

**Article:**

Anna Oberle, Franziska Hanzer, Felix Kokocinski, Anna Ennemoser, Luca Carli, Enrico Vaccari, Markus Hengstschläger, Michael Feichtinger.

Evaluation of Nanopore Sequencing on Polar Bodies for Routine Pre-Implantation Genetic Testing for Aneuploidy

Clin Chem 2024; 70(5): 747–58. <https://doi.org/10.1093/clinchem/hvae024>

Guests: Drs. Anna Oberle and Michael Feichtinger the Wunschbaby Institut Feichtinger in Vienna, Austria.

Bob Barrett:

This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett.

With increasing maternal age, the probability of embryos with chromosomal abnormalities increases, which in turn increases the risk of early pregnancy loss and age-related infertility. In women undergoing in vitro fertilization, pre-implantation genetic testing for aneuploidy can identify affected embryos, thereby improving pregnancy and live birth rates while reducing the number of miscarriages. DNA for preimplantation genetic testing can be obtained through a variety of different approaches, but each has its limitations. Blastomere biopsy can damage the embryo and is no longer routinely performed. In many European countries, trophoctoderm biopsy is restricted by law. As a result, polar body biopsy is often the only option, but analysis using current test methods is not cost-effective. Is there a way to move beyond these challenges? Can new technologies make the benefits of preimplantation genetic testing for aneuploidy accessible to all?

A new research article appearing in the May 2024 issue of *Clinical Chemistry* evaluates nanopore sequencing as a possible solution for routine testing for embryo aneuploidy. In this podcast, we welcome the article's lead and senior authors. Dr. Anna Oberle is Associate Head of the Genetic Laboratory at the Wunschbaby Institut Feichtinger in Vienna, Austria. She focuses on developing novel genetic approaches for reproductive health. Dr. Michael Feichtinger is a Reproductive Medicine Specialist and the Medical Director of the Wunschbaby Institut Feichtinger. He is a Board member of the Austrian Association of Reproductive Medicine and Endocrinology and has published extensively on various topics in reproductive medicine.

So, Dr. Feichtinger, let's start with you. Just how is this study novel?

Michael Feichtinger: Well, our study was the first one to compare systematically sequencing-based aneuploidy screening on polar bodies with

our traditional approach, the so-called array CGH sequencing, with the new nanopore sequencing to evaluate if this method is feasible for routine diagnostics. Therefore, we generated a standardized protocol, including our own optimized bioinformatic data analysis, which automatically called the results, and we compared these results to routine diagnostics. And additionally, we calculated the time and cost of the analysis because out of literature we expect non-routine diagnostics.

Bob Barrett: And why use polar bodies for aneuploidy screening rather than trophoctoderm cells?

Michael Feichtinger: Well, polar bodies have been used for pre-implantation genetic testing in the rather early days in the 1990s. However, there is also clinical reasons to not forget this kind of diagnostic technique because most aneuploidies result from advanced maternal age and the material we get from the polar bodies represents the euploidy of the oocytes. So, paternal aneuploidies only play a minor role, and what can happen after fertilization is that mitotic aneuploidies causing mosaicism. This is a rather a big challenge in testing blastocysts, and actually we know out of the literature that many blastocysts have false positive results, and through polar body biopsy, we don't have this issue. And, last but not least, polar body biopsy is a minimal invasive procedure. So, we do not biopsy the embryo directly, which can cause reduction in implantation rates and maybe even pregnancy complications. So, this technique is much less invasive than the traditional trophoctoderm biopsy.

Bob Barrett: Thank you so much. Dr. Oberle, let's go to you. For this study, you analyzed pooled polar bodies. What are the advantages of pooling instead of analyzing each one separately?

Anna Oberle: The obvious advantage is that we have a lower cost when analyzing both polar bodies together. So, we have lower hands-on time, lower manipulation of the embryo because the embryologists just need to biopsy once to take both polar bodies simultaneously. And also, we get more genetic material in the biopsy, which results in what we see less amplification failure and less no result rates. So, in the literature, when you look at studies using polar body analysis, where both polar bodies are separated, like the ESTEEM trial summarizes, we see a no result or amplification failure rate of up to 20%. In our experience, when pooling polar bodies, we see less than 2% amplification failure and no result rate.

The disadvantages of pooling both polar bodies are that the aneuploidies are not as clear as when looking at both polar bodies separately. The analysis needs to be much more distinct, so we need to be able to differentiate between the

presence of two, three, and four chromatids in the analysis. In comparison of the differentiation which needs to be done between zero, one, or two chromatids present when analyzing the polar bodies separately. But if properly validated, using of pooled polar bodies saves time, and costs, and could even make polar body based PGT-A clinically more attractive or even cost efficient, which was not the case when analyzing both polar bodies separately.

Bob Barrett: What differences did you see between your nanopore sequencing results and the reference method? And what are the reasons for these discordances?

Anna Oberle: We actually did see some differences. So, from our 102 samples that we compared, we found discordant classification of euploid and aneuploid in three samples, and in all three cases, it was a false-negative result where the reference method detected one aneuploid chromosome and our nanopore-based algorithm detected a euploid profile. But in all three cases, these changes were visible in our nanopore profile as well but were not automatically detected as aneuploid.

The same was true in some other discordances on chromosomal level where we mainly see discordant results in the small and varied GC-rich chromosomes like chromosome 19, 21, and 22, but also on chromosome 9. We saw some discordances which could possibly be because of heterochromatic variants, which are very difficult to amplify and sequence. But most of these discordances were mainly due to threshold settings. So, sometimes we saw a slight elevated or reduced chromosomes in the profile that were not automatically called either in our algorithm or also in the reference method, which is a typical phenomenon when analyzing like single cells or not even the material of a single cell, which is the case for polar bodies. Sometimes it's really challenging to correctly determine all aneuploidies, especially for profiles which have more than three aneuploid chromosomes. So, that's why we introduced the declaration of multiples, so the mild distribution of multiple chromosomes that makes sense. So, in all cases, the changes that were discordant were visible, but just the threshold setting was a little bit different. So, the clinical interpretation by a diagnostic expert might be required as we also suggested in our manuscript, and like it is also common practice for clinical PGT-A diagnostics already.

Bob Barrett: What are the advantages of nanopore sequencing compared to the current gold standard technology?

Anna Oberle: So, the current gold standard is next-generation sequencing and the main advantages of nanopore sequencing for our short-read PGT-A application are mainly the costs. So,

nanopore sequencing technology has very low investment costs and also low running costs, which makes it especially attractive for small labs or even small clinics. Also, the nanopore technology has a very straightforward workflow, very fast sequencing, and is really flexible.

So, for example, sequencing success can be tracked live and if something goes wrong, you can act immediately and start a new run without wasting one day, order the whole flow cell, which would be the case for Illumina sequencing, and also the flow cells can be used very flexible so you can for example, use just one sample or sequence up to 24 samples simultaneously in one flow cell. And then these flow cells can be reused until the pores are exhausted.

Also, nanopore sequencing technology has a lot of advantages for other applications like for haplotype phasing or breakpoint mapping for example, for monogenetic disease detection or the detection of structural rearrangements, or also the detection of de novo assembly. So, the advantages of nanopore sequencing are their long and ultra-long reads that can cover highly repetitive regions, copy number variations or structural variations, where short-read sequencing would not be possible. And additionally, it's also possible to sequence so-called native sequencing, which simultaneously allows the detection of methylation, which can be used, for example for the detection of Angleman's syndrome, which is an imprinting disorder. So, nanopore sequencing has really a lot of advantages depending on the applications.

Bob Barrett: What's the most challenging aspect of nanopore sequencing technology?

Anna Oberle: So, it's a rather new technology and a young, still developing technology, and the challenges are mainly because there are no standardized kits, there are no IVD-certified kits with standardized protocols available. So, every application that uses nanopore sequencing needs to be validated properly. So, all the workflows and the protocols need to be validated. And since the technology is still advancing very quickly, also new versions of kits are released, new flow cells are released frequently, which additionally require validation work, which can be very extensive. Additionally, also there are no standardized bioinformatic analysis tools out there. So, for each application, the bioinformatic analysis pipeline need to be created and of course also properly validated, which is the most challenging.

Bob Barrett: Finally, Dr. Feichtinger is the technology ready for routine testing?

Michael Feichtinger: Well, what we have seen within the last years is that the sequencing accuracy and quality of kits and flow cells was getting better and better. The kits that we use are reliable and the technical support of Oxford Nanopore is very professional. So, if the specific workflow plus, importantly, the bioinformatic data analysis is properly validated for each application, then definitely we believe that it's ready for primetime, yes.

Bob Barrett: That was Dr. Michael Feichtinger and Dr. Anna Oberle from the Wunschbaby Institut Feichtinger in Vienna, Austria. They co-authored a research article on the use of nanopore sequencing for preimplantation genetic testing for aneuploidy in the May 2024 issue of *Clinical Chemistry*, and they've been our guests for this podcast on that topic. I'm Bob Barrett. Thanks for listening.