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Polar Body Analysis – Current Clinical Practice and New Developments for Preimplantation Genetic Screening and Diagnosis

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Polar body analysis is an established procedure identifying oocytes with a high risk of an inherited disorder or with an individual mostly age related chromosomal aneuploidy. New insight into human oocyte meiotic division has come along with controversial discussions about the benefits of the application for screening or diagnostic purpose. New diagnostic methods are requested to prove their value for this very unique material and exclusive approach. **J Reproduktionsmed Endokrinol 2009; 6 (1): 32–4.**

Key words: polar body analysis, aneuploidy, chromosomal translocation, FISH, CGH array, preimplantation genetic screening, PGS, PGD

■ Evolution of Polar Body Analysis

Polar body (PB) analysis has emerged as the ethically tolerated alternative of preimplantation genetic diagnosis (PGD) or preimplantation genetic screening (PGS) which is in practice since the early 1990s [1]. PGD was developed to avoid pregnancies with a significant high risk for miscarriage, abortion or morbidity due to monogenetic disorders or chromosomal rearrangements in the family. It is regarded as the earliest form of prenatal diagnosis by direct testing of embryonic material. PGD is performed on one or two blastomeres taken from the embryo. PB analysis is an indirect method to evaluate the oocyte, which is representing only the maternal contribution to the embryo. Meiotic nondisjunction of chromosomes due to advanced maternal age attribute considerably to a poor pregnancy outcome. Aneuploidies in oocytes have been known for many years to be one of the key factors for unsuccessful artificial reproductive technique (ART) cycles particularly for women above at least 38 years or more [2]. Large series of aneuploidy screening have shown that more than at least 50 % of oocytes carry aneuploidies of one or more chromosomes, depending on maternal age and a variety of other factors, most of which are not known [3]. The aetiology of aneuploidies following the meiotic divisions still seems rather unclear. Whereas advanced

maternal age patients suffer more often from premature sister chromatid separation, patients with recurrent reproductive failure seems to be prone for chromosomal nondisjunction [4]. In due course PGD and PB analysis evolved to a screening method for aneuploidy diagnostics for advanced maternal age patients undergoing in vitro fertilisation (IVF). IVF is necessary in any case for PB analysis or PGD even for fertile couples with the family genetic burden. The logic was to make it available for infertile patients, with an individually increased risk. Even without family risk patients long for better chances in IVF outcome. Furthermore the specialists of the reproductive medicine units are looking for means to improve poor pregnancy rates and decrease high miscarriage rates in couples beyond the age of 38.

Today according to the ESHRE data collection, which was set up and expanded over the last 10 years 65 % of all PGD cycles were performed for aneuploidy screening [5]. That includes the vastest data collection on the topic with more than 3300 cycles from 39 centres for 2005. It also includes 334 cycles with PB analysis [6]. The pregnancy rates however are disappointing, even in the patient's cohort with a family running genetic burden (monogenetic or chromosomal rearrangement). For all indications the overall pregnancy rate per oocyte retrieval was 19 % and per embryo transfer 26 % [6].

The benefits for aneuploidy screening are controversial [7] since quite some time, particularly because there are no significant increased pregnancy rates noted, neither for PB analysis nor for PGD. One randomised control trial (RCT) lately proved "PGD performed" to be less successful than "PGD not performed" in regard to pregnancy rates. More than 400 patients (36–41 years of age) were recruited and evaluated in more than 800 cycles, leading to ongoing pregnancy rates of 25 % for "PGD performed" vs 37 % in "PGD not performed" [8]. A multitude of reasons were discussed, i.e. damage to the embryo, undetected mosaicism, wrong chromosomes tested, etc., but most of these seem not to apply for PB analysis [8, 9]. However PB analysis does not show the paternal genetic input, but this is estimated to be neglectable for aneuploidy screening. So far no RCT could prove a more beneficial outcome for patients undergoing PB analysis. However one study reported a markedly improved outcome in IVF more than 70 % ongoing pregnancy rate by testing both polar bodies of the oocyte and one blastomere of the respective embryo [10]. Even vitrification did not seem to compromise this enhancement [11].

■ Performance of PB Analysis

Both meiotic divisions of the oocytes succeed in the extrusion of the first and the second PB respectively. The chromosomal content is divided ideally by

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half, which leaves the first PB with a set of 23 haploid double-stranded chromosomes and the second PB with a set of 23 single-stranded chromosomes. Both PB can be extracted individually or in one step, following the opening of the Zona pellucida by laser light or chemical treatment.

One or both PBs are transferred on slides or into tubes and can be analysed by several methods. Irrespective of the following analytical steps, the analysis remains an indirect diagnostic approach, since the oocyte by itself is not analysed at all. PB biopsy was first described in 1990 by Verlinsky et al. [12] at the Reproductive Genetics Institute in Chicago, which still is the centre with the most experience in the world. Apart from this, there are only a handful centres outside Germany which perform PB analysis. In Germany according to a questionnaire and the results published in 2006 [13] there were 9 centres sharing their experiences, but there are certainly more ART clinics performing PB screening as an optional service. In the near future the German registry for IVF (DIR) aims to include the PB analysis and results in their database. But as yet there is no conclusive evidence of the success rates of PB analysis in Germany or elsewhere.

Certainly the most common way for aneuploidy screening is the fluorescence in situ hybridisation (FISH) method, using 5 probes for the chromosomes 13, 16, 18, 21 and 22. A few centres (including ours) extended the analysis to a set of up to 10 probes by performing two adjacent rounds of FISH. First round for chromosomes 13, 16, 18, 21, 22 and X, and second round for chromosomes 3, 7, 8 and 15, but this could vary. The result of the analysis needs to be finalised approx. 20 h after sperm injection, just right in time for the pronuclei morphological evaluation and before merging of the two pronuclei.

FISH is also a standard procedure for individual patients with maternal translocations and a high risk of unbalanced segregation of parts of the translocated chromosomes. Many years back first noted but still controversial whether there is an additional higher risk for non-disjunction of whole chromosomes in those cases [14, 15]. During first meiosis the diploid chromosomes recombine by lying next to each other tight. Translocated chromosomes have to bend to find their homologous chromosome by forming quadrivalents. In theory there are 16 different segregation options

for such a quadrivalent, the majority of those will never lead to embryonic development, but most are present and found in secondary oocytes. This results in a high frequency of detection of unbalanced first PB and oocytes respectively. Translocation carriers need to be aware and counseled attentively about these circumstances, particularly because those patients mostly do not need an ART procedure to become pregnant. But very often they are found to be subfertile, reflecting a poor oocyte constitution, perhaps comparable to oligozoospermia in male translocation carriers [16]. For the FISH analysis probes corresponding to sequences on either side of the chromosomal breakpoints of the translocated chromosomes are chosen individually. If two rounds of FISH are time wise possible additional testing for chromosome aneuploidy is recommended.

■ Outcome of PB Analysis

After several years of performing PB analysis the outcome in regard to ongoing pregnancy rates are still not convincing, even those per transfer. One of the reasons could be because the interpretation of FISH analysis on PBs is not sim-

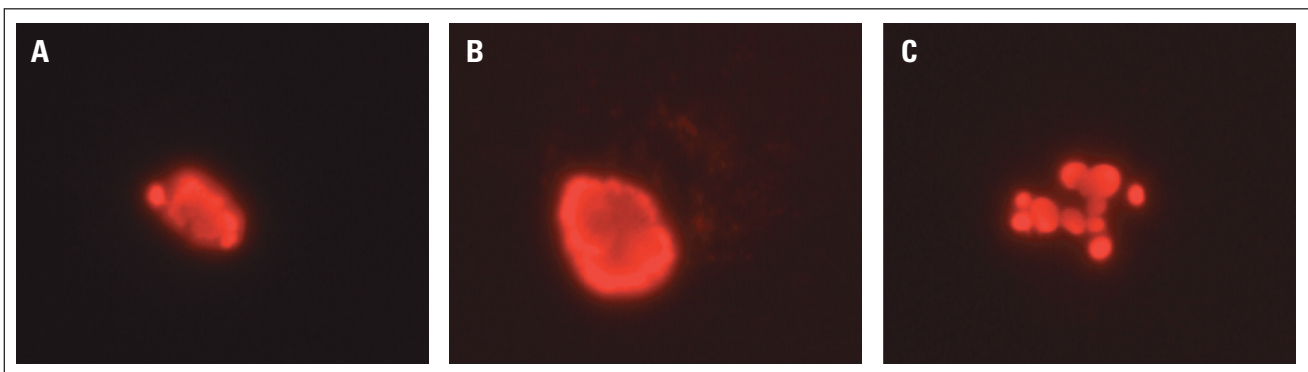


Figure 1: Different polar body shapes following fixation on slide and Propidium Iodine staining. **A, B:** compact polar body; **C:** segmented polar body.

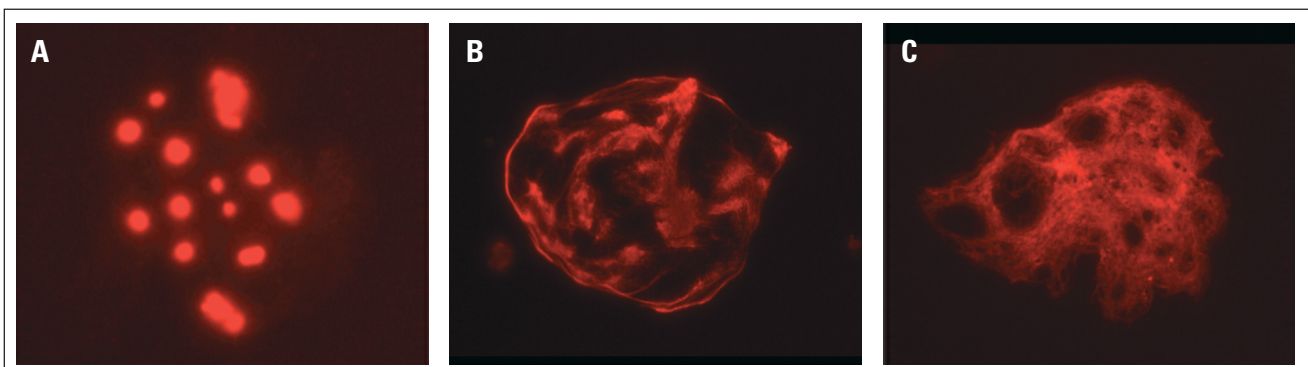


Figure 2: Different polar body conditions following fixation on slide and Propidium Iodine staining. **A:** fragmented polar body; **B, C:** disintegrated polar body.

ple. The optimal timing of PB extraction depends on a variety of features, first on the time of extrusion of the PBs which can be very different, but also on the speed of degrading and secondly, but not least importantly on the integrity of the oocyte. PBs fixed on slides can be evaluated morphologically and a wide variety of shapes and conditions will be discovered (Figs. 1, 2). According to our experience, only a fraction is ideal for further analysis. Suboptimal PBs will lead to questionable results after FISH analysis. Meaning, that out of 10 PBs on average only 3 might be optimal, about 4 questionable and 3 not analyzable. Further influence rests on the FISH probes depending on their sequence lengths and binding capacity. Different levels of laboratory experience with the method may also have detrimental effects on the outcome [17]. All material is unique and cannot be repeated or statistically interpreted. In conclusion the FISH method on PBs has its limits for aneuploidy screening and for translocation diagnostics, which may restrict statistically significant benefits in terms of increased pregnancy rates. Particularly in advanced maternal age patients a significantly higher rate on ART cycles without embryo transfer can be noted, reflecting at least obvious benefits in terms of decreased miscarriage rates [18].

■ New Developments in PB Analysis

Due to the above mentioned restrictions new procedures have been developed further to analyse single cells and in particular PBs. PBs are not equivalent to any other single cell. The first PB carries a haploid set of chromosomes, whereas the second PB only carries one chromatid of each of the 23 chromosomes. New methods for analysis demand a serious amount of DNA needing a whole genome amplified specimen from the original material, i.e. first or second PB. Whereas the comparative genomic hybridisation (CGH) compares loss or gain of chromosomal material on regular metaphase spreads, the CGH array analysis offers a spread of multiple sequences covering the whole genome in various resolutions on a glass slide (Chip).

CGH has been tested for applicability in our laboratory, but has – except for

the moderate costs compared to CGH array – the major dilemma not being able to be fitted in the required time frame. The result of each oocyte is based upon the analysis of 5–10 metaphase spreads for first and second PB separately. Metaphase spread evaluation, even in a semi-automatic set-up, requires up to 5 min. Regardless of a maximum reduce hybridisation time, testing of one cycle with an average of 10 oocytes is too cumbersome and takes up to 8 h.

CGH array analysis however can be performed in the required time frame, since the chip will be scanned and reported automatically [19]. All those procedures in common is the first step, which is the PB amplification. Regarding the very inconsistent material one has to handle to start off with, this still needs to be improved. To our knowledge, there has been no proving of consistent amplification of single PBs by any of the possible methods for whole genome amplification yet. In our laboratory standard procedures have been identified to try to uniform the process as much as possible. A clinical study to evaluate the clinical applicability and the pregnancy rates per embryo transfer has been started this month.

With the CGH array method the detection of gain or loss of all chromosomes is possible and desired. This may lead to an even lower rate of embryo transfer per started cycle. Furthermore younger patients with an unexpected high or nearly exclusive rate of aneuploid oocytes may be identified and counseled appropriately to avoid unnecessary further treatment. However it may hopefully contribute to the aim of finding the best embryo for a single embryo transfer regimen.

■ Practical Aspects

PB analysis could not show benefits for ongoing pregnancy rates by using the FISH method as yet. A decreased miscarriage rate goes along with an increased rate of no embryo transfer cycles. New methods for PB analysis are in approach and need to prove their value for clinical applicability and outcome.

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