Preimplantation genetic testing: polar bodies, blastomeres, trophectoderm cells, or blastocoelic fluid?

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Objective: To investigate the blastocoelic fluid (BF) for the presence of DNA that could be amplified and analyzed; the extent to which its chromosomal status corresponds to that found in trophectoderm (TE) cells, polar bodies (PBs), or blastomeres; and the identification of segmental abnormalities.
Design: Longitudinal cohort study.
Setting: In vitro fertilization unit.
Patient(s): Fifty-one couples undergoing preimplantation genetic screening or preimplantation genetic diagnosis for translocations by array-comparative genomic hybridization on PBs (n = 21) or blastomeres (n = 30).
Intervention(s): BFs and TE cells were retrieved from 116 blastocysts, whose chromosome status had already been established by PB or blastomere assessment. Separate chromosome analysis was performed in 70 BFs.
Main Outcome Measure(s): Presence of DNA in BFs, evaluation of the chromosome condition, and comparison with the diagnosis made in TE cells and at earlier stage biopsies.
Result(s): DNA detection was 82%, with a net improvement after refinement of the procedure. In 97.1% of BFs, the ploidy condition corresponded to that found in TE cells, with one false positive and one false negative. The rate of concordance per single chromosome was 98.4%. Ploidy and chromosome concordance with PBs were 94% and 97.9%, respectively; with blastomeres, the concordances were 95% and 97.7%, respectively. Segmental abnormalities, which were detected in PBs or blastomeres of 16 blastocysts, were also identified in the corresponding BFs.
Conclusion(s): BF represents to a good extent the blastocyst ploidy condition and chromosome status when compared with TE cells. If the proportion of clinically useful BFs is improved, blastocentesis could become the preferred source of DNA for chromosomal testing. (Fertil Steril® 2016;105:676–83. ©2016 by American Society for Reproductive Medicine.)
Key Words: Blastocyst, blastocoel, blastomere, polar bodies, preimplantation genetic screening

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After compaction, the embryo starts to form a cavity in a process whereby cells differentiate into inner-cell mass (ICM) and trophectoderm (TE) and separate and migrate to distinct locations. In a normally developing embryo, TE cells give rise to the extraembryonic tissues that make up the placenta and the amnion, while ICM, made of undifferentiated embryonic stem cells, becomes the embryo proper (1). After polarization of TE cells and the formation of a belt-line seal made by tight junctions, the accumulation of blastocoelic fluid (BF) transported by TE cells begins (2, 3). As far as cavitation progresses, the accumulation of the fluid and the continuous cell mitoses produce enlargement of the blastocyst and thinning of the zona pellucida (ZP). At this point, hatching of the blastocyst occurs through a natural breach in the ZP, making the embryo ready to implant.

The BF represents the natural medium supporting the development of

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the ICM and gives rise to the yolk sac (1, 4). Since both the differentiation process and the process of self-renewal are guided by proteins (5), the identification of metabolites and proteins in BFs is not surprising (6, 7). A recent study identified 286 proteins in the BF including heat shock proteins, ZP proteins, vitamin D–binding protein, retinol-binding protein, and proteins regulating ciliary assembly and function (7). In addition, eight previously uncharacterized proteins were found, which have not yet been assigned a function. In all, the distribution of these proteins according to their biological function was quite similar to that found in the cells forming the corresponding blastocyst. These findings suggest the derivation of BF proteins from the blastocyst cells through transcellular transport and/or through the release of components from ICM and TE cells (8). It is now believed that the BF plays a critical role in supporting cellular processes during embryo development other than merely providing a compartment for cell migration (9).

Beside proteins, DNA has also been found in BFs, whose origin is still under investigation (10–12). It could be free DNA or in particulate forms like microparticles, which are membrane-bound vesicles containing nuclear molecules released by membrane blebbing during cell death and replication (13). Whatever the form is, this DNA can be submitted to amplification and analyzed to identify mutations responsible for genetic diseases or chromosomal abnormalities in preimplantation genetic screening (PGS) or preimplantation genetic diagnosis (PGD) programs (11, 12).

We already presented our preliminary data in a report where DNA was found and analyzed for aneuploidy in 39 out of 51 tested BFs accounting for an efficiency of 76.5% (12).

In this study, we extended our experience with the aims of [1] verifying the presence of DNA in BFs, [2] estimating whether its chromosomal status corresponded to the ploidy condition predicted by polar bodies (PB) or blastomeres and TE cells, and [3] investigating whether segmental abnormalities could also be detected in BFs.

MATERIALS AND METHODS

Plan of the Study

This study included 51 couples (maternal age 38.1 ± 3.2 years) undergoing 24-chromosome array-comparative genomic hybridization (-CGH) in PBs (21 patients) or blastomeres (30 patients) in a PGS/PGD for a translocation program. Indications for PGS were advanced maternal age (n = 26) or repeated IVF failures (n = 13); PGD for translocation (n = 12) included both Robertsonian (n = 1) and reciprocal (n = 11) translocations.

To address the first aim of this study, 116 blastocysts from these couples were investigated for the presence of DNA in the BF. To address the second aim, 70 of the amplified BFs underwent 24-chromosome analysis, and the results obtained were compared with those from the corresponding PBs or blastomeres and TE cells. This analysis was done only on those samples that also had chromosomal results available from PBs or blastomeres and TE cells. Finally, to address the third aim of the study, 16 BFs were analyzed for their chromosomal condition including segmental anomalies, and the results obtained were correlated with those from the other stages.

The study included blastocysts with a chromosome status already defined by PB or blastomere analysis generated by patients, who signed a consent form allowing further chromosomal analysis on their supernumerary blastocysts. These blastocysts were of two types: [1] Euploid, eventually destined to cryopreservation. The BF was aspirated and the collapsed blastocyst was immediately vitrified; and [2] aneuploid and therefore nontransferrable. After BF aspiration, TE cells were biopsied from the reexpanded blastocysts for separate chromosomal analysis. Owing to a national regulation that does not allow embryo donation for research, whole embryos (WE) were only tested when they turned out to be nonviable after extended culture (14). The study was approved by our Institutional Review Board (IRB no. 20110503).

Biopsy Procedures

Biopsy of PBs, blastomeres, TE cells, and BF was performed as described elsewhere in Hepes-buffered medium supplemented with protein (5 mg protein/mL) under oil (LifeGlobal Media) (12).

Briefly, PB1 and PB2 were sequentially biopsied after mechanical opening of the ZP. PB1 was removed immediately before intracytoplasmic sperm injection (ICSI), and PB2 was removed 6–9 hours later. Blastomere biopsy was performed at approximately 62–64 hours after ICSI in regularly developing embryos at the 6- to 8-cell stage (14). The ZP was opened by making a small hole using a laser (Saturn; Research Instruments) through which a nucleated blastomere was aspirated.

TE biopsy was performed by excising via laser pulses 3–5 cells, which had herniated through the breach previously opened in the ZP at the time of PB or blastomere biopsy.

Each biopsy was transferred to 0.2 mL polymerase chain reaction (PCR) tubes with 1 μL phosphate buffered saline kept on ice, spun, and stored at −80°C until further processing for chromosomal analysis (15).

BFs were aspirated from expanded blastocysts using an ICSI pipette paying great attention to avoid the aspiration of any cell. The retrieved fluids were transferred into empty PCR tubes kept on ice, which were spun and stored at −80°C. A volume of approximately 0.01 μL BF was retrieved from each blastocyst.

In case of nonviable blastocysts, the WE was transferred to a PCR tube with 1 μL buffer kept on ice and processed as indicated above.

Whole Genomic Amplification (WGA) and Array-CGH

WGA was performed in a class II Laminar flow cabinet using a PCR library–based method (SurePlex, Illumina). DNA amplification was determined by loading 5 μL of the final reaction onto a 1.5% agarose gel and was defined as “strong,” “weak,” or “failed” according to the band shape and intensity. An aliquot of the amplified DNA was used for 24-chromosome

copy number analysis by a-CGH (24Sure Microarray, Illumina) with dedicated software (Bluefuse Multi v4.0, Illumina) following the manufacturer's instructions. The euploid/aneuploid status of the corresponding oocyte or embryo was predicted (16). Visualization and reporting of aneuploidy was on a per-chromosome basis. In case of embryos generated by reciprocal translocation carriers, a specific kit was used (24Sure+ PGD Microarray, Illumina). All analyses of BFs and TE cells were performed by operators blinded to the previous results.

Assessment of the Chromosome Status

The chromosome status was assessed in 70 BFs and corresponding PBs or blastomeres and TE cells.

To evaluate the results from the different stages, the first indicator was the ploidy condition that defined the correspondence among the different stages of each embryo in terms of euploidy or aneuploidy. The ploidy condition was defined as concordant when all stages indicated the same ploidy condition and as discordant in the opposite situation. This indicator has clinical relevance, since the ploidy condition is the criterion guiding embryo selection in a PGS/PGD for translocation program.

The second criterion was the chromosome concordance, a term defining the proportion of correspondence of all studied chromosomes among the different stages of each embryo. Full concordance indicated that all single chromosomes corresponded in the different stages. Partial concordance included cases where only some aneuploid chromosomes corresponded in the different stages. Finally, null concordance referred to cases where none of the chromosomes called in any of the biopsies found correspondence in the others. This indicator has a biological relevance, as it provides information on the distribution of chromosome abnormalities, including segmental anomalies, throughout the different stages of development.

RESULTS

The BF was aspirated from 116 blastocysts. After WGA, there was DNA amplification in 95 samples (82%), of which 85 had strong amplification and 10 had weak amplification.

To investigate possible criteria able to predict the presence of DNA in the BF, results throughout time, blastocyst morphology, and day of biopsy were analyzed.

When results were stratified throughout time, a learning curve was evident, with a net improvement in the amplification rate of the most recently collected samples (Fig. 1A). From the combined analysis of blastocyst morphology and day of biopsy (expressed as hours postinsemination) there was a tendency indicating that expanded blastocysts on day 5 had the highest chances of having DNA in the BF (Fig. 1B).

Of the 95 samples with positive amplification, 87 were processed for a-CGH, including 82 with strong amplification and five with weak amplification. Informative results were obtained in 82 samples (94%), 80 from DNA with strong amplification (80/82, 98%) and two from DNA with weak amplification (2/5, 40%).

In the following sections, we present the results from 70 amplified BFs for which data were also available for PBs or blastomeres and TE cells.

Concordance between the Chromosome Condition in BF and That Predicted by PBs, Blastomere, and TE Biopsy

For 70 blastocysts, chromosome data were available for the BF and corresponding biopsies (PB or blastomere and TE). The results obtained were compared with those obtained in PBs (n = 34) or blastomeres (n = 36), as well as with those from TE cells (Supplemental Table 1 and Supplemental Table 2).

According to PB or blastomere results, 11 embryos were diagnosed as euploid and 59 as aneuploid. The BF showed a ploidy concordance of 94.3% (66/70), with no false positives (all 11 euploid embryos were also euploid for the BF) and four false negatives (of the 59 aneuploid embryos, four were euploid for the BF). Sensitivity and specificity were 0.93 and 1, respectively; accuracy was 0.94, with a positive test predictive value of 1 and a negative test predictive value of 0.73.

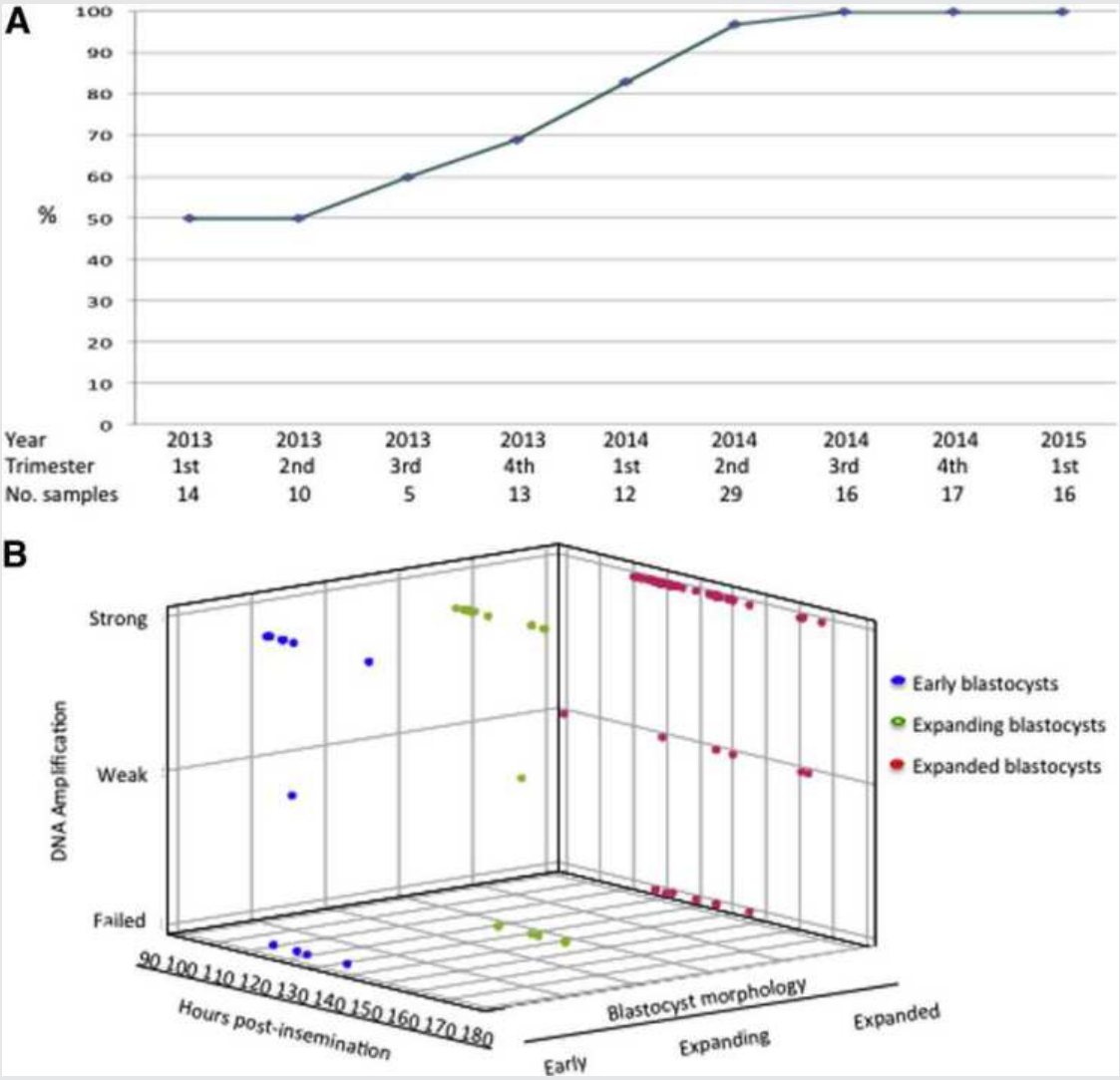
The results from TE analysis reported 14 euploid and 55 aneuploid (one TE result was noninformative). The BF demonstrated a ploidy concordance of 97.1% (67/69) with one false positive (one of 14 TE euploid embryos classified as aneuploid by BF) and one false negative (one of 55 TE aneuploid embryos classified as euploid by BF). Sensitivity and specificity were 0.98 and 0.93, respectively; accuracy was 0.97, with a positive test predictive value of 0.98 and a negative test predictive value of 0.93.

Table 1 reports in detail the cases with ploidy discordance. In four sets (5.7%, 4/70), results from the BF were discordant with those obtained from the chromosome analysis done at previous stages. Two of these cases had been predicted to be aneuploid by PB biopsy, but in agreement with BF results, both TE cells and WE were euploid. The two cases predicted to be aneuploid by blastomere biopsy were also euploid according to the corresponding BFs. In one of these sets, no result was obtained by TE cells, while the WE confirmed the aneuploidy predicted by the blastomere. In the other set, both TE cells and WE presented a condition of aneuploidy involving the same chromosome as in the blastomere, but with an opposite type of aneuploidy.

When comparing the results from BF and TE cells, two ploidy discordances were found (Table 1). In one of these (already listed above, as the BF was also discordant with the prediction made by blastomere analysis), the BF was discordant with TE cells and WE. In the second discordant case, TE cells were euploid, conversely to the chromosome status detected in all other biopsies, indicating that a diagnosis made on the basis of TE cells would have produced a false-negative result.

Regarding chromosome concordance, BFs had a chromosome concordance of 97.9% with PBs, 97.7% with blastomeres, and 98.4% with TE cells, with full concordance being the prevailing condition in 73.5%, 81%, and 81% of embryos, respectively.

FIGURE 1



Presence of DNA in 116 BFs detected by WGA. (**A**) The percentage of BFs resulting in positive amplification increased significantly throughout time, indicating a learning curve. (**B**) Blastocyst morphology and hours postinsemination were related to DNA amplification. Expanded day 5 blastocysts showed the highest chances of having DNA in the BF.

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The results of concordance are detailed in Supplemental Table 3 and summarized in Supplemental Figure 1.

Segmental Abnormalities

In 16 of the analyzed blastocysts, the presence of segmental abnormalities was detected either in PBs (n = 3) or in blastomeres (n = 13; Fig. 2). Nine of these embryos were generated by carriers of a reciprocal translocation, and the remaining seven by patients with a normal karyotype.

The nine embryos from translocation carriers had been diagnosed by blastomere as unbalanced for the chromosomes involved in the translocation (Supplemental Table 2). In all cases, the segmental abnormality was also evident in the corresponding BF, showing total concordance in seven cases (an example is shown in Fig. 2A) and partial concordance in the remaining two, where an additional abnormality appeared in BFs. When looking at the results from TE cells and WEs, one TE was diagnosed as euploid, being ploidy discordant with the results obtained from the other biopsies (Table 1; Fig. 3). Therefore, in this case a diagnosis based on the result from TE cells would have been a false negative.

In the seven embryos generated by patients with a normal karyotype, three segmental abnormalities were predicted by PBs (Fig. 2B) and four by blastomeres (Supplemental Table 2). They were all confirmed by BF, TE cells, and WEs, resulting in five sets with full concordance. In the remaining two sets, the concordance was partial.

TABLE 1

Cases with ploidy discordance between BF and the analysis done at previous stages and between BF and TE cells.

ID	PB1	PB2	Blastomere	Prediction of the blastocyst chromosome status	BF	TE cells	WE
33	Loss 6 Loss 22	Gain 22		Gain 6	Euploid	Euploid	Euploid
50	Loss 18 Loss 19	Gain 18 Loss 15		Gain 19 Gain 15	Euploid	Euploid	Euploid
45			Gain 9	Gain 9	Euploid	Loss 9	Loss 9
54			Loss 2	Loss 2	Euploid	No Result	Loss 2
60			Gain 10p11.23->10q26.3 Gain 20 Loss 16	Gain 10p11.23->10q26.3 Gain 20 Loss 16	Gain 10 Gain 20 Loss 16	Euploid	Gain 10 Gain 20 Loss 16

Note: In the first four sets, BFs were discordant with the results predicted by PBs (PB1 and PB2), but they were concordant with corresponding TE cells and with the WE. BFs were discordant also in the following two cases compared with the prediction made by blastomere analysis. In one of these cases, the WE had the same profile as the blastomere, while in the second both TE and WE gave the same result that involved the same chromosome predicted to be aneuploid by the blastomere, but with an opposite type of aneuploidy. In the last case, no. 60, the ploidy discordance involved TE cells that were euploid conversely to the chromosome status detected in all other biopsies. The term "Gain" indicates hyperhaploidy; the term "Loss" indicates hypohaploidy.

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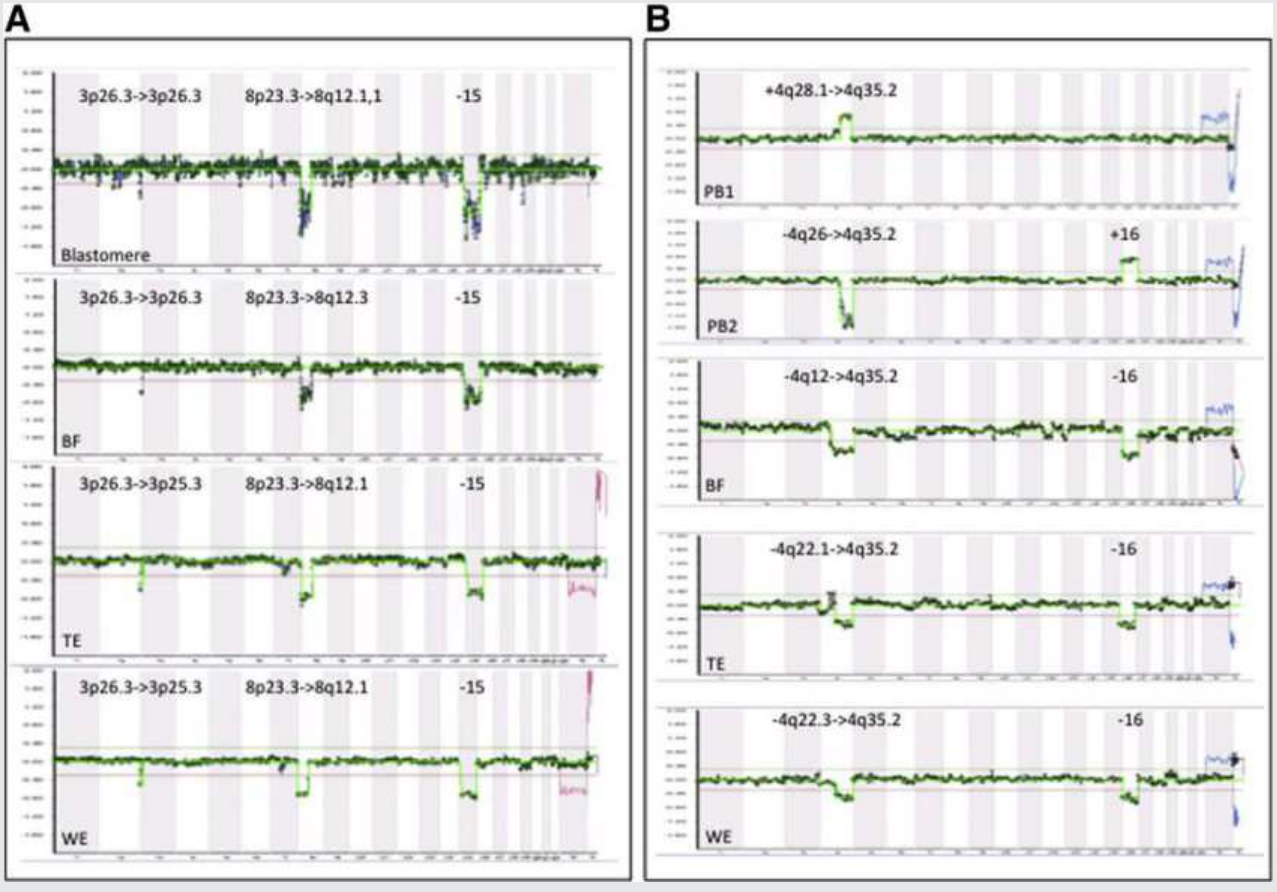
DISCUSSION

Blastocyst culture is now widely used in ART as a method for efficient selection of viable embryos. Additional advantage is represented by the possibility to perform TE biopsy for both PGS and PGD of single-gene disorders and translocations. This approach seems to preserve embryo viability and to

FIGURE 2



Detection of segmental abnormalities. (**A**) Embryo generated from a carrier of the translocation 46XX,t(3;8)(p25;q12) with an unbalanced rearrangement for chromosome 8 and aneuploidy for chromosome 15 (-15) evidenced by the result in the blastomere. The same condition was detected in BF, TE, and the WE. The analyses of both blastomere and BF were performed by 24Sure+, while 24Sure was used for TE cells and WE. (**B**) Detection of segmental abnormality in a couple with a normal karyotype. The PBs predicted an embryo with a condition unbalanced for chromosome 4 and aneuploid for chromosome 16. This condition was also detected in BF, TE, and WE.

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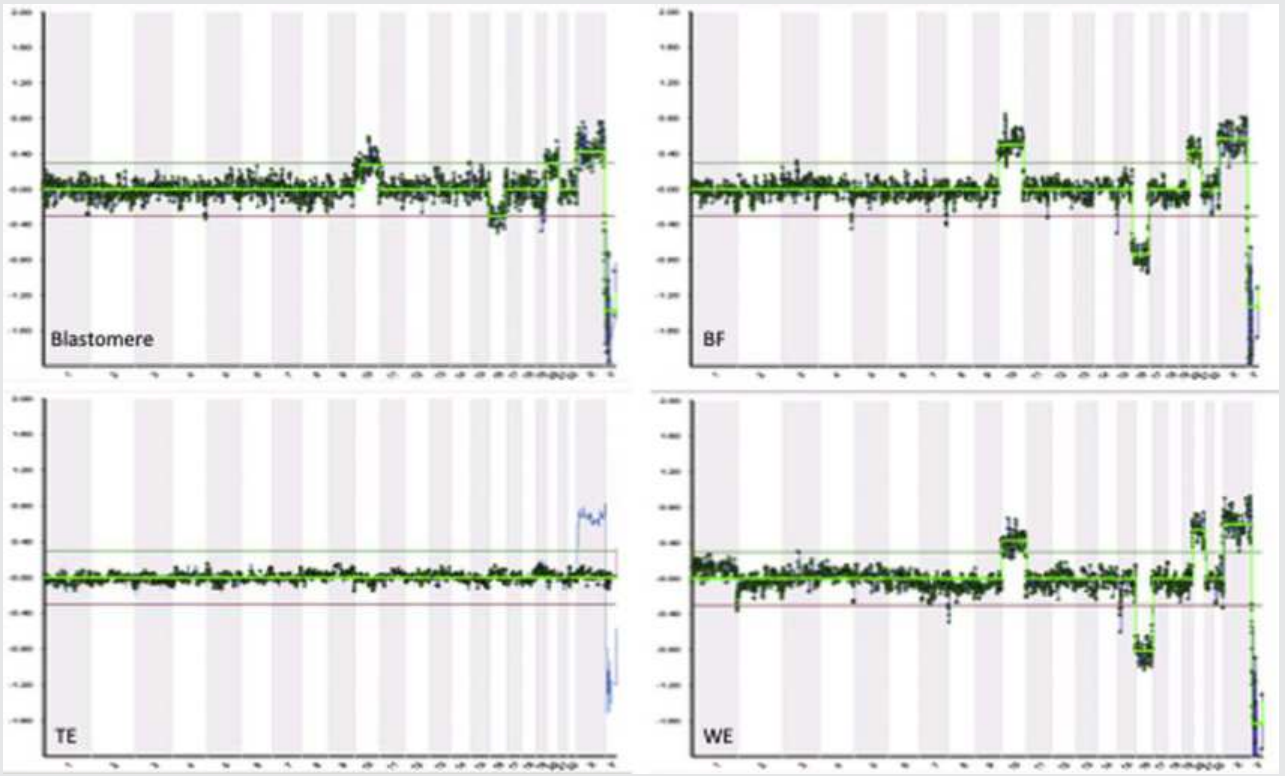
FIGURE 3



Detection of segmental abnormality in a carrier of the translocation 46XX,t(5;10)(q13;p12). The embryo, as evidenced by the result from the blastomere, had the unbalanced rearrangement for chromosome 10 (+10p11.23-10q26.3) associated with aneuploidy for chromosomes 20 and 16 (+20; −16). The same condition was detected in BF and the WE, although here the whole chromosome 10 was in gain, while TE cells turned out to be euploid.

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provide the most reliable results for PGS associated with an improved clinical outcome (17–20).

A major advantage of blastocyst biopsy is that multiple cells can be retrieved from each embryo without touching the embryonic mass. Therefore, the invasiveness of the procedure, if not null, is certainly reduced compared with blastomere biopsy, with the additional benefit of a lower degree of mosaicism (21, 22). Blastocentesis, that is, the aspiration of the fluid contained in the blastocoelic cavity, could represent a biopsy procedure with an even lower effect on blastocyst integrity.

The first relevant finding of our study is the presence of DNA in almost 100% of the samples analyzed in the last year (first aim of our study). Looking at the detection of DNA from the 116 BFs investigated, it is evident that we experienced a learning curve that led to a substantial improvement of our performance (Fig. 1). To identify the reasons for this progress, several factors were evaluated, including the blastocyst stage in relation to the time of development. Blastocysts with an expanded cavity on day 5 were those showing the highest probability of having successful DNA amplification from BF. This is not surprising, especially if we consider that in most cases in which failed amplification occurred, DNA seemed not to be absent, but highly fragmented, as suggested by the smear in the agarose gel when it was electrophoresed for 15 minutes (12). We can postulate that the BF from these blastocysts contained small fragments of DNA derived from dead cells generated by apoptosis, a process known to occur even in good-quality blastocysts (23). It is also possible that the quantity of DNA was so small that successful amplification could not occur. With this in mind, we are working on the possibility that a different protocol of amplification could improve the overall performance. As an additional point, the step of tubing is certainly critical, and we believe that the direct transfer of the retrieved BF from the ICSI pipette into cold PCR tubes followed by immediate short spinning is the approach providing the highest DNA recovery.

Once it was established that it was possible to recover DNA from the BF at an acceptable proportion, the next step was to verify its reliability in assessing the blastocyst chromosome status (second aim of our study). Therefore, we selected two types of reference samples: [1] the results from the biopsies done at previous stages, either PBs or blastomeres, coming from our PGS or PGD for translocation programs; and [2] the results from TE samples as this type of biopsy now represents the most common strategy used for PGD and PGS. In some cases we also analyzed the WE

(ICM and TE cells together), being well aware that some forms of mitotic anomalies could be reciprocal and consequently lead to a condition of apparent euploidy for the involved chromosomes.

A high degree of ploidy concordance was found when comparing the results from either PBs or blastomeres with those derived from BFs, supporting the good prediction power that biopsies done at initial stages have on the developing blastocysts. This is in contrast with other reports indicating the poor predictive value of biopsies done at early stages (24, 25). We want to underline that in our study all embryos had a regular development and that no severe male factor cases were included, which could have increased the incidence of postzygotic abnormalities, either contributed by the sperm or by early cleavage divisions (26, 27). Following this strategy, the meiotic abnormalities (provided that both PBs are assessed) and the abnormalities originating at initial mitoses are likely to be found at early stages (28, 29).

Nevertheless, the general tendency today is to perform TE biopsy for PGS and PGD purposes, and for this reason we took results from TE cells as the main reference to evaluate the results of the corresponding BFs. As represented in Supplemental Table 3, the ploidy concordance was 97.1% (67/69), with a concordance per chromosome of 98.4%. In the vast majority of cases, BFs and TFs were either fully concordant (81%, 56/69) or partially concordant (16%, 11/69), indicating that BF is a good reflection of the TE chromosome condition. The observed partial chromosome concordance could be a consequence of the preferential allocation of aneuploid cells to the BF or to the TE compartment. Both mechanisms have been proposed to occur in the framework of aneuploidy rescue after both meiotic and mitotic errors, which can lead to mosaicism (30, 31). The biological significance and clinical implications of mosaicism confined to BF has not been yet elucidated, but for blastocysts with mosaicism the resulting implantation rate has been reported to be substantially lower compared with the transfer of uniformly euploid blastocysts (32). We know from our experience that mosaicism sometimes can be already predicted when using a-CGH, but it is much more evident with next generation sequencing (NGS). Therefore, we can expect further improvement in the characterization of DNA extracted from BFs by performing NGS, and this is part of our immediate future plans.

Finally, two BFs showed ploidy discordance with TE cells (Table 1). In the first of these cases, a euploid BF was associated with two opposite aneuploid conditions for the same chromosome in the blastomere when compared with TE cells and WE. This could be due to a mitotic error that occurred at very early stages, resulting in aneuploidy for chromosome 9 with one trisomic and one monosomic cell line, the last one prevailing over the other.

In the second discordant case, an embryo from a translocation carrier with a rearrangement involving chromosome 10, the blastocyst was diagnosed as euploid (or balanced) based on TE cells, but the corresponding BF showed the same abnormality detected in the blastomere and in the WE (Fig. 3). Because it is a translocation case involving chromosomes 5 and 10, most abnormalities affecting chromosome 10 would be expected to have a meiotic origin and therefore to be present in all embryonic cells. Nevertheless, the resulting euploidy in TE cells suggests the presence of euploid/aneuploid mosaicism presumably at a very low ratio. We cannot exclude the possibility, although not very likely, that the chromosome 10 abnormality seen in this case was unrelated to the translocation but was the consequence of a mitotic error. These findings suggest that we cannot expect any biopsy, including TE cells, to fully represent the totality of the embryo, although when used in PGS programs, TE cell biopsy leads to extremely positive outcomes (17–20). Based on these results, we can anticipate blastocentesis to provide a similar clinical benefit, possibly even superior owing to its modest invasiveness.

It was of special interest to find that segmental abnormalities can be detected in BFs (third aim of our study), not only those associated with translocation carriers but also those that originated de novo (Fig. 2). The number of sets that we could analyze was relatively small ($n = 16$), but we had ploidy concordance in all cases when comparing the results from PBs or blastomeres with those obtained from BFs. More important, all BFs showed the segmental abnormality predicted by the analysis done at previous stages. The reproducibility of segmental abnormalities throughout the different stages of preimplantation embryos as detected in multiple independent tests is an indirect demonstration of the existence of these anomalies as real events occurring during cell divisions.

Other investigators have reported on the chromosome analysis of DNA retrieved from BF. While the amplification rate seems not to be far from the results that we reported initially (12), the prediction value was much lower compared with ours. Several reasons can explain these diversities. First of all, we have studied embryos showing a regular development (14), while other investigators mostly used discarded embryos donated for research (33, 34). It is possible that not enough attention has been paid to embryo morphology and development, but although the association between blastocyst formation and aneuploidy is known to be weak, some studies reported a higher incidence of multiple aneuploidies in blastocysts with poor morphology (35–37). Hence, it is difficult to expect high concordance results throughout the different stages of development from the analysis of poor-quality embryos. In addition, we would avoid systematically taking the WE as the only reference because, as already mentioned, the occurrence of mosaicism consequent to mitotic nondisjunction could be undetected by this approach (31, 34).

In conclusion, the results reported in the present study reinforce the potential use of BF as a source of DNA for PGS or PGD for translocations, although the reason for the detected discordances has not been yet demonstrated to be related to the biology of preimplantation embryos. It is worth noting that BF aspiration, being less invasive than alternative methods for the sampling of embryo DNA, has potential cost advantages. Highly experienced staff, trained in embryo biopsy, are probably not essential, and costly laser equipment is not needed. Needless to say, the proportion of samples producing DNA suitable for a-CGH analysis needs to be optimized.

Currently, additional research is ongoing with the aim to characterize the proteome content of the BF (7, 38). Optimization of the techniques able to analyze the BF molecules of embryonic origin could open up a novel methodology to assess embryo viability.

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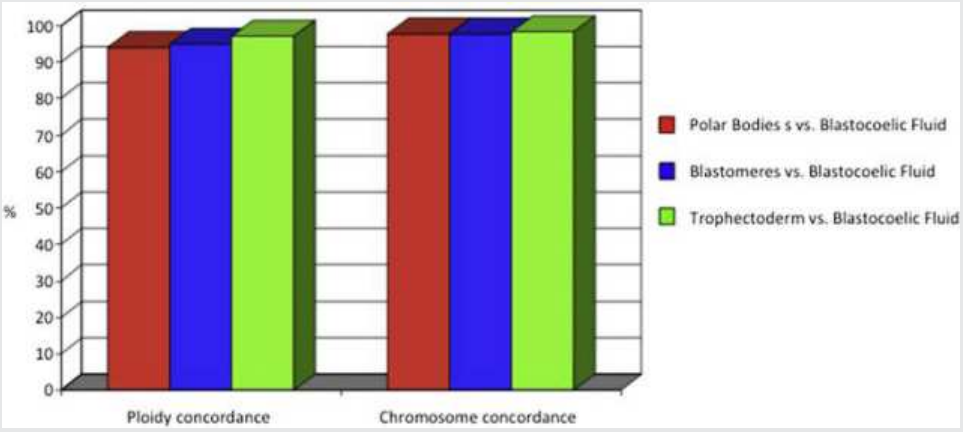
SUPPLEMENTAL FIGURE 1



Results of ploidy and chromosome concordance. No differences were found when comparing the results from BFs with those predicted by PBs, blastomeres, and TE cells.

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SUPPLEMENTAL TABLE 1

List of abnormalities detected by BF in 54 embryos from patients with a normal karyotype.

ID	PB1	PB2	Blastomere	Prediction of the blastocyst status	BF	TE
1	Gain 15 Loss 16	Loss 12		Gain 12,16 Loss 15	Gain 12,16 Loss 15	Gain 12,16 Loss 15
2	Gain 15	Loss 2		Gain 2 Loss 15	Gain 2	Gain 2
3	Euploid	Gain 22		Loss 22	Loss 17,22	Loss 17,22
4	Gain 8	Gain 15,22		Loss 8,15,22	Loss 8,15,22	Loss 8,15,22
5	Gain 9 Loss 1	Gain 1,16,22 Loss 9		Loss 16,22	Gain 16 Loss 15	Gain 16,22
6			Loss 14	Loss 14	Loss 14	Loss 14
7			Gain 5,10,17,21 Loss 1,6,14,15	Gain 5,10,17,21 Loss 1,6,14,15	Gain 8,13,21 Loss 5,17,18,X	Gain 21
8			Euploid	Euploid	Euploid	Euploid
9	Euploid	Gain 16		Loss 16	Loss 9,16	Loss 9,16
10	Loss 19	Euploid		Gain 19	Gain 19	Gain 19
11	Gain 4,5,6,7,9,11, 12,15,19,20,X Loss 1,2,3,8,10, 13,14,16,18	Gain 1,2,3,8,10, 13,14,16,18 Loss 4, 5,6,7,9,11, 12,15,19,20,X		Euploid	Euploid	Euploid
12	Gain 4,19	Gain 5 Loss 4		Loss 5,19	Gain 2,8 Loss 5,13,14	Gain 2,8,17, Loss 5,10,13,19
13	Euploid	Euploid		Euploid	Euploid	Euploid
14			Euploid	Euploid	Euploid	Euploid
15			Euploid	Euploid	Euploid	Euploid
16			Loss 16	Loss 16	Loss 16	Loss 16
17	Euploid	Loss 21		Gain 21	Gain 21	Gain 21 Loss 1
18	Euploid	Gain 15		Loss 15	Loss 1,15	Loss 15
19	Euploid	Gain 19,21 Loss 18,22		Gain 18,22 Loss 19,21	Gain 18,22 Loss 19,21	Gain 18,22 Loss 19,21
20	Euploid	Loss 15		Gain 15	Gain 15	Gain 15
21	Loss 9,19	Gain 19 Loss 18		Gain 9,18	Gain 9,18	Gain 9,18
22	Gain 20 Loss 21	Euploid		Gain 16 Loss 20	Gain 16 Loss 20	Gain 16 Loss 20
23	Gain 15	Loss 15		Euploid	Euploid	Euploid
24	Euploid	Euploid		Euploid	Euploid	Euploid
25	Loss 22	Loss 14		Gain 14,22	Gain 14,22	Gain 14,22
26	Gain 16 Loss 13	Loss 16		Gain 13	Gain 13	Gain 13
27	Loss 15[a]	Gain 15[a]		Loss 15	Loss 15	Loss 15
28	Euploid	Gain 13		Loss 13	Loss 13	Loss 13
29			Loss 8,16	Loss 8,16	Loss 8,16	Loss 8,16
30			Gain 2,7, 21, Loss 10	Gain 2,7,21 Loss 10	Gain 2,7,21 Loss 10	Gain 2,7,21 Loss 10,11,16
31			Gain 8,16	Gain 8,16	Gain 8,16	Gain 8,16
32	Gain 21 Loss 13	Gain 22		Gain 13 Loss 21,22	Gain 13 Loss 21,22	Gain 13 Loss 21,22
33	Loss 6,22	Gain 22		Loss 6	Euploid	Euploid
34	Gain 15,21	Gain 22		Loss 15,21,22	Loss 15,21,22	Gain 10 Loss 15,21,22
35	Loss 9	Euploid		Gain 9	Gain 9	Gain 9
36	Gain 18 Loss 16	Gain 16,18 Loss 22		Gain 22	Gain 22	Gain 22
37	Gain 13	Loss 13		Euploid	Euploid	Euploid
38	Gain 21 Loss 18	Gain 18 Loss 8		Gain 8 Loss 21	Gain 8 Loss 21	Gain 8 Loss 21
39			Loss 14,20	Loss 14,20	Loss 20	Loss 14,20
40	Loss 15	Gain 15 Loss 17		Gain 17	Gain 17	Gain 17
41			Loss 16	Loss 16	Loss 16	Loss 16
42			Loss 22	Loss 22	Loss 22	Loss 22
43			Gain 22	Gain 22	Gain 22	Gain 22
44			Loss 22	Loss 22	Loss 22	Loss 22
45			Gain 9	Gain 9	Euploid	Loss 9
46			Gain 8,22	Gain 8,22	Gain 8,22	Gain 8,22
47			Euploid	Euploid	Euploid	Euploid
48			Loss 11	Loss 11	Loss 11	Loss 11

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SUPPLEMENTAL TABLE 1

Continued.

ID	PB1	PB2	Blastomere	Prediction of the blastocyst status	BF	TE
49			Euploid	Euploid	Euploid	Euploid
50	Loss 18,19	Gain 18 Loss 15		Gain 15,19	Euploid	Euploid
51			Loss 21	Loss 21	Loss 21	Loss 21
52			Euploid	Euploid	Euploid	Euploid
53			Loss 21	Loss 21	Loss 21	Loss 21
54[a]			Loss 2	Loss 2	Euploid	No Result

Note: Embryos with segmental abnormalities are excluded from this table and listed in Supplemental Table 2. Results of the analyses from other stages are also listed: PBs (PB1 and PB2), blastomeres, and TE cells. The term "Gain" indicates hyperhaploidy; the term "Loss" indicates hypohaploidy. The four cases involving BF ploidy discordance are ID nos. 33, 45, 50, 54.

[a] The condition of hypohaploidy for chromosome 2 was confirmed by the analysis of the WE.

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SUPPLEMENTAL TABLE 2

List of segmental abnormalities detected by BF in nine embryos from translocation carriers and seven embryos from patients with a normal karyotype.

ID	Karyotype	PB1	PB2	Blastomere	BF	TE	WE
57	46XX,t(3;8) (p25;q12)			Loss 3p26.3->3p26.3 Loss 8p23.3->8q12.1 Loss 15	Loss 3p26.3->3p26.3 Loss 8p23.3->8q12.3 Loss 15	Loss 3p26.3->3p25.3 Loss 8p23.3->8q12.1 Loss 15	Loss 3p26.3->3p25.3 Loss 8p23.3->8q12.1 Loss 15
58	46XX,t(3;8) (p25;q12)			Gain 8q12.1->8q24.3 Loss 3p26.3->3p26.3	Gain 8q12.1->8q24.3 Loss 3p26.3->3p26.3	Gain 8q12.1->8q24.3 Loss 3p26.3->3p26.3	Gain 8q12.1->8q24.3 Loss 3p26.3->3p26.3
60	46XX,t(5;10) (q13;p12)			Gain 10p11.23->10q26.3 Gain 20 Loss 16	Gain 10 Gain 20 Loss 16	Euploid	Gain 10 Gain 20 Loss 16
61	46XX,t(5;10) (q13;p12)			Loss 5q14.3->5q35.3	Gain 7q11.23->7q36.3 Loss 5q14.3->5q35.3	Loss 5q14.3->5q35.3	Loss 5q14.3->5q35.3
68	46XY,t(4;18) (q31.1;p11.3)			Gain 18p11.32->18p11.31 Loss 4q31.1->4q35.2	Gain 18p11.32->18p11.31 Loss 4q31.1->4q35.2	Gain 18p11.32>18p11.31 Loss 4q31.1->4q35.2	Gain 18p11.32->18p11.31 Loss 4q31.1->4q35.2
64	46XY,t(4;18) (q31.1;p11.3)			Gain 4q31.1->4q35.2	Gain 4q31.1->4q35.2	Gain 4q31.1->4q35.2	Gain 4q31.1->4q35.2
69	46XY,t(16;20) (p13.3;q13.1)			Gain 20q13.12->20q13.33 Gain 22	Gain 20q13.13->20q13.33 Loss 19	Gain 20q13.12->20q13.33 Loss 18	Gain 20q13.13->20q13.33 Loss 19
70	46XY,t(1;4) (p13;q13)			Gain 1p36->1p31.3 Loss 4q24->4q35.2 Loss 11	Gain 1p36->1p31.3 Loss 4q24->4q35.2 Loss 11q14.1->11q25	Gain 1p36->1p31.3 Loss 4q24->4q35.2 Loss 11q14.1->11q25	–
67	46XX,t(9;10) (p24;q11.2)			Gain 18 Gain 20 Loss 10q11.21->10q26.3	Gain 18 Gain 20 Loss 10q11.21->10q26.3	Gain 18 Gain 20 Loss 10q11.21->10q26.3	–
55	Normal	Gain 9q21.1->9q34.3 Loss 9p24.3->9q13 Loss 15	Loss 9		Gain 9p24.3->9q13 Gain 15	Gain 9p24.3->9q13 Gain 15	Gain 9p24.3->9q13 Gain 15
56	Normal	Gain 4q28.1->4q35.2	Gain 16 Loss 4q26->4q35.2		Loss 4q13.1->4q35.2 Loss 16	Loss 4q22.1->4q35.2 Loss 16	Loss 4q22.3->4q35.2 Loss 16
59	Normal	Gain 7q11.23->7q36.3 Loss 4 Loss 7p22.3->7q11.22	Loss 7		Gain 4 Gain 7p22.3->7q11.23	Gain 4 Gain 7p22.3->7q11.23	Gain 4 Gain 7p22.3->7q11.23
62	Normal			Gain 11p15.5->11q13.4 Gain 21 Loss 22	Gain 11p15.5->11q13.2 Gain 11q13.3->11q25 Gain 21 Loss 22	Gain 11 Gain 21 Loss 22	Gain 11p15.5->11q13.2 Gain 21 Loss 22
63	Normal			Gain 2p22.3->2q37.3 Loss 2p25.3->2p22.3 Loss 9	Loss 2p25.3->2p22.3 Loss 9	Gain 22 Loss 2p25.3->2p22.3	Gain 2 Gain 14 Gain 17 Loss 9
65	Normal			Gain 3p26.3->3p11.1 Loss 3p11.1->3q29 Loss 22	Gain 16p13.3->16p12.1 Gain 19 Loss 3p26.3->3p11.1 Loss 22	Gain 3p26.3->3p11.1 Loss 3p11.1->3q29 Loss 22	Gain 11 Gain 16 Loss 3p26.3->3p11.1 Loss 22
66	Normal			Gain 13 Loss 9p24.3->9p21.2	Gain 13 Loss 9p24.3->9p21.2	Gain 13 Loss 9p24.3->9p21.2	Gain 13 Loss 9p24.3->9p21.2

Note: Results of the analyses from other stages are also listed: PBs (PB1 and PB2), blastomeres, TE cells, WE. The term "Gain" indicates hyperhaploidy; the term "Loss" indicates hypohaploidy. The case involving ploidy discordance between BF and the other biopsies is ID no. 60.

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SUPPLEMENTAL TABLE 3

Chromosome concordance between 70 BFs and corresponding oocytes or embryos at the cleavage stage and blastocysts.

	Concordance			
Variable	**Full**	**Partial**	**Null**	**Total**
BFs vs. PBs				
No. of embryos (%)	25 (73.5)	7 (20.5)	2 (6)	34
No. of chromosomes (%)	575/575 (100)	148/161 (91.9)	43/46 (93.5)	766/782 (97.9)
BFs vs. blastomeres				
No. of embryos (%)	29 (81)	5 (14)	2 (5)	36
No. of chromosomes (%)	696/696 (100)	102/120 (85.0)	46/48 (95.8)	844/864 (97.7)
BFs vs. TE cells				
No. of embryos (%)	56 (81)	11 (16)	2 (3)	69[a]
No. of chromosomes (%)	1,344/1,344 (100)	242/264 (91.7)	44/48 (91.7)	1,630/1,656 (98.4)

Note: Oocytes were tested on PBs, cleavage-stage embryos on one blastomere, and blastocysts on TE cells. Concordance was expressed per embryo and per single chromosome.
[a] One TE sample did not provide informative results and was excluded from this calculation.

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