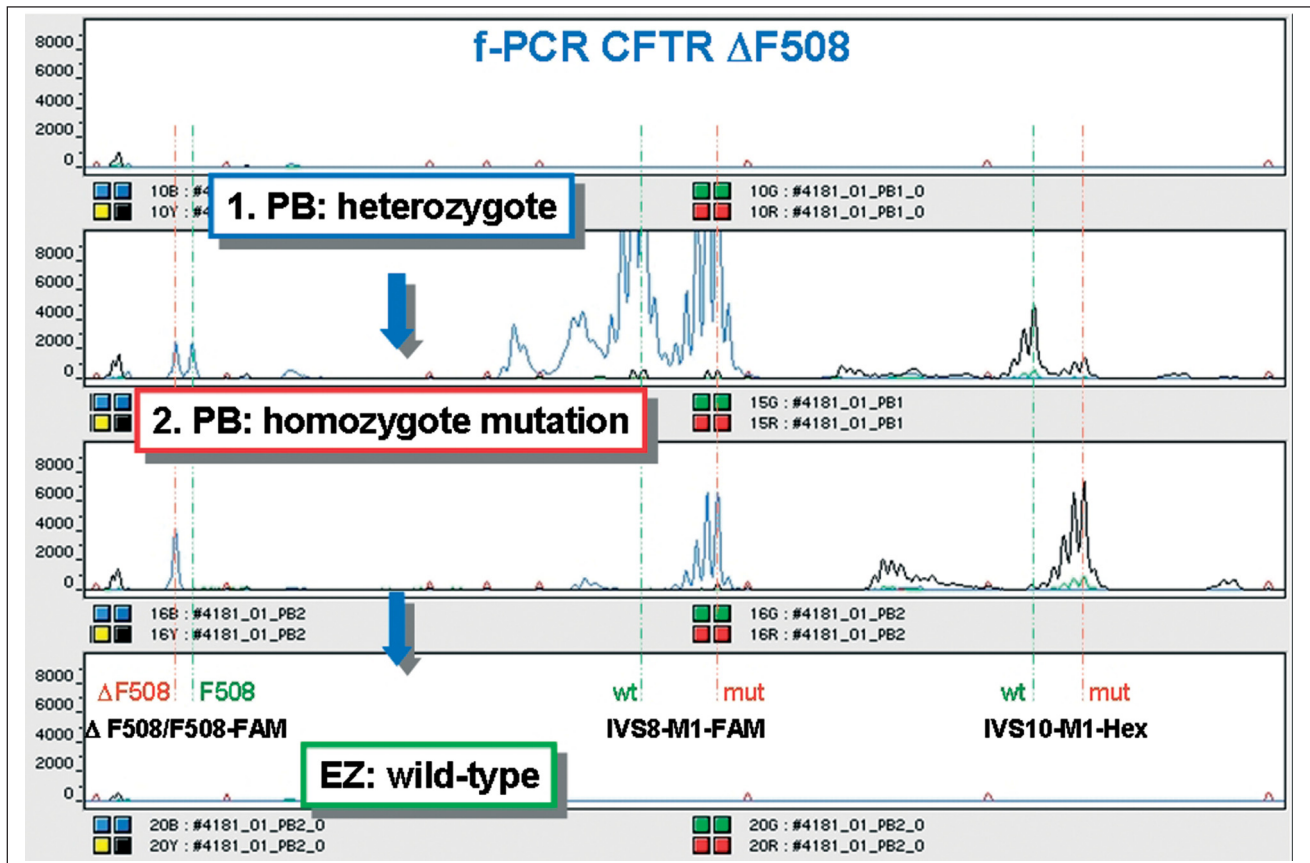


Figure 1: Family-specific assay system with multiplex PCR for direct mutation detection of CFTR Δ F508 and two polymorphic markers located close to the mutation-example of a complete diagnosis for one oocyte: Wash buffer control from first and second PB (first and last row respectively), results of the first and second PB (second and third row), from left to right alleles of the DF508 mutation and wild-type (blue), linked polymorphic marker IVS8-M1-FAM (blue) and IVS10-M1-HEX (black), the mutant alleles are labeled with a dotted red line, the wild-type alleles with a dotted green line, respectively.

deletions, insertions or repeat expansions) an indirect diagnosis can be performed using at least 3, but preferably 4, closely linked polymorphic markers.

For this purpose, commercially available VNTR markers can be selected or new potential polymorphic VNTR markers will be searched based on the known DNA sequences. Suitable markers should be located as closely as possible to the mutation of interest to minimize cross-over events between the mutation and the VNTR markers and should allow for a reproducible amplification starting with a single haploid cell. An efficient and reproducible amplification is necessary in order to get a low allele dropout rate. For the selection of the respective markers the frequency of heterozygous in the population is negligible. The main criterion is whether the particular patient is heterozygote for this marker or not. After selecting markers the allele segregation of these markers within the family has to be established to determine which of the two alleles of the female patient is

linked to the mutation or the wild-type allele, respectively.

In the next step suitable primers are tested in multiplex PCR reactions. Testing and optimization of these multiplex PCR reactions for high reproducibility and amplification efficiency is the most laborious part of the assay set-up. Starting with single cells of a control person and the patient, different parameters of the PCR reaction are optimized (e.g. annealing temperature, magnesium concentration, buffer, different Taq polymerases, several PCR enhancers etc.)

Different methods are available for direct mutation detection. A major criterion in Germany is the tight timeframe. While small insertions and deletions can be easily detected using fluorescently labeled primers to visualize the length polymorphism generated by the mutation, point mutations need a different detection method. In our laboratory a mini-sequencing application (SnapShot kit, Applied Biosystems) is used to get a reli-

able information about the nucleotide at the mutation site within the given timeframe [7].

The final assay should give a reproducible result and even amplification of all alleles of all markers with a low ADO (allele dropout) rate that can be performed for approximately 10–15 first and second polar bodies within a timeframe of approximately 12 hours, respectively. Due to the very small amount of DNA, ADO events are unavoidable. However, precaution can be taken to minimize the number of ADO events. Furthermore, analysis of several polymorphic markers per cell allows detecting ADO events by combining the results of all markers.

Assisted Reproduction

After the establishment of an appropriate PBD assay, regular hormonal stimulation of the patient is scheduled and started preferentially at an ART center close to the patient's hometown in close con-

sultation with the ART center in Regensburg. Oocyte retrieval, polar body biopsy, and embryo transfer will then be performed at the ART center in Regensburg. The polar bodies are extracted sequentially one and ten hours after ICSI. Immediately after extraction, the polar bodies will be transferred to the molecular genetics laboratory. Subsequent cell lysis and setup of the multiplex PCR are carried out adjacent to the IVF laboratory in a separate room, in which no DNA or cyclized PCR products are handled. A comprehensive package of security measures such as sterile disposable operating room laboratory coats, operating room caps and mouth protection ensure that contaminations through laboratory employees (for example hair, dandruff, etc.) do not interfere with the examination results.

No later than 22 hours after oocyte retrieval, the genetic results of first and second polar bodies are available and evaluated in order to recommend those pronuclear stages for continued culture, which do not carry the respective familial specific mutation. Embryo transfer is then performed according to standard recommendations two days after oocyte retrieval. Further counseling of the couple, treatment after transfer, and the pregnancy test can be carried out again by the ART center near the hometown. During pregnancy PND should be offered and was performed for 3 of the 7

pregnancies in our cohort to exclude the remaining recurrence risk after PBD for the respective monogenic disorders.

Discussion

At our own center, we have so far applied PBD for 16 female mutation carriers for nine monogenic disorders (cystic fibrosis, Huntington's disease, tuberous sclerosis, fragile-X-syndrome, neurofibromatosis, Norrie disease, spinocerebellar ataxia type 1, myotonic dystrophy, and dystrophinopathies). In each cycle, both polar bodies were sequentially removed and genetically assessed within 22 hours after oocyte retrieval. In our experience, evaluation of both polar bodies allows to independently confirm the results of the first polar body in case of a hemizygous situation. But, more importantly, oocytes with a heterozygous first polar body can only be diagnosed in the presence of an accurate diagnosis for the second polar body and otherwise would need to be discarded. Table 1 gives the results of 35 PBD cycles performed at our center from 2001–2008 in comparison to the results of 500 PGD cycles for the calendar year 2005 published by the ESHRE PDG Consortium [2]. In our PBD program 261 oocytes were biopsied and 145 oocytes could be diagnosed. The rate of positive pregnancy tests per transfer was similarly as high with 39.1 % as in the PGD group on blastomeres with 38.5 %. In

contrast to the genetic diagnosis on blastomeres, the clinical pregnancy and birth rates after PBD were significantly higher with 30 % per transfer versus 27 % and 23 %, respectively. One third-trimester pregnancy was included in the calculation of the birth rate. Six patients gave birth to 8 healthy children and one patient with neurofibromatosis has a third-trimester ongoing pregnancy. Five children after PBD for cystic fibrosis (1 twin pregnancy) and three children after PBD for Norrie disease (1 twin pregnancy) were born. The good preliminary pregnancy rate after PBD in our patient cohort when compared to PGD may be in part explained by the arguments that (I) the integrity of the embryo is not injured through the removal of the polar bodies and (II) the in vitro culture of the embryos does not have to be extended from two to five or six days as a potential additional interference with the developing embryo. This thesis is supported by recent data from Mastenbroek et al [8], who reported a lower pregnancy rate after PGD on blastomeres in comparison to control groups without PGD. For a successful PGD and PBD with subsequent pregnancy, an adequate number of intact oocytes is necessary. All patients who gave birth to children after PBD at our center were younger than 37 years (median age at conception: 33 years). However, even in those younger women the very particular monogenic disorders, which prompt PBD, may at the same time be associated with a reduced ovarian reserve. In particular heterozygous female carriers of the Fragile-X repeat expansion are known to carry a substantially increased risk for premature hypergonadotropic ovarian failure. Furthermore, PBD for X-chromosomal as well as autosomal recessive disorders requires discarding of substantially more oocytes when compared to PGD, because the paternal genetic contribution can not be considered. In the presence of a reduced number of available oocytes PBD therefore can result in a higher number of cycles without embryo transfer, an important disadvantage of PBD when compared to PGD, which should be discussed with the couple particularly in the setting of older women and/or a known reduced ovarian reserve. We recently reported the first clinical pregnancy after PBD on vitrified oocytes [9]. Whether this additional vitrification of oocytes may be suitable to

Table 1: Results for PGD cycles performed for single gene disorders in Regensburg and in the ESHRE PDG Consortium [2]

	PBD Regensburg	PGD Consortium 2008
cycle to OR	35	500
number infertile	16	106
female age (years)	33	33
COCs	426	6716
injected (MII oocytes)	268	5588
PN stages/fertilized	145	3979
transferred embryos	41	715
cycles to ET	23	405
hCG positive (> 5 IU/l)	9	156
positive heart beat	7	109
clinical pregnancy rate (% per OR)	20	22
clinical pregnancy rate (% per ET)	30	27
number fetal heart beat	9	145
implantation rate ¹	22	20
deliveries ²	7	95
delivery rate (% per ET)	30	23

PGD, preimplantation genetic diagnosis; PBD, polar body diagnosis; OR, oocyte retrieval; COCs, cumulus-oocyte complexes; MII oocytes, metaphase II oocytes; PN stages, pronuclear stage oocytes; ET, embryo transfer. ¹fetal hearts/100 embryos transferred. ²Ongoing third-trimester pregnancy included

collect oocytes from several successive oocyte retrieval cycles before the PBD cycle foremost in older patients with reduced ovarian reserve and hence improves the PBD results still needs to be demonstrated.

We show that PBD for monogenic disorders on first and second polar bodies can be performed under the tight regulations of the German Embryo protection act and today is an accepted option for a small cohort of informed families. The remaining recurrence risk after PBD needs to be addressed and requires offering of conventional PND in a subsequent pregnancy. Further studies are necessary to assess if PBD may perhaps result in a higher pregnancy rate than PGD because of the preserved integrity of the embryo. However, the obvious disadvantages of PBD, in particular the lack of access to the paternal genetic information, should prompt reconsiderations to generate legal premises for PGD in Germany. This may be part of a larger national initiative to prospectively collect and evaluate

data on all PBD cycles in Germany for both monogenic as well as chromosomal conditions and bring together the expertise and efforts of all active German PBD centers to develop national standards covering all medical and methodical aspects of PGD.

■ Practical Aspects

The results of 35 polar body diagnosis (PBD) cycles performed at our center confirm that this method is a valuable tool for preimplantation diagnosis (PGD) in families at risk of transmitting a monogenic disorder. Although our experience with PBD cycles is limited, we found that the delivery rate is higher after PBD compared to PGD with blastomere biopsy (30 vs. 23 %). Furthermore, to increase the number of oocytes available for a PBD cycle in women with a diminished ovarian reserve, vitrification of oocytes collected from several preceding stimulation cycles has been carried out successfully.

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. Goossens V, Harton G, Moutou C, Scriven PN, Traeger-Synodinos J, Sermon K, Harper JC; European Society of Human Reproduction and Embryology PGD Consortium. 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.
3. Gesetz zum Schutz von Embryonen. 13. Dezember 1990 (BGBl. I 1990, S. 2746), aktuelle Fassung 23. Oktober 2001 (BGBl. I 2001, S. 2702).
4. Taupitz J, Kaiser P, Günther HL. Embryonenschutzgesetz. Juristischer Kommentar mit medizinisch-naturwissenschaftlichen Einführungen. Kohlhammer Verlag, Stuttgart, 2008.
5. Polkörperdiagnostik. Stellungnahme des Nationalen Ethikrates. Berlin 16. Juni 2004.
6. (Muster-)Richtlinie zur Durchführung der assistierten Reproduktion – Novelle 2006 –. *Deutsches Ärzteblatt* 2006; 103: 1392–403.
7. Hehr U, Gross C, Paulmann B, Seifert B, Hehr A. Polkörperdiagnostik für monogene Erkrankungen am Beispiel von Chorea Huntington und Norrie-Syndrom. *Medgen* 2004; 16: 37–42.
8. Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, Vogel NE, Arts EG, de Vries JW, Bossuyt PM, Buys CH, Heineman MJ, Repping S, van der Veen F. In vitro fertilization with preimplantation genetic screening. *N Engl J Med* 2007; 357: 9–17.
9. Bals-Pratsch M, Hehr A, Seifert B, Hehr U. Geburt nach Eizell-Vitrifikation und Polkörperdiagnostik für Mukoviszidose. *Geburtsh Frauenheilk*, im Druck.

Mitteilungen aus der Redaktion

Besuchen Sie unsere Rubrik

☒ Medizintechnik-Produkte



Neues CRT-D Implantat
Intica 7 HF-T QP von Biotronik



Artis pheno
Siemens Healthcare Diagnostics GmbH



Philips Azurion:
Innovative Bildgebungslösung

Aspirator 3
Labotect GmbH



InControl 1050
Labotect GmbH

e-Journal-Abo

Beziehen Sie die elektronischen Ausgaben dieser Zeitschrift hier.

Die Lieferung umfasst 4–5 Ausgaben pro Jahr zzgl. allfälliger Sonderhefte.

Unsere e-Journale stehen als PDF-Datei zur Verfügung und sind auf den meisten der marktüblichen e-Book-Readern, Tablets sowie auf iPad funktionsfähig.

☒ Bestellung e-Journal-Abo

Haftungsausschluss

Die in unseren Webseiten publizierten Informationen richten sich **ausschließlich an geprüfte und autorisierte medizinische Berufsgruppen** und entbinden nicht von der ärztlichen Sorgfaltspflicht sowie von einer ausführlichen Patientenaufklärung über therapeutische Optionen und deren Wirkungen bzw. Nebenwirkungen. Die entsprechenden Angaben werden von den Autoren mit der größten Sorgfalt recherchiert und zusammengestellt. Die angegebenen Dosierungen sind im Einzelfall anhand der Fachinformationen zu überprüfen. Weder die Autoren, noch die tragenden Gesellschaften noch der Verlag übernehmen irgendwelche Haftungsansprüche.

Bitte beachten Sie auch diese Seiten:

Impressum

Disclaimers & Copyright

Datenschutzerklärung