Deoxyribonucleic acid detection in blastocoelic fluid: a new predictor of embryo ploidy and viable pregnancy

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Objective: To investigate blastocysts, defined as euploid and aneuploid by trophectoderm (TE) cell analysis, for the presence of DNA in the blastocoelic fluid (BF) detected by whole-genomic amplification (WGA); and to correlate the presence of DNA in BF with the clinical outcome after the transfer of TE-euploid blastocysts.
Design: Retrospective study.
Setting: In vitro fertilization unit.
Patient(s): This study included 91 patients performing preimplantation genetic testing for aneuploidy on TE cells from January 2015 to December 2017. In the case of ET, only single blastocyst transfers were performed.
Intervention(s): Blastocoelic fluids and TE cells were retrieved from 256 blastocysts before vitrification. All blastocysts were diagnosed by array-comparative genomic hybridization (a-CGH) on TE cells. Amplification and a-CGH of DNA from BFs was performed at a later time after TE biopsy and ET.
Main Outcome Measure(s): Whole-genomic amplification of BFs, evaluation of the chromosome condition in BFs and TE cells, and correlation of BF results with the clinical outcome of TE-euploid transferred blastocysts.
Result(s): The incidence of amplification after WGA was significantly lower in BFs from TE-euploid blastocysts (n = 32, 45%) when compared with the aneuploid ones (n = 150, 81%), resulting in 182 BFs with successful DNA amplification. When submitted to a-CGH, informative results were obtained from 172 BFs. Comparison of these results with those from the corresponding TE cells gave a ploidy concordance of 93.6% and a mean number of aneuploid events per sample that was higher in BFs than in TE cells (2.0 vs. 1.4, respectively). After the transfer of 53 TE-euploid blastocysts, the clinical pregnancy rate was 77% in the group with BF-failed amplification, and 37% after BF-successful amplification. The same trend was found for the ongoing pregnancy rate (68% vs. 31.5%, respectively).
Conclusion(s): The presence of DNA in BFs detected by WGA is correlated with the blastocyst ploidy condition defined by TE cell biopsy and with the implantation potential of TE-euploid blastocysts. These findings could have a clinical implication for the selection of the most viable embryo for transfer because, after submitting BFs to WGA, priority would be given to TE-euploid blastocysts with BF-failed amplification. Similarly, BF-failed amplification could be an additional selection criterion to prioritize embryos for transfer even in conventional IVF cycles with blastocysts that were vitrified after BF aspiration. (Fertil Steril® 2019;111:77–85. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Aneuploidy, blastocoel, blastocyst, preimplantation genetic testing, trophectoderm cell biopsy

The presence of DNA in the blastocoelic fluid (BF) from expanded blastocysts has been reported in different studies (1–3). After amplification this DNA can be analyzed with the aim of determining the blastocyst chromosome condition, although the degree to which it is representative of the corresponding embryo ploidy is presently unclear (4).

In two of the most recent studies, BF-DNA sufficient for analysis, assessed by whole-genomic amplification (WGA), ranged from 63% (5) to 82% (6). The subsequent chromosome analysis by array-comparative genomic hybridization (a-CGH) indicated a ploidy concordance with the results of the conventional forms of embryo biopsy ranging from 62% (5) to 97% (6). The reason for this

Received June 15, 2018; revised and accepted September 25, 2018; published online December 5, 2018.
M.C.M. has nothing to disclose. C.A. has nothing to disclose. A.C. has nothing to disclose. C.T. has nothing to disclose. A.P.F. has nothing to disclose. L.G. has nothing to disclose.
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Fertility and Sterility® Vol. 111, No. 1, January 2019 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2018.09.016

divergence could reside in several factors, including the different status of the studied embryos (frozen and donated for research because unsuitable for clinical use vs. fresh, respectively, in the two studies).

Timing of BF aspiration, a process known as blastocentesis, is a factor possibly affecting the amplification outcome (6). Cavitation actually begins on day 4 of development, leading to the formation of the blastocyst, where cells differentiate into the inner cell mass and trophectoderm (TE) lineages (7). At the same time, an active transport of sodium ions out of the TE cells leads to a constant accumulation of water in the blastocoelic cavity that arrives to occupy most of the volume of the blastocyst. Therefore, when blastocentesis is done in expanded blastocysts developed in fresh embryo culture, the retrieved fluid derives from a process that initiated several hours earlier. Conversely, when the BF is aspirated from thawed blastocysts, the short time needed for re-expansion could not be sufficient for having a number of DNA molecules in the cavity sufficient to be successfully amplified.

According to the results currently available, the proportion of BF samples with failed DNA amplification is evidently higher compared with that from TE cells or even from blastomeres, making BF not suitable as an alternative source of DNA for preimplantation genetic testing (4, 8). This can be ascribed to several possibilities, namely DNA is absent; it is present in low quantity and quality owing to DNA fragmentation perhaps caused by cell apoptosis (3); or it is lost owing to technical problems in retrieving such a small BF volume (on the order of nanoliters), particularly during the tubing step.

After successful WGA, comparative analyses have reported on the ploidy concordance between BF and conventional biopsies mainly derived from aneuploid embryos (5, 6). Conversely, very scarce information is available on the DNA content in BFs from euploid embryos, whereas no correlation has ever been done between BF-DNA results and the implantation potential of the TE-euploid transferred embryos.

In this study we extended our experience to TE-euploid blastocysts with the aims of [1] verifying the presence of analyzable DNA in BF after amplification; [2] investigating the rate of concordance between BFs and corresponding TE biopsies, especially in the case of TE-euploid blastocysts; and [3] correlating the results from the BFs' analysis with implantation after single embryo transfer of blastocysts that had been diagnosed as euploid by TE cell analysis. To be consistent with our dataset, the chromosomal analysis was performed by a-CGH.

MATERIALS AND METHODS

Patients from a Preimplantation Genetic Testing for Aneuploidy Program

This study included 256 blastocysts from 91 couples (maternal age 37.7 ± 4 years [mean ± SD]) undergoing 107 cycles of 24-chromosome a-CGH analysis in TE cells in a preimplantation genetic testing for aneuploidy (PGT-A) program. Indications for PGT-A were advanced maternal age (n = 67) or repeated IVF failures (n = 24).

To address the first aim of this study, the BF from 256 blastocysts was submitted to WGA, and a-CGH was then performed on those that amplified successfully. To address the second aim of the study, the concordance between BFs and corresponding TE cells was evaluated in the 172 blastocysts with informative a-CGH results from BFs. For the third aim of the study, the BF results from 53 transferred TE-euploid blastocysts (single embryo transfer) were correlated with implantation.

All patients signed a consent form allowing PGT-A on TE cells and further chromosomal analysis on BFs. Embryos were cultured in LifeGlobal medium (Lifeglobal Group), and only blastocysts of the highest grade were selected for biopsy (9). After TE and BF biopsy, all blastocysts were vitrified (solutions and protocols from Kitazato), and single embryo transfer was performed in a following cycle. Only blastocysts diagnosed as euploid according to the results of TE biopsy were transferred. The analysis of BFs was done at a later time after TE biopsy and ET.

Clinical Pregnancy Outcome

All patients were followed up after ET to have information on the clinical outcome. Clinical pregnancies were defined by the presence of a gestational sac with fetal heartbeat. The ongoing pregnancy rate per transfer was calculated as the number of clinical pregnancies beyond the 22nd week of gestation (10).

Biopsy Procedures

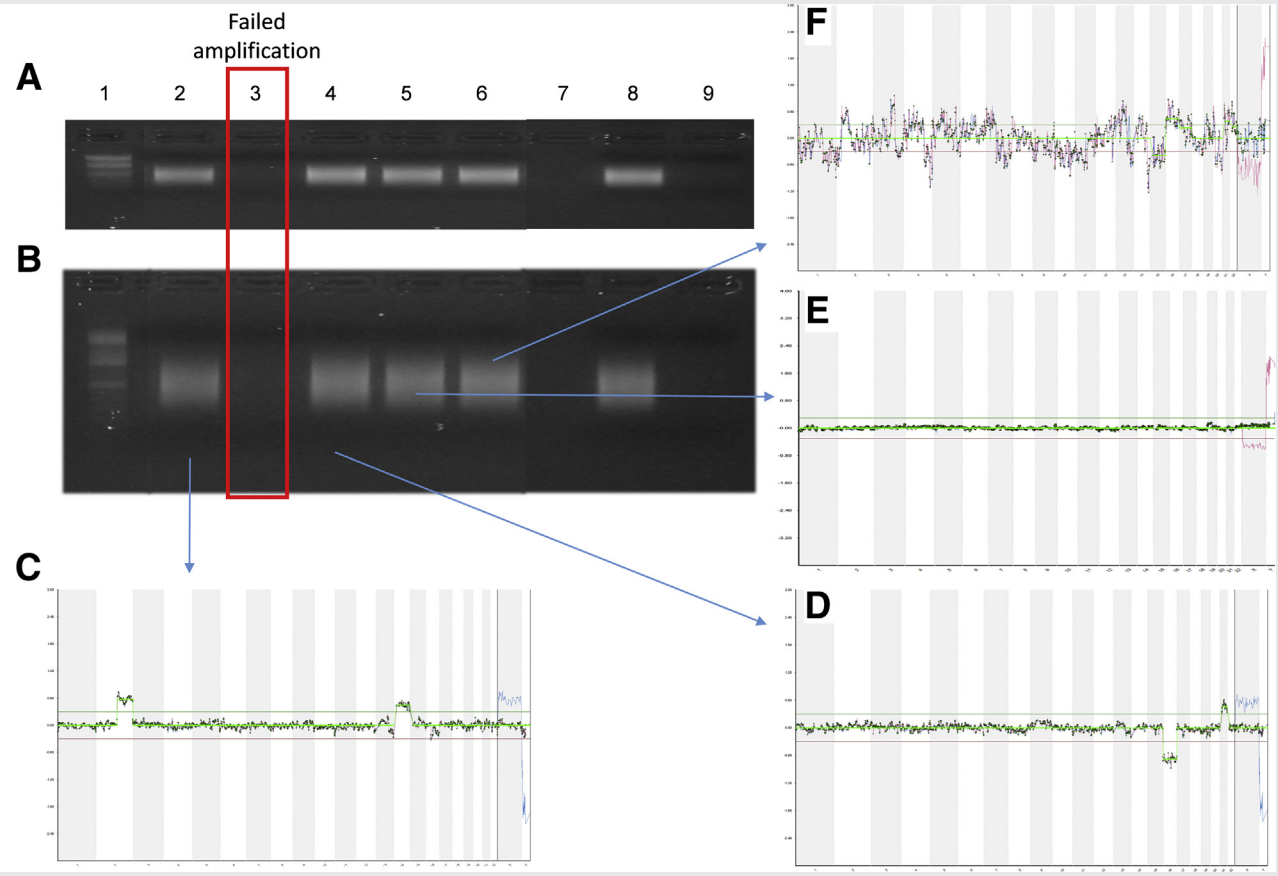
Biopsies of TE cells and BF were performed as already described (2). Briefly, BFs were aspirated from expanded blastocysts using an intracytoplasmic sperm injection pipette. The retrieved fluids were transferred into 0.2-mL polymerase chain reaction tubes containing 1 μL phosphate-buffered saline kept in cold racks and spun immediately after each biopsy. The procedure was repeated for all blastocysts from the same patient, and then BFs were stored at −80°C until further processing for chromosomal analysis. The TE biopsy was performed a few hours later, as soon as the hatching process started. After TE biopsy, blastocysts were cryopreserved, and the chromosome analysis on TE cell biopsies was initiated to assess the blastocyst ploidy status.

WGA and A-CGH

Amplification of DNA was performed by WGA in a class II Laminar flow cabinet (SurePlex, Illumina) and assessed by loading 5 μL of the final reaction onto a 1.5% agarose gel. Successful amplification was indicated by a band with an intensity similar to that of the positive control, whereas in the event of negative amplification, the lane in the agarose gel looked like the negative control (Fig. 1).

After successful amplification an aliquot of the WGA product was labeled for a-CGH (24Sure v3.0 Microarray, Illumina). Results were evaluated by dedicated software (Bluefuse Multi v4.0, Illumina) to predict the euploid/aneuploid status of the corresponding embryo (11). Informative a-CGH results were those for which data interpretation and calling were straightforward. This was not possible in the case of

FIGURE 1



Control of DNA amplification after processing the DNA from five BFs by 1.5% agarose gel electorphoresis. (**A**) After 5 minutes, the bands in agarose gel electrophoresis indicated one failed amplification (lane 3) and four successful amplifications (lanes 2, 4, 5, and 6). Lane 7 was empty, lane 8 was the positive control, and lane 9 the negative control. (**B**) Appearance of the bands after a 15-minute gel electrophoresis run. Lane 1 is the DNA marker ladder. The corresponding profiles after array-CGH indicated two aneuploid DNA (**C** and **D**) and one euploid DNA (**E**). One noninformative profile resulted despite successful amplification (**F**).

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noninformative a-CGH results, for which the obtained profiles did not make it possible to establish the chromosome condition of the corresponding DNA.

All BF analyses were performed by operators blinded to the previous TE cell results as well as to the clinical outcome after ET.

Concordance of the ploidy condition between BF and TE results was defined as full concordance when the status of all chromosomes corresponded in the two biopsies (both classified as euploid or both as aneuploid), and partial concordance when only some aneuploid chromosomes corresponded in the two biopsies (both classified as aneuploid). Finally, null concordance defined those cases in which the ploidy status of one biopsy did not correspond to that predicted by the other biopsy. The concordance per single chromosome was also reported.

For the whole-embryo analysis, the entire embryo was transferred into a 0.2-mL polymerase chain reaction tube following the procedure previously described. The analysis of results for the detection of mosaicism followed the indications given by the a-CGH manufacturer (Illumina).

Statistical Analysis

Chi-square test or Fisher's exact test were used to compare categoric variables, applying the Yates' correction, 2×2 contingency tables. Student's t test with unequal variances was applied to quantitative variables, one-way analysis of variance.

Ethical Approval

The study was approved by our institutional review board (no. 20110503).

RESULTS

PGT-A Program

As shown in Table 1, a total of 256 BFs were submitted to WGA. These BFs originated from blastocysts that had already been diagnosed as euploid (n = 71) or aneuploid (n = 185) according to the analysis done by TE biopsy. In all, successful amplification was obtained in 182 BFs (71%), whereas 74

TABLE 1

Blastocoelic fluid outcome of WGA and a-CGH in 256 blastocysts from our PGT-A program.

Variable	Total	Chromosome condition according to TE biopsy		*P* value (euploid vs. aneuploid)
		Euploid	Aneuploid	
No. BFs submitted to WGA	256	71	185	
No. BFs with successful amplification (%)	182 (71)	32 (45)	150 (81)	<.001
No. BFs with failed amplification (%)	74 (29)	39 (55)	35 (19)	<.001
No. total BFs analyzed by a-CGH	182	32	150	
No. informative BFs/analyzed by a-CGH (%)	172/182 (87)	26/32 (81)	146/150 (97)	<.005
No. total noninformative BFs/submitted to WGA (%)	84/256 (33)	45/71 (63)	39/185 (21)	<.001

Note: The corresponding embryos had been previously diagnosed as euploid or aneuploid by TE biopsy.

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(29%) failed to amplify. There was no difference in BF-DNA amplification rates in relation to the time between BF storage at −80°C and the WGA procedure (data not shown).

When comparing the two groups, TE-euploid blastocysts vs. aneuploid blastocysts, the incidence of successful amplification was significantly lower in BFs from euploid blastocysts (n = 32, 45%) when compared with the aneuploid ones (n = 150, 81%, $P<.001$). Conversely, failed BF-DNA amplification was more frequent in TE-euploid blastocysts than in TE-aneuploid blastocysts.

After amplification, 182 BFs were analyzed by a-CGH, yielding informative results in 172 samples (87%; Table 1). The comparison between the two TE ploidy groups indicated that informative results were more frequent in aneuploid embryos (97%) compared with euploid embryos (81%, $P<.005$).

The total failure of informativity from BF biopsy was 63% in euploid blastocysts and 21% in aneuploid blastocysts ($P<.001$). This figure was calculated by adding the number of samples that failed to amplify (39 in the TE-euploid and 35 in the TE-aneuploid group) to the number of noninformative a-CGH profiles (6 in the TE-euploid and 4 in the TE-aneuploid group).

Concordance Between BF and TE Cells

A total of 172 blastocysts had chromosomal results after a-CGH on BFs. The comparison with the data from the corresponding TE a-CGH profiles gave a ploidy concordance of 93.6% (66.3% full and 27.3% partial concordance) and a discordance of 6.4% (Supplemental Table 1, available online). When calculated per single chromosome, the total concordance was 96%. When broken down per ploidy concordance, the concordance per single chromosome decreased from 100% in the cases with total concordance to 86.5% in cases with partial ploidy concordance, and 90% in cases with null concordance.

The mean number of aneuploid chromosomes per sample tended to be higher in BFs than in TE cells (2.0 vs. 1.4, respectively, $P=.01$), and this difference was especially accentuated in the case of partial concordance (4.0 vs. 2.3, $P=.002$).

A total of 11 discordant cases were recorded, including four blastocysts aneuploid after TE analysis with a euploid BF, and seven blastocysts presenting with the opposite condition. None of the four TE-aneuploid blastocysts were transferred, and the analysis of the corresponding whole embryos confirmed the TE-predicted aneuploid status.

Clinical Outcome

Table 2 reports the results from 53 transferred blastocysts that had been classified as euploid according to TE biopsy. In all, 33 clinical pregnancies were obtained, of which 29 were regularly ongoing beyond the 22nd week of gestation, and 4 miscarried. Unfortunately, no information is available on the abortive material. Of the 29 ongoing pregnancies, 20 underwent prenatal diagnosis, and all PGT-A results were confirmed.

TABLE 2

Results from BF analysis by a-CGH in 53 transferred embryos (single embryo transfer) classified as euploid by TE biopsy.

Variable	Total	BF successful amplification	BF failed amplification	*P* value (successful vs. failed amplification)
Maternal age (y), mean ± SD	36.3 ± 4.2	35.4 ± 4.4	36.9 ± 4	
No. transferred blastocysts	53	19	34	
No. euploid BFs	17	17	0	
No. aneuploid BFs	1	1	0	
No. BFs with no result	35	1	34	
No. clinical pregnancies	33	7	26	
No. ongoing	29	6	23	
No. miscarriages	4	1	3	
Total clinical pregnancy rate (%)	33/53 (62)	7/19 (37)	26/34 (77)	.005
Ongoing pregnancy rate (%)	29/53 (55)	6/19 (31.5)	23/34 (68)	.010

Note: The BF results were related to implantation.

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The majority of pregnancies derived from the group with failed BF amplification, accounting for a clinical pregnancy rate of 77% vs. 37% after successful amplification ($P=.005$). The same trend was found for the ongoing pregnancy rate (68% vs. 31.5% respectively, $P=.010$).

In the group of successful amplification, a miscarriage occurred (Table 2). The BF from the corresponding blastocyst was found to be aneuploid, in total discordance with the result from the TE biopsy (Supplemental Fig. 1).

DISCUSSION

The present study is focussed on the characterization of the DNA retrieved from the BF of expanded blastocysts that had already been diagnosed by TE chromosome analysis. Differently from our previous work that was concentrated on aneuploid embryos, we extended the procedure of blastocentesis to embryos that had been classified as euploid by TE cell biopsy.

Overall, the proportion of BF samples that were analysable after WGA was 71%, a figure substantially lower compared with our previous experience (6). Surprisingly, we found a significant difference between TE-euploid and TE-aneuploid blastocysts, with the incidence of failed amplification and of noninformative results after a-CGH being higher in TE-euploid blastocysts (Table 1). This finding implies a different quality or quantity of the DNA content in the corresponding BFs, suggesting an effect of the embryo ploidy condition on the blastocyst internal compartment.

Little is known about the reason why aneuploidy is so frequent in human preimplantation embryos (12–14). What is becoming more and more clear is that, irrespective of its origin, embryos try to react to aneuploidy, although the majority of chromosomal errors are probably unlikely to be corrected, especially if they are present in multiple copies (15). Several processes can contribute to the embryonic aneuploid self-correction, including cellular fragmentation, the formation of micronuclei, blastomere exclusion, and the presence of a stringent cell cycle control that only becomes active after the embryonic genome activation (16–18). In other words, in up to day-4 preimplantation embryos, aneuploid blastomeres can continue cell division resulting in aneuploid daughter cells. However, when reaching the blastocyst stage, there is a drastic change in the embryo's cell cycle control, and the spindle assembly checkpoint becomes able to induce apoptosis, in agreement with what has been reported in the mouse (19, 20). A condition of mosaicism could be a possible consequence in the attempt of aneuploidy correction, and the chances that mosaic embryos have to further develop are strictly related to the proportion of euploid/aneuploid cells, to the type of abnormalities involved, and to the efficiency of the corrective mechanisms (21–23).

According to the results from an experimental mouse model, there are different mechanisms in the blastocyst lineages to control aneuploidy. Whereas in the TE, aneuploid cells exhibit increased cell cycle length and senescence for which they tend to be outcompeted by euploid cells, in inner cell mass they are preferentially eliminated by apoptosis (19). A similar mechanism acts in humans, by which apoptosis occurs more frequently in aneuploid cells in the attempt made by the embryo to recover a euploid, viable condition (20, 24). The blastocoelic cavity, a closed compartment where DNA and proteins of embryonic origin accumulate (3, 25, 26), could represent a sort of collector for different cell products, including those generated by apoptosis. This possibility is supported by a sequencing study reporting that the DNA from the BF was highly fragmented, with the dominant population of fragments (160–220 bp) being very similar to the size of that seen in the circulating plasma of human blood, and typical of apoptotic fragments (3). In view of these findings, we can expect a more frequent presence of DNA, most probably of apoptotic origin, in the BF from aneuploid blastocysts, echoing the attempt made by the embryo to correct its aneuploid condition. Should the elimination of abnormal cells be complete, ploidy discordance between BF and TE would be the consequence. This could have been the case in seven blastocysts having euploid TE and aneuploid BF (Supplemental Table 1). We cannot exclude a priori that the chromosome abnormalities in the BF could derive from cells not incorporated in the TE (although none was noticed during biopsy), which are known to be necrotic and highly aneuploid (27). Similarly, the outgrowth of TE-euploid cells over aneuploid cells in mosaic embryos described in the mouse (19) could contribute to the discordance between BF and TE chromosome results.

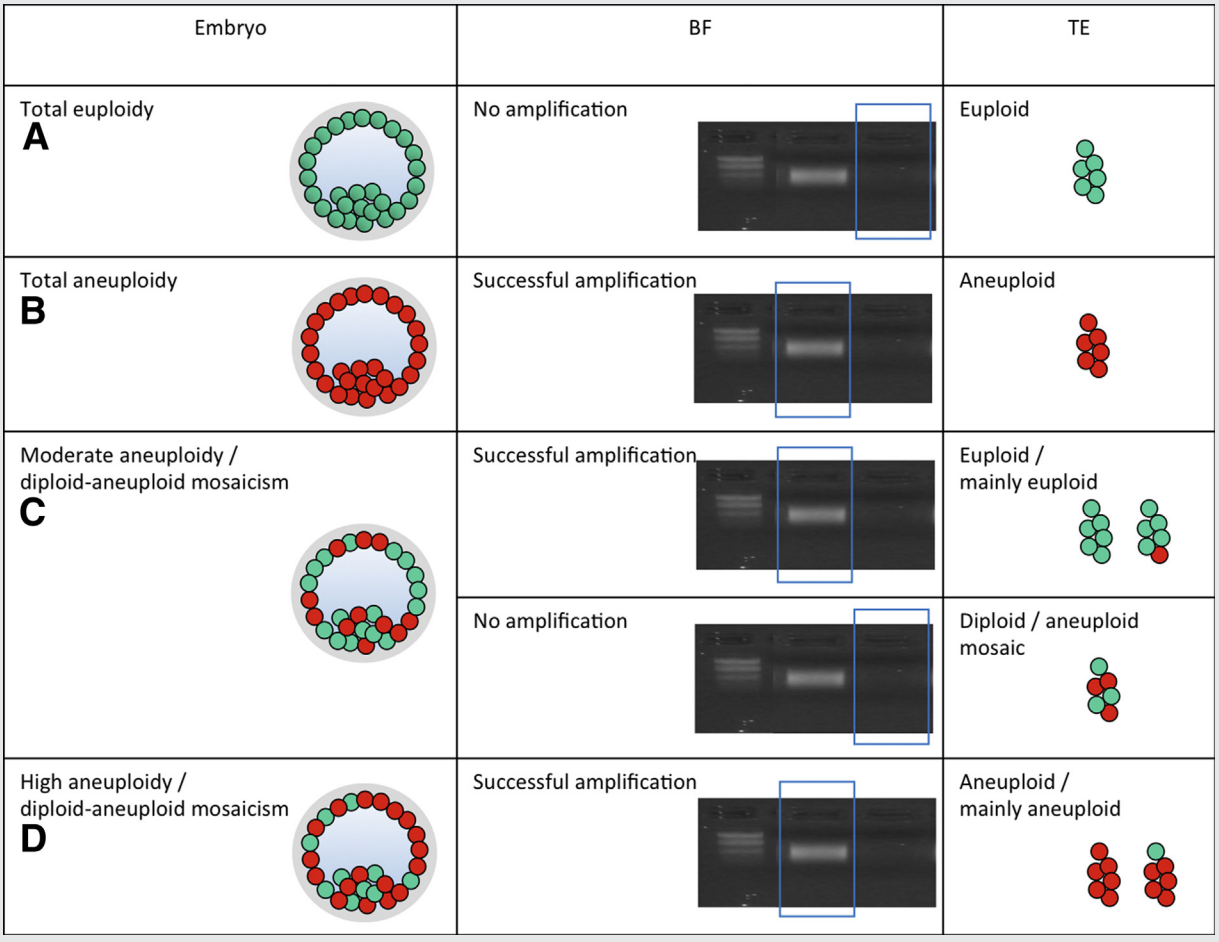
The opposite situation happened in four blastocysts, where an aneuploid TE was coexisting with a euploid BF (Supplemental Table 1). These blastocysts were not transferred, and the analysis of the four corresponding whole embryos confirmed the results predicted by the TE cells. In this case the presence of a euploid BF could reflect a defective transition from the mechanism of DNA repair pathways toward the cell cycle control and apoptosis (28).

Conversely, necrosis or apoptosis could be less likely to occur, or to occur to a lesser extent, in euploid blastocysts that probably are in the quiet metabolic state that is normally associated with an increased viability potential (the quiet embryo hypothesis from Leese) (29). All these considerations could support the data from our study, although we did not prove directly that the DNA in the BF was originated by apoptosis.

Additional reflections derived from the clinical outcome of the transferred TE-euploid embryos. Although a minor proportion of blastocysts with successful BF amplification implanted, the total and ongoing pregnancy rates were significantly higher after the transfer of the blastocysts with failed BF-DNA amplification (Table 2). Interestingly enough, one BF from the group with successful amplification resulted in an aneuploid a-CGH profile, and this blastocyst gave rise to a pregnancy that ended in a miscarriage (Supplemental Fig. 1). In this case, the combination of the BF and TE results suggests the occurrence of mosaicism that was reflected in the extrusion of aneuploid DNA in the BF, as a possible cause of the spontaneous abortion.

In all, the clinical outcome of the TE-euploid transferred embryos suggests that the grade of BF amplification after

FIGURE 2



Proposed model of ploidy condition in human blastocysts and TE biopsy in relation to the BF amplification after WGA. (**A**) In euploid blastocysts, lack of BF amplification is the most likely condition, compatible with the embryo quiet metabolism hypothesis. The chances of implantation are high. (**B**) In aneuploid blastocysts, successful BF amplification could reflect the blastocyst attempt to correct the aneuploid condition. (**C**) Different scenarios can be postulated if the blastocyst is moderately aneuploid. Successful BF amplification may result after the elimination, possibly by apoptosis, of aneuploid cells into the blastocoelic cavity. This process could be efficient to the point that TE cells give a euploid or mainly euploid profile. The chances of implantation of the resulting embryo depend on the grade of mosaicism and on the amount of energy spent to rescue an aneuploid condition. If the elimination of aneuploid cells is very modest or not occurring at all, aneuploid cells will remain in the embryo, and TE cells would be mosaic. Also in this case, the grade of mosaicism plays a critical role in determining the probability of implantation, but the embryo could have the advantage of not having wasted much energy in removing aneuploid cells to the BF. (**D**) If the incidence of aneuploidy is preponderant, the elimination of aneuploid cells results in successful BF amplification, but TE cells are expected to be completely or mainly aneuploid.

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WGA could have a predictive value regarding the corresponding blastocysts' viability, but before drawing solid conclusions, we need to expand our dataset and to validate the results in a prospective study. In addition, further characterization of the DNA in the BF would permit definition of its origin and nature.

On the basis of the current experience, we can suggest different scenarios that relate our preliminary findings to the possible mechanism of aneuploidy rescue. As represented in Figure 2, we can expect euploid blastocysts to be in a quiet metabolic state. Therefore, they do not undergo extensive apoptosis, and the DNA in the BF is absent or present in extremely reduced quantity. The resulting blastocysts have high probability of ongoing implantation.

Conversely, an abundance of DNA in the BF could be indicative of embryos confronting the presence of aneuploid cells. If this process of aneuploid cell elimination is very efficient, TE cells can give a euploid or mainly euploid profile. The resulting chances of implantation would depend on the grade and type of mosaicism and on the amount of energy spent to rescue an aneuploid condition. If the extrusion of aneuploid cells is very modest or almost absent, aneuploid cells will remain in the embryo, and TE cells would be mosaic. Also in this case, the grade and type of mosaicism is closely related to the probability of implantation, but the embryo would have the advantage of not having wasted much energy in extruding aneuploid cells to the BF.

Should these hypothetical considerations reflect a biological condition, we can conclude that the fate of the blastocyst will depend on the grade of diploid/aneuploid mosaicism, on its capacity of rescuing an aneuploid or prevalently aneuploid condition, and on the energy spent on this transition. The use of next generation sequencing instead of a-CGH would improve identification and quantification of mosaicism in BF and TE to possibly substantiate the above considerations.

Besides unravelling a peculiar aspect of early embryogenesis, these findings, if confirmed, could translate into a clinical advantage in the approach of selecting the best embryo for transfer. On the basis of these considerations, after submitting BFs to WGA, priority would be given to TE-euploid blastocysts with failed BF amplification. A similar approach could be adopted in patients without indications for PGT-A where the BF, retrieved from expanded blastocysts before vitrification, could be submitted to WGA, and the results used to grade embryos for transfer. Our preliminary data after 52 single blastocyst transfers in no-PGT-A patients indicate, in case of BF-DNA amplification failure (n = 30), 77% clinical pregnancy rate (n = 23) and 70% ongoing pregnancy rate (n = 21), compared with 37% clinical pregnancy rate (n = 8) and 18% ongoing pregnancy rate after 22 successful BF-DNA amplification.

Considering that the quantity of DNA in the BF can be extremely low, a more sophisticated technique for its detection, from those available nowadays, could be of great value (30). Despite disappointing results, it would also be interesting to investigate the DNA of embryonic origin detected in spent culture medium to verify whether it reflects the condition of that found in the BF (30–32).

In conclusion, our recent data propose blastocentesis as an additional tool aimed at contributing to the knowledge of early embryogenesis. The presence of genomic DNA in the BF could be a reflection of the struggle engaged by preimplantation embryos against the condition of aneuploidy that is extremely common in the early phases of human embryo development. The marginalization of aneuploid nuclei into the blastocoelic cavity could represent a strategy toward a euploid condition. For this reason, this event tends not to occur when the blastocyst is fully or prominently euploid. Therefore, failure to detect DNA from the BF after WGA could be a criterion to prioritize embryos for transfer.

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Detección de ácido desoxirribonucleico en fluido de blastocele: un nuevo factor predictor de ploidía embrionaria y gestación viable.

Objetivo: Investigar los blastocistos, definidos como euploides o aneuploides según el análisis celular de trofoectodermo (TE), mediante la presencia de ADN en el fluido del blastocele (BF) detectado por amplificación genómica completa (WGA); y correlacionar la presencia de ADN en BF con el resultado clínico tras el transfer de blastocistos TE-euploides.

Diseño: Estudio Retrospectivo.

Entorno: Unidad de fecundación in vitro.

Paciente (s): Este estudio incluyó 91 pacientes que realizaron test genético preimplantacional para aneuploidías en células TE desde enero 2015 hasta diciembre 2017. En caso de ET (*transferencia embrionaria*), solo se realizó transfer de blastocisto único.

Intervención (s): Los fluidos de blastocele y las células de TE fueron extraidos de 256 blastocistos antes de la vitrificación. En todos los blastocistos se realizó el diagnóstico mediante arrays de hibridación genómica comparada (a-CGH) en las células de TE. La amplificación y el a-CGH de DNA de las BFs se realizó en un tiempo posterior, tras la biopsia de TE y el ET.

Principales medidas de resultado: La amplificación genómica completa de BFs, la evaluación de la condición cromosómica de las BFs y las células TE y la correlación de los resultados de BF con el resultado de TE-de blastocistos euploides transferidos.

Resultado (s): La incidencia de amplificación tras WGA fue significativamente inferior en BFs de blastocistos TE-euploides (n= 32, 45%) en comparación con los aneuploides (n = 150, 81%), con resultado de 182 BFs con amplificación de DNA con éxito. Al enviarlas a a-CGH, se obtuvieron resultados informativos de 172 BFs. La comparación de estos resultados con los de las correspondientes células de TE dio una concordancia de ploidía del 93.6% y un número medio de hallazgos aneuploides por muestra que fue superior en BFs que en células TE (2.0 vs. 1.4, respectivamente). Tras el transfer de 53 blastocistos TE-euploides, la tasa de gestación fue del 77% en el grupo con fallo de amplificación de BF y del 37% tras amplificación con éxito de BF. Se encontró la misma tendencia para tasa de gestación clínica viable (68% vs. 31.5%, respectivamente).

Conclusión (s): La presencia de ADN en BFs detectado mediante WGA se correlaciona con la condición ploide del blastocisto definido mediante biopsia de células de TE y con el potencial de implantación de los blastocistos TE-euploides. Estos hallazgos podrían tener una implicación clínica para la selección del embrión más viable para transfer, porque, tras enviar las BFs a WGA, debería darse prioridad a los blastocistos TE-euploides con amplificación de BF fallida. De modo similar, la amplificación fallida de BF-podría ser un criterio adicional para priorizar embriones para transfer incluso en FIV convencional con blastocistos que fueron vitrificados tras aspiración de BF.