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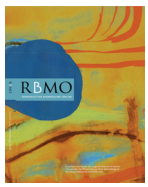

ARTICLE

Functional assessment of donated human embryos for the generation of pluripotent embryonic stem cell lines

BIOGRAPHY

Ianaê I Ceschin obtained her MSc from the Institute of Biosciences at University of São Paulo, and is currently a preimplantation genetic counsellor. She has extensive experience in the field of embryology, and her research involves genetic counselling and the derivation of human embryonic stem cell lines.

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KEY MESSAGE

Of the 23 donated embryos, 16 were considered viable for human embryonic stem cell derivation, of which 15 harbouring genetic abnormalities and poor morphology quality displayed no capacity for further in-vitro cell expansion. Such embryos may be unsuitable for clinical use and the derivation of human embryonic stem cell lines.

ABSTRACT

Research question: Can discarded embryos at blastocyst stage, donated to research because of genetic abnormalities and poor morphological quality, become a reliable source of human embryonic stem cell (HESC) lines?

Design: This study was consecutively conducted with 23 discarded embryos that were donated to research between February 2020 and April 2021. All embryos, except one, were morphologically evaluated and underwent trophectoderm biopsy for preimplantation genetic testing using next-generation sequencing (NGS), and then vitrified. After warming, the embryos were placed in appropriate culture conditions for the generation of HESCs, which was functionally assessed with immunofluorescence and flow cytometry for pluripotency capacity and spontaneous in-vitro differentiation. Cytogenetic assessment of the HESC was conducted with multiplex ligation-dependent probe amplification, and micro array comparative genomic hybridization.

Results: From the 23 embryos initially included, 17 survived warming, and 16 of them presented viability. Overall, the embryos presented poor morphological quality after warming. Only the previously untested embryo was capable of generating a new HESC line. Further characterization of this line revealed fully functional, euploid HESCs with preserved pluripotency, becoming a useful resource for research into human development and therapeutic investigation.

Conclusions: None of the donated blastocysts with poor morphological quality in association with genetic abnormalities detected by NGS had the capacity for further in-vitro expansion to originate pluripotent HESC lines. This finding seems to provide extra support to genetic counselling on the suitability of this type of embryo for clinical use.

INTRODUCTION

Unlike induced pluripotent stem cells, human embryonic stem cells (HESCs) are bona-fide pluripotent cells that do not require genetic or molecular manipulation to acquire such a property. The natural ability to differentiate into cells from the three embryonic germ layers, makes HESC the gold standard in the study of pluripotency. They have become a precious source of differentiated human cells for research in early embryonic development, degenerative conditions, disease modelling and treatment, drug screening and toxicology (*Eguizabal et al., 2019*). The first HESC cell lines were

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KEYWORDS
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derived from the inner cell mass (ICM) of good-quality embryos in blastocyst stage that had been generated for IVF purposes and later donated to basic research (*Thomson, 1998*). Later, less controversial alternatives for HESC line derivation have been sought with the use of blastocysts with genetic abnormalities detected during the preimplantation stage (*Ben-Yosef* et al., *2008*). Embryos showing morphological abnormalities, aneuploidy identified by different preimplantation genetic testing (PGT) methods, or both, may be either discarded or donated to research purposes, depending on the legislation of each country.

Although the derivation of HESC lines from embryos with poor morphological quality has been shown to be possible, success rates have been low, ranging between 4.1% and 25% (*Mateizel* et al., *2006*; *Lerou et al., 2008*; *Liu et al., 2009*; *O'Leary et al., 2012*). Similiarly, several studies have also reported on the derivation of HESC lines from embryos presenting genetic abnormalities detected with the use of fluorescence in-situ hybridization (FISH) (*Lavon et al., 2008*; *Mandal et al., 2013*; *Huang et al., 2015*). Although the embryos had been characterizard as aneuploid by PGT-A, the hESC lines derived in those studies were composed of cells with an euploid karyotype. Those findings suggest that the cytogenetically abnormal embryo may have been misdiagnosed owing to false-positive FISH results, the presence of chromosomal mosaicism, or both (*Baart et al., 2006*). Mosaicism is a biological event and its detection may depend on the accuracy of the genetic test used, the developmental stage of the embryo during the biopsy and the presence of technical artifacts (*Takeuchi, 2020*).

Recently, more sensitive methods, such as the microarray comparative genomic hybridization (aCGH) and next-generation sequencing (NGS) offer the possibility of simultaneously analysing all the 23 pairs of chromosomes (*Coonen* et al., *2020*). They also allow the detection of mosaicism and segmental mutations with greater precision, and, depending on the detection limit established for each platform, their accuracy in the detection of mosaicism can be similar (*Coonen* et al., *2020*). Some studies have also reported on the derivation of pluripotent HESC lines from embryos with aneuploidy as detected by aCGH (*Fonseca et al., 2015*) and NGS (*Zhou et al., 2018*). Despite the higher accuracy of these methods, the possibility that mosaicism may also have been present in the inner cell mass and trophectoderm, or only in the trophectoderm, still exists (*Vera-Rodriguez and Rubio, 2017*). The probability of obtaining a HESC line from a mosaic embryo is higher compared with embryos with single chromosomal aneuploidy. *Zhou et al. (2018)* reported on the derivation of two HESC lines from embryos with de-novo segmental aneuploidy, which has been associated with mitotic errors, and is more likely to have occurred in mosaic embryos (*Girardi et al., 2020*). The successful derivation of euploid HESC lines from embryos with segmental aneuploidy reported by *Girardi et al. (2020)* seems to indicate that selective pressure may have favoured viable euploid cells during in-vitro expansion of mosaic embryos. Selective pressure is a well-known phenomenon by which cells with euploid content have an expansion advantage compared with aneuploid cells in mosaic embryos (*Lavon et al., 2008*; *Singla et al., 2020*).

Studies assessing the effect of naturally occurring genetic defects and their association with morphological quality on the viability and pluripotency of HESC lines are still required. Embryos subjected to PGT by NGS and identified as carriers of genetic abnormalities can be of great value to better ascertain their developmental progress. The use of these pluripotent cells in basic research can provide novel insights in the field of human preimplantation development. Moreover, better understanding the capability of embryos to generate HESC lines may also have a practical effect on genetic counselling. Therefore, the present study reports on the attempt to derive HESC lines from embryos with genetic abnormalities detected by NGS methods and which also present poor morphological quality.

MATERIALS AND METHODS

The present study was analysed and duly approved by the Ethics Research Committee of the Biosciences Institute at the University of São Paulo (CAAE number: 30686420.8.0000.5464; first version: 20 April 2020; second version: 12 November 2020). The study conforms to the Declaration of Helsinki for medical research involving human subjects. All embryos used in the present study were donated to research in accordance with the Brazilian Bio-safety Law 11.105/2005, and written informed consent was obtained from biological parents.

The present study was consecutively conducted with 23 embryos that were donated to research between February 2020 and April 2021. Of the 23 donated embryos, 16 embryos were aneuploid, five embryos presented high-risk haplotype for one specific target mutation in gene *COL1A2*, one embryo demonstrated DNA amplification failure with no informative result, and one embryo, untested for genetic abnormalities, was donated after long-term freezing storage (over 3 years).

Quality analysis, trophectoderm biopsy and preimplantation genetic testing analysis

In every case, mature oocytes were fertilized with intracytoplasmic sperm injection (*Palermo et al., 1992*), and resulting embryos cultured in GV Blast medium (Ingámed, Maringá, Brazil) in an incubator with humidified atmosphere with 5.0% CO_2 and 20% O_2 at 37°C, until day 5 or 6 after fertilization (*Almodin et al., 2010*). When reaching blastocyst stage, the embryos were morphologically evaluated (*Gardner and Schoolcraft, 1999*) before being biopsied for PGT analysis (*Kokkali et al., 2020*), vitrified, and stored in liquid nitrogen (*Almodin et al., 2010*).

The biopsied cells were washed in phosphate buffered saline (PBS) (Ingámed, Maringá, Brazil), placed into sterile polymerase chain reaction tubes, frozen at −20°C, stored and shipped to the genetic laboratory (Igenomix, São Paulo, Brazil) for PGT analysis by NGS. For those embryo samples that were subjected to PGT for aneuploidy (PGT-A), the results were categorized as euploidy (aneuploidy, present in up to one chromosome); complex aneuploidy (present in two to five chromosomes); chaotic aneuploidy (present in more than five chromosomes) and segmental aneuploidy (duplications and deletions over 10 Mb). For samples showing no DNA amplification, the result was reported as non-informative. Mosaicism level was determined as follows: euploid embryo, less than 30% of cells estimated as aneuploid; mosaic embryo, between 30% and 70% of cells estimated as aneuploid; and aneuploid embryo, above 70% of cells estimated as aneuploid (*García-Pascual et al., 2020*).

In those cases of potential genetic disease caused by a known target mutation, PGT

for monogenetic disease was carried out by assaying the pathogenic variant linked to the matching haplotype markers (*Carvalho et al., 2020*). Segregation analysis using short tandem repeat (STR) markers was conducted on DNA samples of related family members to establish matching allele markers (haplotype). The gene *COL1A2* was evaluated with the direct and indirect method using quantitative fluorescence polymerase chain reaction. For the direct method, polymerase chain reaction amplification was carried out in the region c.1477G>C, whereas, for the indirect method, polymerase chain reaction was carried out on the STR linked to the gene *COL1A2*. Samples were categorized as high risk or low risk for haplotype inheritance of the targeted mutation. In samples presenting high risk for haplotype inheritance, no PGT-A analysis was conducted.

Post-warming quality analysis and embryo processing

As the donated blastocysts became available, they were warmed and cultured in optimal conditions (*Almodin et al., 2010*). Twenty-four hours after warming, the embryos were morphologically evaluated in a Diaphot 300 inverted fluorescence phase contrast microscope (Nikon, Tokyo, Japan). Embryos showing more than 50% of degenerated cells were considered as not having survived the procedure. Only surviving embryos had their morphological pattern evaluated for viability. Embryos were considered viable when over 50% expanded blastocoel was present (*Gardner and Schoolcraft, 1999*).

Embryo processing varied depending on the characteristics of blastocyst. ICM and embryo quality. Embryos with a well-defined ICM were subjected to ICM dissection with a high-precision laser pulse (*Turetsky et al., 2008*). The ICM was then transferred to a pre-treated culture dish for cell expansion. Embryos with undefined ICM, or poor morphological quality, had their zona pellucida removed by laser pulse and the whole embryo without the zona pellucida was transferred to a pre-treated culture dish for cell expansion.

Culture matrices and human embryonic stem cell derivation

Recombinant 521 laminin (Biolamina, Sundbyberg, Sweden) and E-cadherin (R&D Systems, Minneapolis, MN, USA) diluted at 9 to 1 ratio with PBS (Ingámed, Maringá, Brazil) was used as the basement membrane matrix. Culture dishes were coated at 37°C for 2 h at a concentration of 13.5 μg/ml 521 laminin and 1.5 μg/ml E-cadherin (*Rodin et al., 2014*). A warmed mTeSR™ Plus medium (STEMCELL Technologies, Vancouver, Canada) was used (*Ludwig et al., 2006*), and cells were plated in controlled atmosphere with 5% CO_2 and 5% O_2. The first medium change occurred at 48 h by replacing one-half of the volume with fresh medium. Afterwards, the totality of the medium was changed every 48 h. The first cell culture passage was carried out mechanically 9 days from initial cell plating. The HESC colonies were cut and transferred to a new pre-treated plate, in the same dish area, and cultured with medium supplemented with 10 μM ROCK inhibitor Y-27632 (STEMCELL Technologies, Vancouver, Canada).

New HESC colonies were passaged at every 5–12 days for the first few weeks. From passage 4 on, as the cell culture became more stable and homogeneous, the passages were conducted in a split ratio of 1:2 with a dissociation reagent composed of a Matrigel® basement membrane matrix (Corning Life Sciences, Nork York, USA), supplemented with 10 μM ROCK inhibitor Y-27632 (STEMCELL Technologies, Vancouver, Canada) (*Bajpai et al., 2008*). Once established, the HESC line was maintained using chemical dissociation, the Matrigel matrix and culture medium with 1:500 Normocin (InvivoGen, San Diego, CA, USA).

The cells were cryopreserved in CryoStor® CS10 cell freezing medium (STEMCELL Technologies, Vancouver, Canada) according to manufacturer's instructions (*Ramos et al., 2014*). Briefly, the cells in cryopreservation medium were progressively cooled after the incubation programme: −20°C for 2 h, followed by −80°C for 24 h and final storage in liquid nitrogen at −196°C.

Human embryonic stem cell characterization

Cell pluripotency was determined by the expression of the markers OCT4A, NANOG, SOX2 and SSEA4, using immunofluorescence microscopy and flow cytometry. For the immunofluorescence analysis, the HESC line at passage 7 was cultured in Nunc Lab Tek chamber slides (ThermoFisher, Waltham, MA, USA) and fixed with 4% paraformaldehyde for 1 h. The cells were then incubated overnight at 4°C with primary Rabbit mAb (Cell Signalling, Danvers, MA, USA) at the following dilutions: 1:400 for OCT4A, 1:200 for NANOG, 1:400 for SOX2, and with 1:10 Alexa Fluor® 488 Mouse anti-SSEA-4 (BD Biosciences, Franklin Lakes, NJ, USA). The next day, cells were rinsed with PBS three times and incubated with 1:1000 Alexa Fluor® 488 - Anti-Rabbit secondary antibody and 1μg/ml of DAPI solution (ThermoFisher, Waltham, MA, USA). The slides were mounted in VECTASHIELD® antifade mounting medium (Vector Laboratories, Newark, CA, USA) and analysed under a Zeiss 800 inverse laser scanning confocal microscope (Zeiss, Aalen, Germany).

For flow cytometry, cells at passage 7 were fixed with 4% paraformaldehyde for 1 h and incubated for 24 h at 4°C with primary Rabbit mAb (Cell Signalling, Danvers, MA, USA) at the following dilutions: 1:200 for OCT4A, 1:100 for NANOG, 1:300 for SOX2 and with 1:10 Alexa Fluor® 488 Mouse anti-SSEA-4 (BD Biosciences, Franklin Lakes, NJ, USA). After being rinsed three times, the cells were incubated with 1:1000 Alexa Fluor® 488 - Anti-Rabbit secondary antibody (ThermoFisher, Waltham, MA, USA). The analysis was conducted with 5000 events acquired in a FACSAria II flow cytometer (BD Bioscience, Franklin Lakes, NJ, USA), and the results were analysed with FlowJo™ Software (BD Biosciences, Franklin, NJ, USA).

As negative controls for the immunofluorescence and flow cytometry, a mouse isotypic antibody conjugated to Alexa-Fluor 488 was used (Supplementary Figure 1).

Embryoid body formation and characterization by immunofluorescence

Spontaneous in-vitro HESC differentiation was analysed by embryoid body formation and characterization (*Itskovitz-Eldor et al., 2000*). The HESC were dissociated and plated at a concentration of 9000 cell/well using mTeSR™ Plus medium (STEMCELL Technologies, Vancouver, Canada) in an ultra-low attachment round bottom 96-well plate (Corning, New York, USA). On day 4 after seeding, the differentiation medium was implemented, composed of 80% DMEM high glucose (Gibco, Billings, MT, USA), 20% fetal bovine serum (Gibco, Billings, MT, USA), Glutamax 100X (1:100) Gibco, Billings, MT, USA), and Antibiotic-Antimycotic Solution (100x) (1:100) (Gibco, Billings, MT, USA). After 1 week of growth with differentiation medium, the embryoid bodies, containing cells derived from the

three germ layers, were analysed by immunofluorescence. Embryoid bodies were fixed with 4% paraformaldehyde for 15 min at room temperature. After fixation, embryoid bodies were incubated with primary antibodies at a 1:1000 dilution overnight at 4°C. The primary antibodies used were as follows: rabbit anti-Brachyury (T) (Albacam, Cambridge, UK), for mesoderm cells; mouse anti-FOXA4 (Albacam, Cambridge, UK), for endoderm cells; anti-PAX6 (Albacam, Cambridge, UK), for ectoderm cells. Subsequently, embryoid bodies were rinsed three times using PBS (1x) and incubated with Alexa Fluor® 647 Anti-Rabbit and Alexa Fluor® 488 Anti-Mouse secondary antibodies (ThermoFisher, Waltham, MA, USA) at a dilution of 1:1000 for 1 h at room temperature. Incubated embryoid bodies were rinsed three times using PBS (1x) and transferred to a plate with PBS (1x). Images were immediately acquired using a Zeiss 800 inverse laser scanning confocal microscope (Zeiss, Oberkochen, Germany).

Molecular cytogenetic analysis

The presence of aneuploidy in the HESC was evaluated by multiplex ligation-dependent probe amplification (MLPA) and aCGH. Genomic DNA was extracted from the HESC line (at passage 7) using a QIAamp DNA Mini Kit (QIAGEN, Germantown MD, USA), according to manufacturer's protocol. MLPA was carried out using SALSA® MLPA® Probemix P070 Subtelomeres Mix 1 (MRC-Holland, Holland) to evaluate copy number variation (CNV), the deletion and duplication of subtelomeric regions (*Ahn et al., 2007*). An assay using a 180 K oligonucleotide array platform (Agilent Technologies, Santa Clara, CA, USA) was also conducted to investigate the presence of rare CNVs in the entire genome, and the analysis was conducted using the Nexus Copy Number software (Biodiscovery, El Segundo, CA, USA).

RESULTS

Embryo quality and genetic abnormalities

The donated embryos used in the present study were heterogeneous in their morphology, the type of PGT analysis and PGT results (TABLE 1). Of the 23 embryos initially evaluated, six did not survive the vitrification–warming procedure, one embryo survived but presented no viability, and 16 embryos were found to be viable and suitable for further manipulation. Approximately 60% (14/23) of blastocysts morphologically analysed before the trophectoderm biopsy had poor morphological quality (BC and CC), whereas eight embryos were graded as medium quality (B1 and BB), and one embryo as top quality (AA). On the other hand, the morphological quality of 15 out of 16 viable blastocysts analysed 24 h after warming were poor (BC and CC), whereas one embryo was graded as top quality (AA).

Of the 16 viable embryos, nine were subjected to ICM dissection and seeded in a pre-treated plate. The remaining seven viable embryos had their zonae pellucidae removed and the whole embryo was seeded in the pre-treated plate. Only four of the 16 embryos plated (25%) showed initial adhesion 48 h after plating (embryo identification: E-4; E-7; E-12; E-18), all of them subjected to ICM dissection. Ninety-six hours after plating, however, only one embryo (E-7) kept cells showing adhesion and expansion capabilities in the following culture time. Those cells were further cultured, deriving a stable HESC line, which was named Si-10 (FIGURE 1).

TABLE 1 CHARACTERIZATION OF THE 23 EMBRYOS EVALUATED IN THE STUDY

Embryo ID	EMQ[a]	NGS	PGT result	EMQ[b]	HESC
E-1	4BB	PGT-A	(−10)	4CC	–
E-2	3BB	PGT-A	(+17 +12 -16 +17)	NS	–
E-3	3BB	PGT-A	(+16 +22)	3CC	–
E-4	4CC	PGT-A	(chaotic)	4CC	–
E-5	B1	PGT-A	(−5p, +7, +22)	BC	–
E-6	5BC	PGT-A	(−19)	5BC	–
E-7	4AA	No	No genetic analysis	4AA	Si-10
E-8	4CB	PGT-M	High risk for *COL1A2 haplotype*	4CC	–
E-9	3CC	PGT-A	(+22)	4BC	–
E-10	4BC	PGT-M	High risk for *COL1A2* haplotype	NS	–
E-11	4CC	PGT-M	High risk for *COL1A2* haplotype	NS	–
E-12	3BC	PGT-A	(−5p, −11p)	3CC	–
E-13	4CC	PGT-A	(−4, −X0)	NS	–
E-14	4CC	PGT-M	High risk for *COL1A2* haplotype	4CC	–
E-15	3BB	PGT-A	(+1, +15, −21)	NV	–
E-16	4CC	PGT-A	(+3, -15, +16, −17, +22)	NS	–
E-17	4CC	PGT-M	High risk for *COL1A2* haplotype	4CC	–
E-18	3BC	PGT-A	(−4)	3BC	–
E-19	2BC	PGT-A	Non-informative	2CC	–
E-20	3BB	PGT-A	(−5, −11, −13)	NS	–
E-21	5CC	PGT-A	(+11 +16)	5BC	–
E-22	6BB	PGT-A	(chaotic)	6CC	–
E-23	3BC	PGT-A	(−1 +22)	3BC	–

[a] Embryo morphological quality before trophectoderm biopsy, vitrification, or both.

[b] Embryo morphological quality 24 h after warming.

E, embryo; EMQ, embryo morphological quality; NGS, next-generation sequencing; NS, did not survive warming; NV, non-viable; PGT-A, preimplantation genetic test for aneuploidy; PGT-M, preimplantation genetic test for monogenic disease. –, no HESC derivation.

Human embryonic stem cell characterization

The pluripotent Si-10 cells exhibited a morphology typical of well-defined HESC colonies (*Kim et al., 2005*), compatible with small undifferentiated cells capable of expanding *in vitro* (FIGURE 2). Expression of classic pluripotency factors was also detected in these cells both with immunofluorescence (FIGURE 3A, FIGURE 3B, FIGURE 3C and FIGURE 3D) and flow cytometry (FIGURE 3E). The latter method revealed that 99.8%, 99.8%, 99.8% and 92.1% of the Si-10 cells were positive for the markers OCT4A, NANOG, SOX2 and SSEA4, respectively.

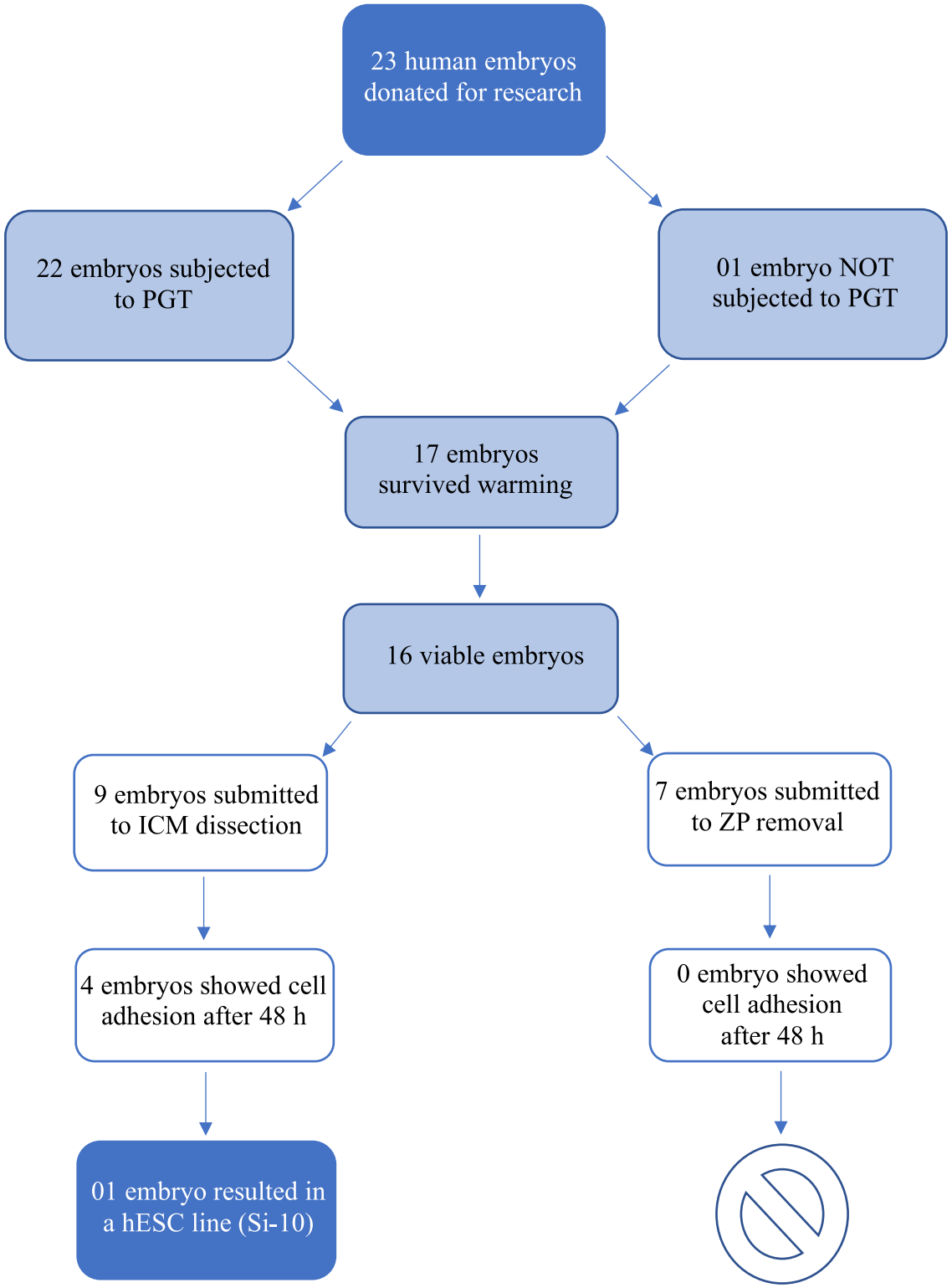

FIGURE 1 The experimental progression of the embryos used in the present study. hESC, human embryonic stem cell; ICM, inner cell mass; PGT, preimplantation genetic testing; ZP, zona pellucida.

Pluripotency of the Si-10 HESC line was also indicated by their capacity to generate embryoid bodies and differentiate *in vitro*. Immunofluorescence analysis of the embryoid bodies detected proteins that are typically expressed in cells of each of the three germ layers: endoderm (FOXA2), mesoderm (Brachyury) and ectoderm (PAX6). The morphological aspect of the embryoid bodies formed after 48 h are presented in FIGURE 4A and after 96 h in FIGURE 4B, and the expression of the germ

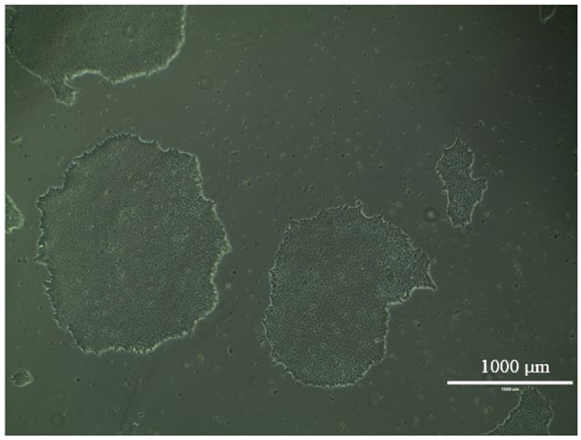

FIGURE 2 Colonies of Si-10 human embryonic stem cell (HESC) line expanded *in vitro*, displaying typical HESC colony morphology (images of Si-10 HESC were captured by the EVOS® FL Cell Imaging System).

layer makers FOXA2, Brachyury and PAX6 in the embryoid body cells, as visualized with immunofluorescence microscopy (FIGURE 4C and FIGURE 4D).

Molecular cytogenetic analysis of the Si-10 human embryonic stem cell line

The MLPA analysis did not detect the presence of gain or loss of subtelomeric chromosomal regions in the Si-10 HESC line (FIGURE 5A). The genome-wide evaluation of relevant CNVs, conducted by aCGH, also did not detect changes (FIGURE 5B). Both methods indicated that the Si-10 HESC line carries an euploid 46, XY karyotype.

DISCUSSION

Despite favourable legislation, many couples are still understandably reluctant to donate their unused embryos for scientific research regardless of their conditions and may, instead, opt for their destruction. Personal, ethical and religious issues play a relevant part in their decision. Such difficulty can only be overcome through proper education and counselling. Couples need to be enlightened on the value carried by each donated embryo and their potential to result in major advances in medicine (*Pennings, 2007*). As HESC have become precious resources for researchers studying developmental cell biology and cell therapy, every effort should be made to secure embryo donations.

The findings of the present study revealed that donated blastocysts with poor morphological quality in association with genetic abnormalities detected by NGS had no capacity for further in-vitro expansion to originate a pluripotent HESC line. Only a supernumerary embryo with good morphological quality and untested for genetic abnormalities produced a new and chromosomally normal HESC line.

The stage of the embryo, isolation conditions and experience of the group are important factors in the derivation of HESC lines. Moreover, differences in morphology quality, the presence of genetic abnormalities, or both, can also reflect on the success rates of HESC derivation (*Peura et al., 2007*). With advancements in embryo culture and the use of more robust methods for the assessment of aneuploidy (NGS and aCGH), genetic counselling has become increasingly more reliable (*Scott et al., 2008*; *Rubio et al., 2019*). The outcome of HESC line derivation may also be an important indicator of the viability and functionality of embryos with genetic and morphological alterations and can contribute to preimplantation counselling.

As part of a rigorous embryo selection programme, all the embryos donated to this study, with the exception of one, had been considered unsuitable for clinical use and discarded owing to the presence of genetic abnormalities in association with poor morphological quality. The survival rate in the present study (73.91%) was similar to a previous report (70.6%), when poor-quality embryos were also vitrified (*Aran et al., 2012*). Higher survival rates (95%), however, have been reported for good-quality embryos vitrified with the same technique (*Almodin et al., 2010*; *Cobo et al., 2012*). Differences between results seem to indicate that poor-quality embryos are more sensitive to the vitrification–warming procedure than good-quality embryos (*Ren et al., 2012*).

The concept of aneuploidy involves the genomic status of a cell with abnormal chromosome content. There are two main types of aneuploidies: numerical, which is related to gains and losses of the whole chromosome, and segmental. These genetic abnormalities may affect cell expansion behaviour (*Verlinsky et al., 2009*), and present different incidence and origin. The de-novo segmental aneuploidy suggests that sub-chromosomal abnormalities may be caused by mitotic cell division. The incidence of segmental aneuploidy at the preimplantation stage has been reported to be up to 15.6%, and not dependent on female age, suggesting a mosaicism-related event (*Babariya et al., 2017*). *Girardi et al. (2020)* showed a low concordance rate between trophectoderm and ICM analysis in samples tested for PGT-A conducted with NGS. In contrast, whole chromosome aneuploidy suggests errors of meiotic origin with incidences up to 80% depending on the woman's age (*Franasiak* et al., 2014). Moreover, high concordance rates between trophectoderm and ICM in re-biopsies have also been observed (*Girardi et al., 2020*). Hence, whole chromosome aneuploidy tends to have a more deleterious effect in embryos and consequently in their expansion capacity.

Trisomies and monosomies, which are the most common type of whole aneuploidy, occur at a relatively similar frequency, with chromosomes 15, 16, 18, 21 and 22 being the most frequently affected (*Fragouli et al., 2013*). Monosomies are related to embryo implantation failure and early miscarriages. Previous studies have shown that embryos with autosomal chromosome monosomies are not viable and tend not to survive in culture (*Biancotti et al., 2012*). Although trisomies have also been associated with early pregnancy loss, some of these embryos may results in a live birth (*Rodriguez-Purata et al., 2015*). It has been assumed that the derivation of HESC lines from embryos with trisomy is possible, depending on the extra chromosome that may give the cell some growth advantage (*Lavon et al., 2008*). In fact, HESC lines from embryos presenting chromosome 12,

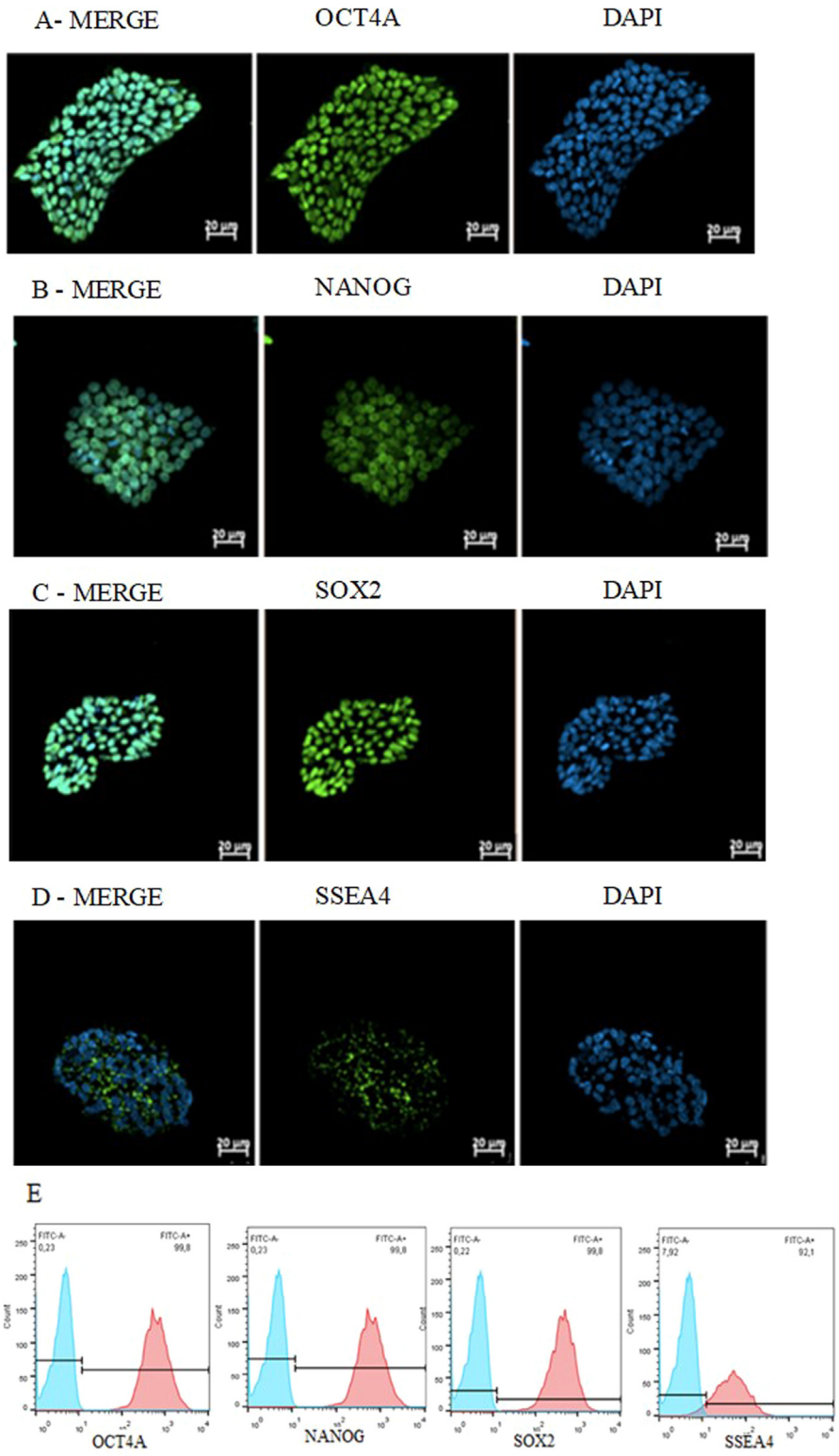

FIGURE 3 Expression of pluripotency markers in Si-10 human embryonic stem cell (HESC). (A) OCT4A; (B) NANOG; (C) SOX2; and (D) SSEA4 expression was detected by immunofluorescence; (E) was detected by flow cytometry. The heterogeneity of DAPI staining intensity in the nuclei observed in **Figure 3** can be explained by how the colonies were disposed on the chamber slides.

20 and 21 trisomy, diagnosed with blastomere analysis, have already been generated (*Biancotti et al., 2012*).

Alfarawati et al. (2011) found that trisomies were common in embryos with high morphological quality, whereas monosomies were generally found in embryos with poor-quality morphology. Complex aneuploidy has also been more

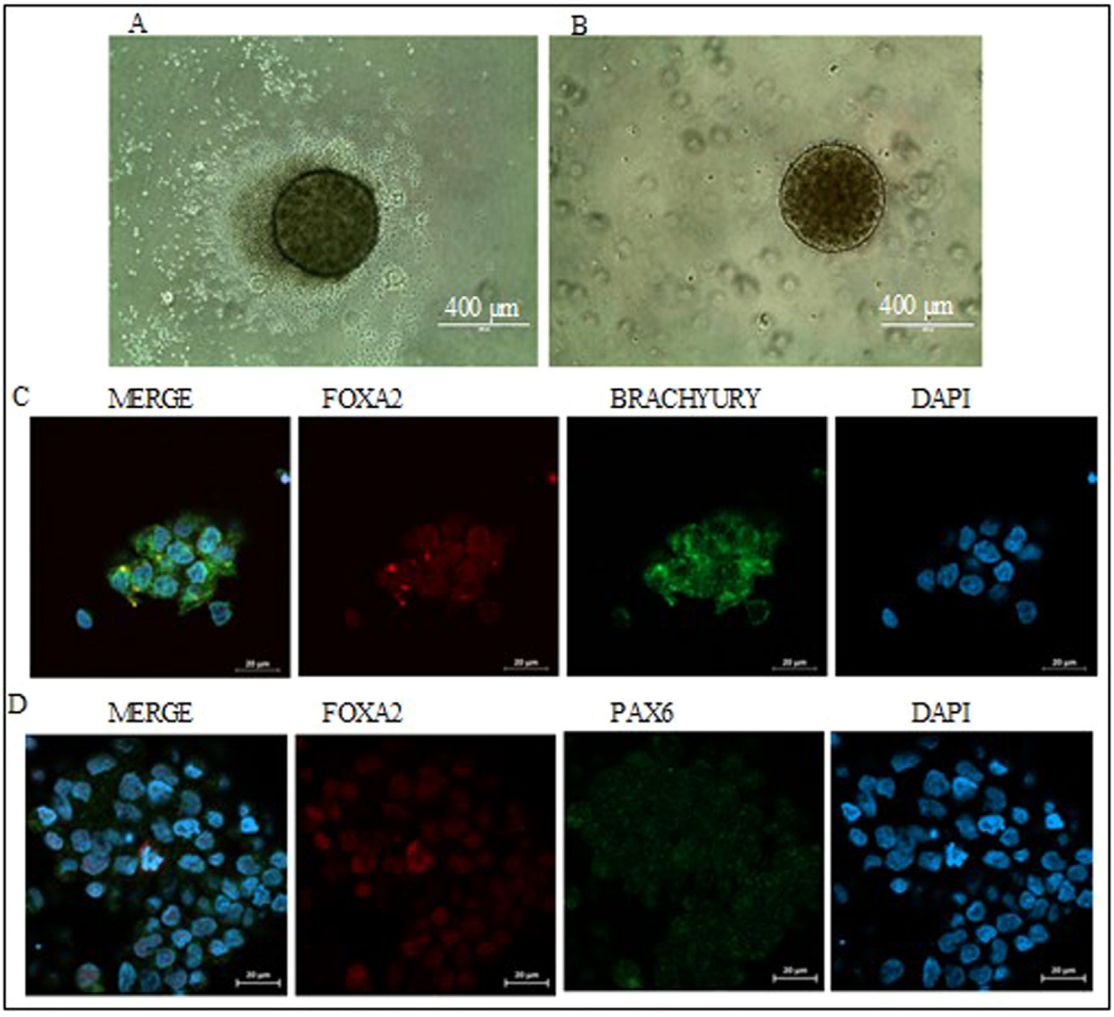

FIGURE 4 Embryoid bodies generated by Si-10 human embryonic stem cell (HESC) *in vitro*. Embryoid body morphology after (A) 48 h and (B) 96 h; (C) immunofluorescence assay for detection of FOXA2 and PAX6 expression in embryoid body cells (endodermal and ectodermal lineage markers, respectively); and (D) immunofluorescence assay for detection of FOXA2 and Brachyury expression in embryoid body cells (endodermal and mesodermal markers, respectively). The heterogeneity of DAPI staining intensity in the nuclei observed in **Figure 4** can be explained by how the colonies were disposed in the dish.

commonly observed in embryos with poor morphological quality (*Capalbo et al., 2014*). Because of the variation in the methodologies used in HESC derivation, and inconsistencies in the reporting of embryo quality and stage of development, however, effective comparisons between the work carried out by different laboratories are difficult (*Cortes et al., 2009*). Associations between aneuploidy and low morphological quality of embryos from cleavage to blastocyst stage have been the focus of interest of some research groups (*Munné et al., 2005*; *Capalbo et al., 2014*). A higher proportion of grade A in ICM and trophectoderm analysis, as well as a higher rate of blastocele expansion (5 and 6) has been reported for euploid blastocysts (*Alfarawati et al., 2011*). On the other hand, grade C on trophectoderm analysis has been associated with decreased euploidy rate (*Wang et al., 2018*). Although these findings may suggest that a link between good morphological quality and euploidy rate seems to exist, these observations must be interpreted with caution. A significant number of aneuploid embryos can demonstrate the highest morphologic scores, whereas some euploid embryos may be morphologically poor (*Capalbo et al., 2014*).

In the present study, the five viable embryos in the sample that underwent PGT for monogenetic disease demonstrated a high-risk haplotype pathogenic variation in the gene *COL1A2*. A likely pathogenic variant identified in this gene (*COL1A2*: c.1477G>C) has been associated with osteogenesis imperfecta type IV, a rare autosomal dominant disorder (*Marini et al., 2007*). None of these embryos showed capability to generate a HESC line. Although HESC line from an embryo with *COL1A2* mutation has not been reported, a *COL1A2* mutated induced pluripotent stem cell line has been recently described (*Zheng et al., 2020*). Therefore, one can assume that morphology quality may have been a limiting factor for the derivation of a HESC line in the present study.

Adhesion rate of the embryonic cells to culture plating in the present study was low. In the present study, one embryo with

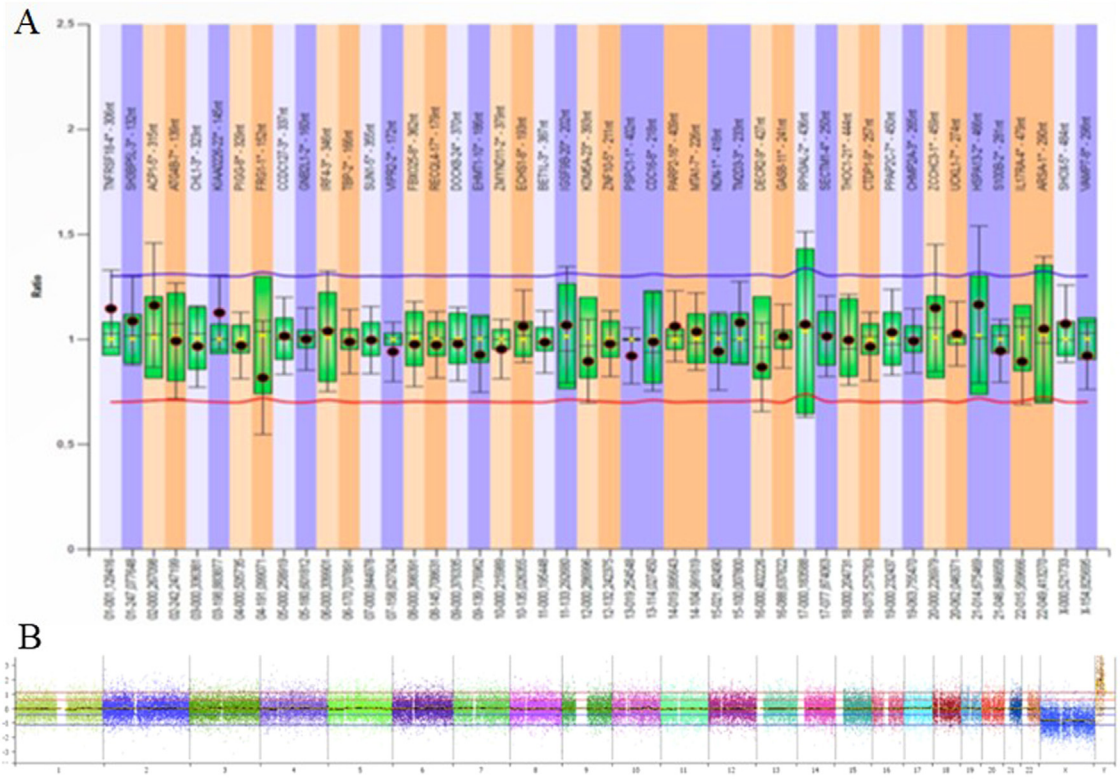

FIGURE 5 Molecular cytogenetic analysis of the Si-10 human embryonic stem cell (HESC) line. (A) multiplex ligation-dependent probe amplification profile of the Si-10 cell line. The interval between the blue and red lines represents the normal (02 alleles) of each gene. The X axis represents the location of the probe, whereas the Y axis represents correct the number of copies or alleles of each gene; (B) array comparative genomic hybridization: the values obtained by the formula Log2 PS/PC were plotted to construct the graphs. The PS is the number of probes in the tested sample, whereas PC refers to the number of probes in the control sample.

chaotic aneuploidy, one showing monosomy 4 and another with partial loss of 5p and 11p demonstrated poor initial adhesion *in vitro*, preventing further HESC line derivation. Embryos with euploidy, trisomy and segmental duplication have been shown to remain viable up to 12 days after fertilization, whereas embryos harbouring monosomies and multiple aberrations remained attached to the plate for 8 days or less (*Popovic et al., 2019*). Those findings suggest the lethality of autosomal monosomies and multiple chromosomal aberrations in preimplantation human embryos, corroborating our data.

The embryo from which the Si-10 HESC cell line was derived had not been subjected to previous manipulation and was the only one to display top-quality morphology. It is important to mention that culture conditions used for this embryo were strictly the same as that used for all the other embryos in the sample, suggesting the suitability of the in-vitro expansion conditions used in the present study. This new HESC line comprised euploid 46, XY cells, indicating that the original embryo was euploid, although mosaicism cannot be ruled out. The lack of PGT information for this embryo might be considered a limitation of our study. Pluripotency of the Si-10 cell line was demonstrated by their in-vitro self-renewing ability, expression of pluripotency markers, and in-vitro spontaneous differentiation with embryoid bodies formation and the generation of three germ layers. These results indicate that the Si-10 HESC line is adequate for research use in the fields of human development, genetics and treatment.

In conclusion, the findings obtained under the conditions of this study seem to demonstrate that embryos diagnosed with poor morphological quality in association with genetic abnormalities detected by NGS displayed no capacity for further in-vitro expansion and derivation of HESC lines. In contrast, a previous study reported on a euploid HESC line derived from an embryo diagnosed at the blastocyst stage by aCGH with multiple abnormalities that also presented poor morphological quality (*Fonseca et al., 2015*). Therefore, the findings in the present study do not necessarily imply that embryos with genetic abnormalities and poor morphology are incapable of generating a HESC line; rather, that they are more likely to pose a higher level of difficulty. Therefore, more studies with embryos considered unsuitable for clinical use owing to genetic abnormalities detected by NGS, but also presenting good morphological quality, are still required to better ascertain the likelihood of these embryos to derive a HESC line.

DATA AVAILABILITY

Data will be made available on request.

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AUTHOR ROLES

II Ceschin was responsible for study design, execution, analysis, manuscript drafting and critical discussion. AP Ceschin was responsible for study design, manuscript drafting and critical discussion. MS Joya was responsible for execution, analysis and critical discussion. TG Mitsugi was responsible for the execution and analysis. LK Nishikawa was responsible for study design, execution and analysis. ACV Krepischi was responsible for study design, execution, analysis and critical discussion. OK Okamoto was responsible for study design, analysis, manuscript drafting and critical discussion. All the authors approved the final version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2022.11.020.

REFERENCES

Ahn, J.W., Mackie Ogilvie, C., Welch, A., Thomas, H., Madula, R., Hills, A., Donaghue, C., Mann, K., 2007. Detection of subtelomere imbalance using MLPA: Validation, development of an analysis protocol, and application in a diagnostic centre. BMC Med. Genet. 8, 1–13.

Alfarawati, S., Fragouli, E., Colls, P., Stevens, J., Gutiérrez-Mateo, C., Schoolcraft, W.B., Katz-Jaffe, M.G., Wells, D., 2011. The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. Fertil. Steril. 95, 520–524.

Almodin, C.G., Minguetti-Camara, V.C., Paixao, C.L., Pereira, P.C., 2010. Embryo development and gestation using fresh and vitrified oocytes. Hum. Reprod. 25, 1192–1198.

Aran, B., Sole, M., Rodriguez-Pizà, I., Parriego, M., Muñoz, Y., Boada, M., Barri, P.N., Izpisúa, J.C., Veiga, A., 2012. Vitrified blastocysts from Preimplantation Genetic Diagnosis (PGD) as a source for human Embryonic Stem Cell (hESC) derivation. J. Assist. Reprod. Genet. 29, 1013–1020.

Baart, E.B., Martini, E., van den Berg, I., Macklon, N.S., Galjaard, R.J.H., Fauser, B.C.J.M., Van Opstal, D., 2006. Preimplantation genetic screening reveals a high incidence of aneuploidy and mosaicism in embryos from young women undergoing IVF. Hum. Reprod. 21, 223–233.

Babariya, D., Fragouli, E., Alfarawati, S., Spath, K., Wells, D., 2017. The incidence and origin of segmental aneuploidy in human oocytes and preimplantation embryos. Hum. Reprod. 32, 2549–2560.

Bajpai, R., Lesperance, J., Kim, M., Terskikh, A.V., 2008. Efficient propagation of single cells accutase-dissociated human embryonic stem cells. Mol. Reprod. Dev. 75, 818–827.

Ben-Yosef, D., Malcov, M., Eiges, R., 2008. PGD-derived human embryonic stem cell lines as a powerful tool for the study of human genetic disorders. Mol. Cell. Endocrinol. 282, 153–158.

Biancotti, J.C., Narwani, K., Mandefro, B., Golan-Lev, T., Buehler, N., Hill, D., Svendsen, C.N., Benvenisty, N., 2012. The in vitro survival of human monosomies and trisomies as embryonic stem cells. Stem. Cell. Res. 9, 218–224.

Capalbo, A., Rienzi, L., Cimadomo, D., Maggiulli, R., Elliott, T., Wright, G., Nagy, Z.P., Ubaldi, F.M., 2014. Correlation between standard blastocyst morphology, euploidy and implantation: An observational study in two centers involving 956 screened blastocysts. Hum. Reprod. 29, 1173–1181.

Carvalho, F., Moutou, C., Dimitriadou, E., Dreesen, J., Giménez, C., Goossens, V., Kakourou, G., Vermeulen, N., Zuccarello, D., De Rycke, M., 2020. ESHRE PGT Consortium good practice recommendations for the detection of monogenic disorders. Hum. Reprod. Open 2020, 1–18.

Cobo, A., de los Santos, M.J., Castellò, D., Gámiz, P., Campos, P., Remohí, J., 2012. Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. Fertil. Steril. 8, 1138–1146 e1.

Cortes, J.L., Sanchez, L., Ligero, G., Gutierrez-Aranda, I., Catalina, P., Elosua, C., Leone, P.E., Montes, R., Bueno, C., Ramos-Mejía, V., Maleno, I., García-Pérez, J.L., Menendez, P., 2009. Mesenchymal stem cells facilitate the derivation of human embryonic stem cells from cryopreserved poor-quality embryos. Hum. Reprod. 24, 1844–1851.

Eguizabal, C., Aran, B., Chuva de Sousa Lopes, S.M., Geens, M., Heindryckx, B., Panula, S., Popovic, M., Vassena, R., Veiga, A., 2019. Two decades of embryonic stem cells: a historical overview. Hum. Reprod. Open 2019, 1–17.

ESHRE PGT-SR/PGT-A Working Group, Coonen, E., Rubio, C., Christopikou, D., Dimitriadou, E., Gontar, J., Goossens, V., Maurer, M., Spinella, F., Vermeulen, N., De Rycke, M., 2020. ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations. Hum. Reprod. Open 2020, hoaa017.

Fonseca, S.A.S., Costas, R.M., Morato-Marques, M., Costa, S., Alegretti, J.R., Rosenberg, C., Alves Da Motta, E.L., Serafini, P.C., Pereira, L.V., 2015. A euploid line of human embryonic stem cells derived from a 43,XX,dup(9q),+12,-14,- 15,-18,-21 embryo. PLoS One 10, 1–9.

Fragouli, E., Alfarawati, S., Spath, K., Jaroudi, S., Sarasa, J., Enciso, M., Wells, D., 2013. The origin and impact of embryonic aneuploidy. Hum. Genet. 132, 1001–1013.

Franasiak, J.M., Forman, E.J., Hong, K.H., Werner, M.D., Upham, K.M., Treff, N.R., Scott, Jr, R.T., 2014. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil. Steril. 101, 656–663.

García-Pascual, C.M., Navarro-Sánchez, L., Navarro, R., Martínez, L., Jiménez, J., Rodrigo, L., Simón, C., Rubio, C., 2020. Optimized NGS approach for detection of aneuploidies and mosaicism in PGT-A and imbalances in PGT-SR. Genes 11, 1–10.

Gardner, D.K., Schoolcraft, W.B., 1999. Culture and transfer of human blastocysts. Curr. Opin. Obstet. Gynecol. 11, 307–311.

Girardi, L., Serdarogullari, M., Patassini, C., Poli, M., Fabiani, M., Caroselli, S., Coban, O., Findikli, N., Boynukalin, F.K., Bahceci, M., Chopra, R., Canipari, R., Cimadomo, D., Rienzi, L., Ubaldi, F., Hoffmann, E., Rubio, C., Simon, C., Capalbo, A., 2020. Incidence, Origin, and Predictive Model for the Detection and Clinical Management of Segmental Aneuploidies in Human Embryos. Am. J. Hum. Genet. 106, 525–534.

Huang, B., Jiang, C., Chen, A., Cui, Y., Xie, J., Shen, J., Chen, J., Cai, L., Liao, T., Ning, S., Jiang, S.W., Fan, G., Qin, L., Liu, J., 2015. Establishment of human-embryonic-stem-cell line from mosaic trisomy 9 embryo. Taiwan J. Obstet. Gynecol. 54, 505–511.

Itskovitz-Eldor, J., Schuldiner, M., Karsenti, D., Eden, A., Yanuka, O., Amit, M., Soreq, H., Benvenisty, N., 2000. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol. Med. 6, 88–95.

Kim, H.S., Oh, S.K., Park, Y.B., Ahn, H.J., Sung, K.C., Kang, M.J., Lee, L.A., Suh, C.S., Kim, S.H., Kim, D.W., Moon, S.Y., 2005. Methods for Derivation of Human Embryonic Stem Cells. Stem Cells 23, 1228–1233.

Kokkali, G., Coticchio, G., Bronet, F., Celebi, C., Cimadomo, D., Goossens, V., Liss, J., Nunes, S., Sfontouris, I., Vermeulen, N., Zakharova, E., De Rycke, M., 2020. ESHRE PGT Consortium and SIG Embryology good practice

recommendations for polar body and embryo biopsy for PGT†. Hum. Reprod. Open 2020, 1–12.
Lavon, N., Narwani, K., Golan-Lev, T., Buehler, N., Hill, D, Benvenisty, N., 2008. Derivation of Euploid Human Embryonic Stem Cells from Aneuploid Embryos. Stem Cells 26, 1874–1882.
Lerou, P.H., Yabuuchi, A., Huo, H., Takeuchi, A., Shea, J., Cimini, T., Ince, T.A., Ginsburg, E., Racowsky, C., Daley, G.Q., 2008. Human embryonic stem cell derivation from poor-quality embryos. Nat. Biotechnol. 26, 212–214.
Liu, W., Yin, Y., Long, X., Luo, Y., Jiang, Y., Zhang, W., Du, H., Li, S., Zheng, Y., Li, Q., Chen, X., Liao, B., Xiao, G., Wang, W., Sun, X., 2009. Derivation and characterization of human embryonic stem cell lines from poor quality embryos. J. Genet. Genomics 36, 229–239.
Ludwig, T.E., Levenstein, M.E., Jones, J.M., Berggren, W.T., Mitchen, E.R., Frane, J.L., Crandall, L.J., Daigh, C.A., Conard, K.R., Piekarczyk, M.S., Llanas, R.A., Thomson, J.A., 2006. Derivation of human embryonic stem cells in defined conditions. Nat. Biotechnol. 24, 185–187.
Marini, J.C., Forlino, A.., Cabral, WA., Barnes, A.M., San Antonio, J.D., Milgrom, S., Hyland, J.C., Körkkö, J., Prockop, D.J., De Paepe, A., Coucke, P., Symoens, S., Glorieux, F.H., Roughley, P.J., Lund, A.M., Kuurila-Svahn, K., Hartikka, H., Cohn, D.H., Krakow, D., Mottes, M., Schwarze, U., Chen, D,, Yang, K., Kuslich, C., Troendle, J., Dalgleish, R., Byers, P.H., 2007. Consortium for osteogenesis imperfecta mutations in the helical domain of type I collagen: regions rich in lethal mutations align with collagen binding sites for integrins and proteoglycans. Hum. Mutat. 28, 209–221.
Mandal, A., Mathew, S., Saha, D., Viswanathan, C., 2013. Establishment, characterization, and differentiation of a karyotypically normal human embryonic stem cell line from a trisomy-affected embryo. Vitr. Cell. Dev. Biol. Anim. 49, 15–26.
Mateizel, I., De Temmerman, N., Ullmann, U., Cauffman, G., Sermon, K., Van de Velde, H., De Rycke, M., Degreef, E., Devroey, P., Liebaers, I., Van Steirteghem, A., 2006. Derivation of human embryonic stem cell lines from embryos obtained after IVF and after PGD for monogenic disorders. Hum. Reprod. 21, 503–511.
Munné, S., Velilla, E., Colls, P., Bermudez, M.G., Vemuri, M.C., Steuerwald, N., Garrisi, J., Cohen, J., 2005. Self-correction of chromosomally abnormal embryos in culture and implications for stem cell production. Fertil. Steril. 84, 1328–1334.
O'Leary, T., Duggal, G., Lierman, S., Van den Abbeel, E., Heindryckx, B., De Sutter, P., 2012. The influence of patient and cohort parameters on the incidence and developmental potential of embryos with poor quality traits for use in human embryonic stem cell derivation. Hum. Reprod. 27, 1581–1589.
Palermo, G., Joris, H., Devroey, P., Van Steirteghem, A.C., 1992. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340, 17–18.
Pennings, G., 2007. The ethics od using embryos in research. Reprod. Biomed. Online 14, 92–97.
Peura, T.T., Bosman, A., Stojanov, T., 2007. Derivation of human embryonic stem cell lines. Theriogenology 67, 32–42.
Popovic, M., Dhaenens, L., Taelman, J., Dheedene, A., Bialecka, M., Sutter, P.De, Chuva De Sousa Lopes, S.M., Menten, B., Heindryckx, B., 2019. Extended in vitro culture of human embryos demonstrates the complex nature of diagnosing chromosomal mosaicism from a single trophectoderm biopsy. Hum. Reprod. 34, 758–769.
Ramos, T.V., Mathew, A.J., Thompson, M.L., Ehrhardt, R.O., 2014. Standardized cryopreservation of human primary cells. Curr. Protoc. Cell. Biol. 64, A.3I.1-A.3I.8.
Ren, X., Liu, Q., Chen, W., Zhu, G., Li, Y., Jin, L., Zhang, H., 2012. Selection and vitrification of embryos with a poor morphological score: A proposal to avoid embryo wastage. J. Huazhong. Univ. Sci. Technol. - Med. Sci. 32, 405–409.
Rodin, S., Antonsson, L., Niaudet, C., Simonson, O.E., Salmela, E., Hansson, E.M., Domogatskaya, A., Xiao, Z., Damdimopoulou, P., Sheikhi, M., Inzunza, J., Nilsson, A.S., Baker, D., Kuiper, R., Sun, Y., Blennow, E., Nordenskjöld, M., Grinnemo, K.H., Kere, J., Betsholtz, C., Hovatta, O., Tryggvason, K., 2014. Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment. Nat. Commun. 5, 1–13.
Rodriguez-Purata, J., Lee, J., Whitehouse, M., Moschini, R.M., Knopman, J, Duke, M., Sandler, B., Copperman, A., 2015. Embryo selection versus natural selection: How do outcomes of comprehensive chromosome screening of blastocysts compare with the analysis of products of conception from early pregnancy loss (dilation and curettage) among an assisted reproductive technolog. Fertil. Steril. 104, 1460–1466 e12.
Rubio, C., Rodrigo, L., Garcia-Pascual, C., Peinado, V., Campos-Galindo, I., Garcia-Herrero, S., Simón, C., Gardner, D.K., 2019. Clinical application of embryo aneuploidy testing by next-generation sequencing. Biol. Reprod. 101, 1083–1090.
Scott, R.T., Miller, K.A., Olivares, R., Su, J., Fratterelli, J.L., Treff, N.R., 2008. Microarray based 24 chromosome preimplantation genetic diagnosis (mPGD) is highly predictive of the reproductive potential of human embryos: a prospective blinded non-selection trial. Fertil. Steril. 90, S22–S23.
Singla, S., Iwamoto-Stohl, L.K., Zhu, M., Zernicka-Goetz, M., 2020. Autophagy-mediated apoptosis eliminates aneuploid cells in a mouse model of chromosome mosaicism. Nat. Commun. 11, 1–15.
Takeuchi, K., 2020. Pre-implantation genetic testing: Past, present, future. Reprod. Med. Biol. 20, 27–40.
Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., Jones, J.M., 1998. Embryonic stem cell lines derived from human blastocysts. Science 282, 1145–1147.
Turetsky, T., Aizenman, E., Gil, Y., Weinberg, N., Shufaro, Y., Reve, I A., Laufer, N., Simon, A., Abeliovich, D., Reubinoff, B.E., 2008. Laser-assisted derivation of human embryonic stem cell lines from IVF embryos after preimplantation genetic diagnosis. Hum. Reprod. 23, 46–53.
Vera-Rodriguez, M., Rubio, C., 2017. Assessing the true incidence of mosaicism in preimplantation embryos. Fertil. Steril. 107, 1107–1112.
Verlinsky, Y., Zech, N.H., Strelchenko, N., Kukharenko, V., Shkumatov, A., Zlatopolsky, Z., Kuliev, A., 2009. Correlation between preimplantation genetic diagnosis for chromosomal aneuploidies and the efficiency of establishing human ES cell lines. Stem. Cell. Res. 2, 78–82.
Wang, A., Kort, J., Behr, B., Westphal, L., 2018. Euploidy in relation to blastocyst sex and morphology. J. Assist. Reprod. Genet. 35, 1565–1572.
Zheng, Z., Lu, W., Pei, Z., Chen, J., Yang, T, Luo, F., 2020. Generation of an induced pluripotent stem cell line (CHFUi001-A) from an osteogenesis imperfecta patient with COL1A2 mutation. Stem Cell Res 47, 3–6.
Zhou, S., Cheng, D., Ouyang, Q., Xie, P., Lu, C., Gong, F., Hu, L., Tan, Y., Lu, G., Lin, G., 2018. Prevalence and authenticity of de-novo segmental aneuploidy (>16 Mb) in human blastocysts as detected by next-generation sequencing. Reprod. Biomed. Online 37, 511–520.

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