

Polar body biopsy – Advantages of the Eppendorf micromanipulation system

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Abstract

A well-established technique which is used in preimplantation genetic diagnostics (PGD) is polar body (PB) biopsy. The polar bodies of the oocyte are extruded at the conclusion of the meiotic division; normally the first polar body is noted after ovulation; the second polar body is observed 2–3 h following entry of the sperm into the oocyte. Removal of the first and second polar bodies takes place 6–12 hours after the performance of intracytoplasmic sperm injection (ICSI). The biopsy of the polar bodies

is followed by detection of certain chromosomes using fluorescence *in-situ* hybridization (FISH) or detection of all chromosomes by comparative genomic hybridization. Using the Eppendorf micromanipulation system with manual injectors and the electronic TransferMan® 4m micromanipulators in combination with the OCTAX Laser Shot™ System, fast and sensitive handling of the oocyte and polar bodies can be guaranteed.

Introduction

Over the past few decades the mean age of women conceiving their first child has steadily increased. However, advanced maternal age lowers the chance for pregnancy and increases the risk of miscarriage once a woman is pregnant. One major problem strongly correlated to maternal age is the occurrence of numerical chromosomal abnormalities in human oocytes. In women who are 40 years old and older, up to 70% of their oocytes can be chromosomally abnormal [1, 2]. In the context of assisted reproduction treatment, it is possible to identify and exclude such oocytes, thereby increasing the success rate. The underlying technique is the biopsy of the first and second polar bodies (Figure 1), followed by detection of certain chromosomes using fluorescence *in-situ* hybridization (FISH) or detection of all chromosomes by comparative genomic hybridization (CGH). To understand these techniques it is important to be familiar with certain aspects of fertilization and subsequent early embryo development. The cell division that results in sperm and oocytes containing 23 chromosomes each is called meiosis.

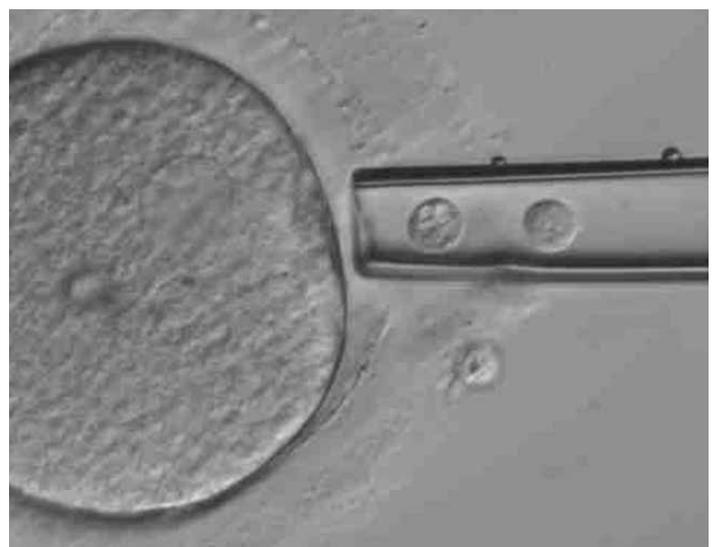


Fig. 1: Biopsy of 1st and 2nd polar bodies

In their resting state, eggs exist in a state of arrested meiosis and still contain all 23 paired chromosomes. During ovulation, meiosis resumes, and the egg extrudes one set of its 23 chromosomes in a small structure called the first polar body. Soon after fertilization occurs, a second polar body, containing 23 maternal chromatids, is expelled. On the day after oocyte retrieval, both polar bodies are visible by light microscopy in the normally fertilized oocyte.

Polar bodies with missing or excess chromosomal material are indicative of an oocyte that contains either an excess of chromosomes, which, after fertilization, results in an embryo with a trisomy, or of an oocyte which is missing chromosomes, which, again after fertilization, results in an embryo with a monosomy. Thus, polar body biopsy provides an indirect diagnosis of the oocyte for aneuploidy testing. This method can also be applied to couples with a balanced translocation of the mother and to couples who are aware of maternal predisposition for a genetic disease that may manifest itself in the child.

Polar body biopsy was first presented in 1990 [3], and several aspects of this technique have been technically refined including the use of lasers and electronic micromanipulators to facilitate the biopsy procedure [4].



Fig. 2: Eppendorf Workstation for IVF

Materials and Methods

Compared to ICSI, polar body biopsy requires additional micromanipulation steps; thus it greatly benefits from the proper instrumentation, especially the Eppendorf TransferMan 4m (Figure 2) with its special features which render the procedure as economic as possible. The use of application specific masks of the TransferMan 4m micromanipulator with predefined as well as freely definable softkey functions eases the individual workflow process. The unique DualSpeed joystick enables precise and intuitive movement along all three axes when handling the oocyte as well as dynamic movements while covering greater distances (see Figure 3). This helps reducing exposure time of the oocytes to stressful conditions outside the incubator, and it minimizes the risk of losing the material of interest.

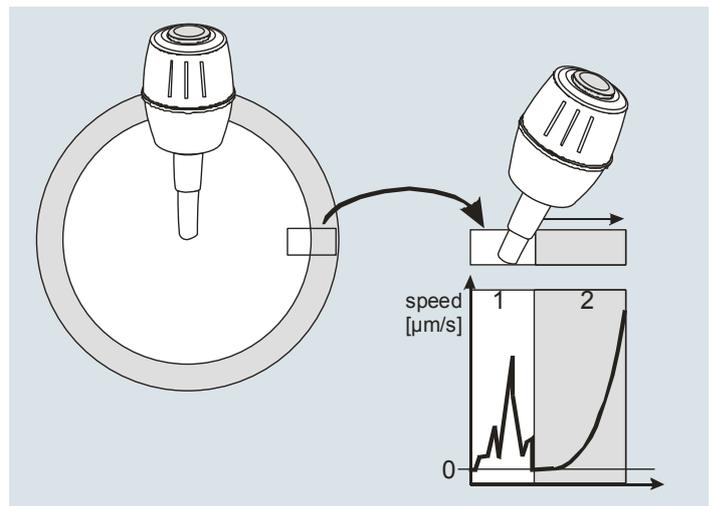


Fig. 3: Movement in direct correlation with the deflection of the joystick. 1: proportional kinetics, 2: area of dynamic movement. The ratio of the dynamic movement may be adjusted according to the proportional range.

Devices for polar body biopsy

- > Inverted microscope with a heated plate and modulation contrast objectives
- > OCTAX Laser Shot™ System (OCTAX, MTG)
- > 2 TransferMan 4m micromanipulators (Eppendorf)
- > CellTram® Air microinjector for holding the embryo (Eppendorf)
- > CellTram® vario microinjector for removal and transfer of the polar body (Eppendorf)
- > Biopsy capillary, for laser-assisted biopsy of polar bodies (recommended: 19 µm inner diameter, 35° tip angle)
- > Holding capillary, for holding of oocytes (recommended: 15 µm inner diameter, 100 µm outer diameter, 35° tip angle)

Polar body biopsy

The most important feature of the TransferMan 4m for polar body biopsy is its ability to store several freely definable capillary positions. Up to five positions can be stored. For the polar body biopsy procedure, as it is performed in our laboratory, one needs to store three user-defined positions; hence the use of the application mask »Cell Transfer« with three softkeys dedicated to position storage is recommended. In addition, using this mask, a vertical limit can be defined to avoid capillary breakage (see Figure 4). One softkey is free for individual settings.

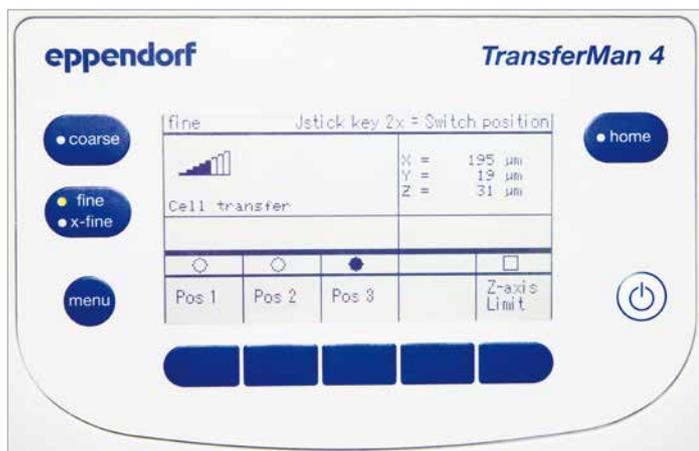


Fig. 4: Application mask »Cell transfer« with three softkeys: for position storage and one for definition of a vertical limit (Z-axis limit) to avoid capillary breakage. One softkey is free for individual programming.

The polar body biopsy is performed in a culture dish. A biopsy capillary is used to transfer the polar bodies directly into a drop of water on a glass slide for the purpose of FISH analysis or into another droplet in the dish for later transfer to PCR tubes if array-CGH is to be performed.

The three user-defined capillary positions are used as follows: position 1B for biopsy/release into adjacent droplets in the case of array-CGH, position 2B for easily replacing

the culture dish with a glass slide, and position 3B for releasing the polar bodies into the water droplets on the slide for later FISH.

Additionally, two positions are reserved for the manipulator used for holding the oocyte: position 1H for holding the oocyte during biopsy and position 2H for the changing of droplets in the dish. Once stored, these positions can be automatically activated by pressing the relevant position button on the device. Alternatively, the positions can be relocated in succession by clicking the joystick key twice (double-click). This set-up enables a fast and economic change from one capillary working position to another, but most importantly it controls the capillary that holds the aspirated polar bodies, thus helping to reduce the rate of lost polar bodies to less than 0.5 %. An overview of the different positions required during the biopsy procedure is shown in Figure 5a–b.

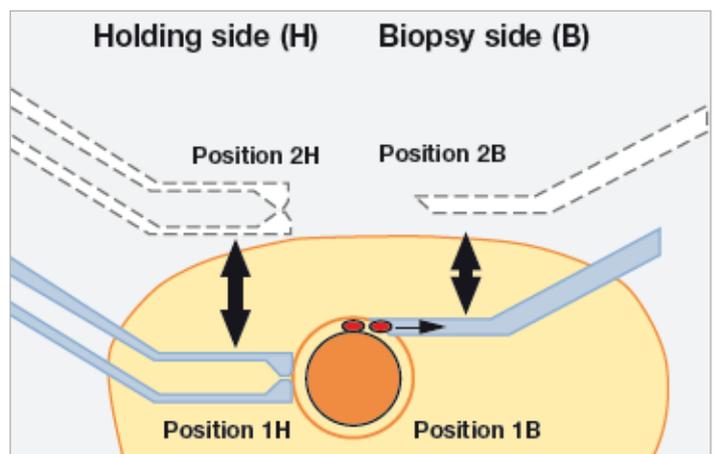


Fig. 5a: Stored positions for polar body biopsy.

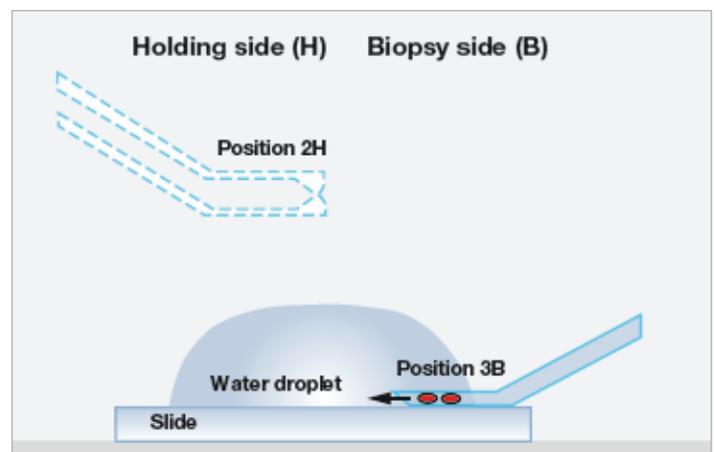


Fig. 5b: Stored positions for polar body biopsy.

For polar body biopsy we recommend setting up a dish with a single droplet that contains PVP and two/three rows of droplets for FISH/array-CGH, 3–5 μL each, which contain buffered culture medium. The buffered culture medium is required for maintenance of the proper pH during manipulation outside the incubator. PVP is used for rinsing the biopsy capillary, which helps to avoid polar bodies sticking to the inner glass wall. The left row of droplets is used for the oocytes (one per droplet) and the right row for sampling of the polar bodies after biopsy. In the case of array-CGH two rows for polar body sampling are required as polar bodies 1 and 2 need to be processed separately. In preparation for biopsy, an oocyte is gently aspirated by the holding capillary and affixed as closely as possible to the bottom of the dish. This position is stored as position 1H. Rotation of the oocyte may be necessary in order to obtain proper alignment of the first and second polar bodies in one focal plane (Figure 6a). This focal plane defines position 1B for the biopsy capillary, and, once adjusted, this position should also be stored. Following laser-assisted opening of the zona pellucida, the biopsy capillary is pushed through the opening of the zona towards the polar body next to the capillary opening. By rotating the knob of CellTram vario both polar bodies are slowly aspirated into the capillary (Figure 6b). To slowly push the capillary through to the polar bodies, slight suction is usually helpful. Once both polar bodies are completely aspirated, the capillary is removed from the zona and the oocyte is released from the holding capillary.

If several oocytes need to be biopsied, the first and second polar bodies can be temporarily stored in the neighboring medium droplets while the biopsy of the next oocyte is performed as previously described. Once all polar bodies are biopsied, it is advisable to first place the oocytes back into the incubator. To do so, both capillaries are brought into a

position outside and far above the culture dish by activating the »home« key, and if required, the motors are swiveled out to the side of the microscope. Now the dish can be removed safely so that the oocytes may be transferred to another culture dish.

Transfer of polar bodies to a slide for later FISH analysis

The dish still holding the polar bodies is placed back on the microscope stage, the motors are swiveled back in and the biopsy capillary is lowered automatically by pressing the »home« key again. Next the capillary can be moved directly into position 1B by pressing »Pos 1«. The polar bodies corresponding to oocyte 1 are then aspirated into the biopsy capillary. Next, the capillary is moved to position 2B (by pressing »Pos 2«), the culture dish is removed, and a glass slide holding a 0.2 μL droplet of pure water is placed under the capillary. The biopsy capillary still holding the polar bodies is lowered into the water droplet so that it just touches the glass surface. This position is stored as position 3B. The first and second polar bodies are carefully released into the droplet, and the capillary is first drawn back and then brought into position 2B by pressing »Pos 2«. The small volume ensures that the polar body will attach to a small area on the slide and the fluid will dry fast, thereby reducing the risk of dislocation from the slide. Even so, the drying process must be observed under a stereo microscope, and the final location of the polar body after air-drying must be circled on top of the slide using a diamond marker. This procedure can be repeated until all polar bodies have been transferred to the slide. With some experience, 4 to 6 polar bodies can be placed within an area of approx. 10 mm, each encircled using a diamond marker [5]. Because all relevant capillary positions have been stored during the first round, further manipulation of polar bodies is less time consuming.

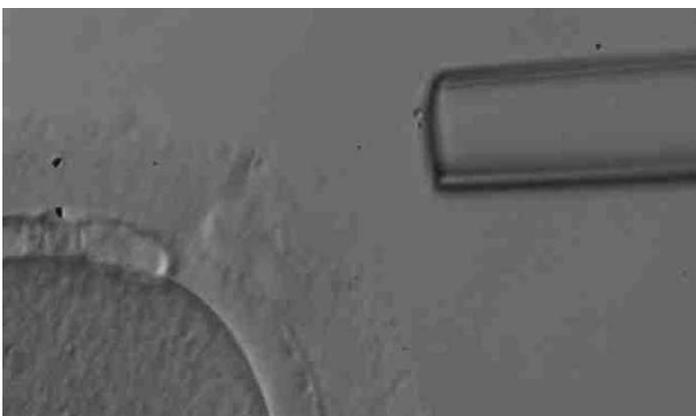


Fig. 6a: First and second polar bodies are aligned properly in one focal plane

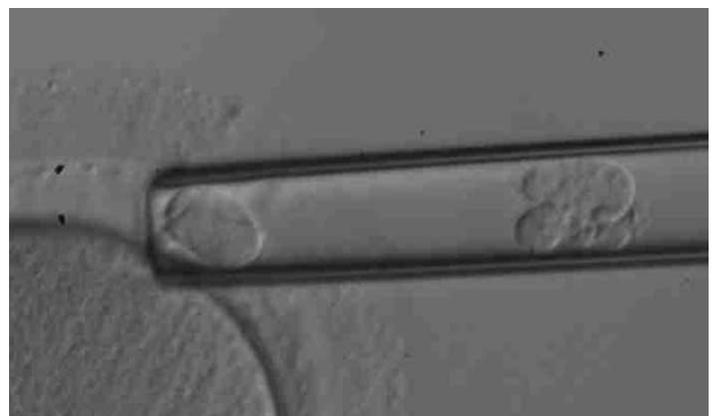


Fig. 6b: Both polar bodies are slowly aspirated into the capillary.

Transfer of polar bodies into a PCR tube for later array-CGH analysis

For the transfer into PCR tubes polar bodies must be transferred one by one into PCR tubes pre-filled with PBS. As PCR tubes are narrow, the transfer cannot be performed using the biopsy capillary; instead the use of stripper tips is recommended. A detailed description of the transfer as well as the array-CGH procedure is provided by Magli et al., 2012 [6].

Fluorescence *in-situ* hybridization (FISH)

For FISH analysis, the dried polar bodies are fixed by the addition of 2 x 10 μ L ice-cold methanol: acetic acid (3:1), followed by incubation in methanol at room temperature for another 5 min. The slides are dried, and the FISH probe for chromosomal detection is directly applied to the slide, which is then covered by a cover slip and sealed with rubber cement. The slide is placed into the Mastercycler[®] nexus flat. Co-denaturation of the probe and the genomic DNA as well as subsequent hybridization is performed using the Mastercycler nexus at the time and temperature settings indicated by the manufacturer of the chromosome probe. Following hybridization, unbound probe is washed off, and the FISH signals can be evaluated using a fluorescence light microscope equipped with appropriate filter sets (Figure 7).

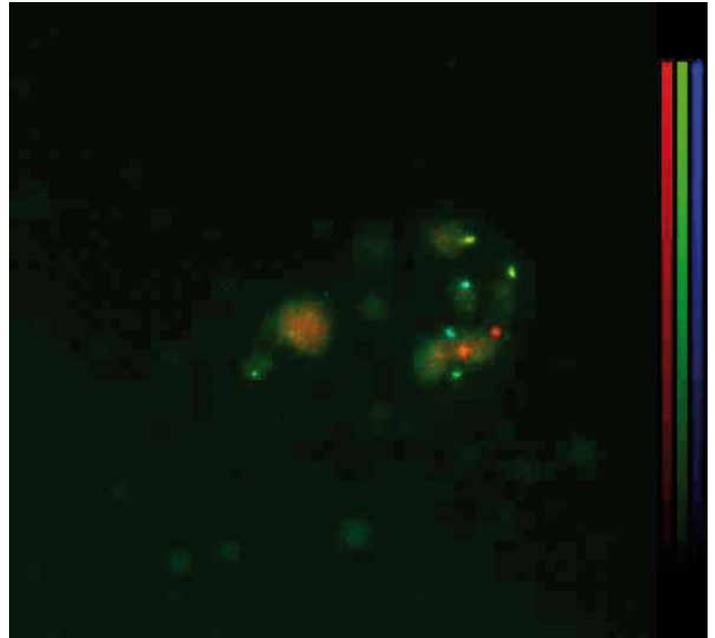


Fig. 7: FISH signals of chromosomes 13, 18, 21, 22

Results and Discussion

Each chromosome should show two signals in the 1st polar body and one signal in the 2nd polar body. Approx. 70% of all chromosomal disorders are detectable in the 1st polar body; however, a disorder may occur in the formation of the 2nd polar body.

A frequent problem when judging FISH results is the occurrence of chromatin degeneration which can be identified by speckled signals. Interestingly, this phenomenon occurs most often when using LSI probes. Nevertheless, it is still possible to draw conclusions about the respective chromosomes, since early segregation of chromatids means that the regions with speckled signals are also separated.

The main problem with polar body biopsy is the fragmentation of polar bodies, particularly the first polar body, which can be seen in Figure 6b. Since each fragment can contain chromosomes, it is crucial that all fragments be removed during biopsy.

Here, sensitive handling is required which can be perfectly executed with the intuitive proportional fine control of the TransferMan 4m micromanipulator in combination with the fine wheel of the CellTram vario. Any movement of the hand is directly transferred to the biopsy capillary, without any delay or even tiniest stutter.

In addition to the removal of all fragments, identifying the number and re-location of the fragments is critical, because they can disconnect while drying and move to different areas of the slide. Therefore it is absolutely necessary to compile a drawing. Otherwise the risk is quite high that signals in small fragments are disregarded which will lead to a false diagnosis.

Depending on the results of the FISH or the array-CGH analysis, chromosomally normal oocytes can be selected for further culture and transfer.

Ordering Information

Description	Order no. International
TransferMan® 4m¹ , Micromanipulator with DualSpeed™ joystick for direct and dynamic movement control	5191 000 015
Microscope adapter , Adapter for micromanipulators, available for different inverse microscopes of major brands	Available upon request
CellTram® 4m Air^{1,2} , Pneumatic, manual microinjector with gear, for holding of oocytes or biopsy of polar bodies	5196 000 021
CellTram® 4m Oil^{1,2} , Hydraulic, manual microinjector with gear, for holding of oocytes or biopsy of polar bodies	5196 000 048
Antivibration Pad , Pads in different weight ranges available, for protecting micromanipulation workstations from external vibrations	Available upon request
Mastercycler® nexus flat , PCR cycler with thermoblock for 4 slides	6335 000 011

¹This product is registered in Europe as a medical device (according to Medical Device MDD 93/42/EEC).

For distribution in your country, please contact your local sales organization.

²The CellTram 4m Air and CellTram 4m Oil replace the CellTram Air and CellTram vario.

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