Cell Stem Cell
Brief Report

HLA-Matching Potential of an Established Human Embryonic Stem Cell Bank in China

Ge Lin,[1,2,4,5] Yubin Xie,[3,5] Qi OuYang,[1,5] Xiaobing Qian,[4] Pingyuan Xie,[1] Xiaoying Zhou,[4] Bo Xiong,[4] Yueqiu Tan,[1,4] Wen Li,[1,4] Leiyu Deng,[1] Jing Zhou,[1] Di Zhou,[1] Lili Du,[1] Dehua Cheng,[4] Yi Liao,[4] Yifan Gu,[1] Shuoping Zhang,[4] Tiancheng Liu,[1] Yi Sun,[1,2] and Guangxiu Lu[1,2,4,*]
[1]Institute of Reproductive & Stem Cell Engineering, Central South University, Changsha, 410078, China
[2]National Engineering and Research Center of Human Stem Cell, Changsha, 410078, China
[3]Changsha Blood Center, Changsha, 410001, China
[4]Reproductive & Genetic Hospital of CITIC-Xiangya, Changsha, China
[5]These authors contribute equally to this work
*Correspondence: lugxdirector@yahoo.com.cn
DOI 10.1016/j.stem.2009.10.009

Previous studies have performed internal matching of HLA and ABO blood type data from either cadaveric organ donors (Taylor et al., 2005) or a cord blood bank (Nakajima et al., 2007) to estimate that a theoretical collection of approximately 150–170 human embryonic stem cell (hESC) lines with various HLA genotypes or a collection of ten lines homozygous for the common HLA types will suffice to provide HLA-matches for populations in the UK (Taylor et al., 2005) and Japan (Nakajima et al., 2007). In this report, we have established a hESC bank containing 188 hESC lines mainly derived from abnormal and poor-quality in vitro fertilization (IVF)-derived embryos and have evaluated the actual HLA-matching capacity of the bank with respect to the regional population. We find that the current bank could provide a beneficial match for 24.9%–56.3% of the local population, regardless of ethnic differences.

Although hESCs are less susceptible to immune reaction (Drukker et al., 2006), these cells and their derivatives can express human leukocyte antigen (HLA), and the consequent major histocompatibility complex (MHC) incompatibility can cause allograft rejection after transplantation (Bradley et al., 2002; Drukker and Benvenisty, 2004). The previously theoretical calculations did not clarify in actual situation (1) whether the embryos from in vitro fertilization (IVF) treatment can provide adequate HLA diversity for matching; (2) whether the presumptive populations used for modeling calculations in the Taylor et al. and Nakajima et al. studies accurately reflect existing populations; and (3) whether diversity in the ethnic composition present in different geographical regions influences the number of hESC lines required for HLA matching. Moreover, the destruction of large numbers of normal human embryos to obtain cell lines is an ethically controversial issue; thus, we undertook this study to assess whether lines isolated from embryos unsuitable for IVF embryo transfer (ET) could offer a suitable HLA-match for the regional population from which the banked lines were derived.

A total of 692 blastocysts obtained from various abnormal embryos were collected after obtaining written informed consent from both gamete donors and the approval of the hospital ethical committee. The collected blastocysts were not suitable for IVF-ET because they were obtained from poor-quality embryos on day 3 postfertilization, had an abnormal number of pronuclei at the zygote stage, showed abnormalities in the preimplantation genetic diagnosis (PGD), or were obtained from artificially parthenoactivated oocytes. A serum-free mechanical derivation method was adopted to facilitate the potential clinical application of these cell lines (see Supplemental Experimental Procedures available online), and 188 hESC lines were successfully established.

We observed that the quality of the inner cell mass (ICM) of individual blastocysts correlated with the derivation efficiency. The rate of successful isolation of hESC lines was significantly higher when we used blastocysts with high- or medium-quality ICMs (grade A and B, as previously described [Gardner et al., 2000]). In our study, 69 cell lines were derived from 170 blastocysts with grade A ICMs (40.6%); 99 cell lines were derived from 332 blastocysts with grade B ICMs (29.8%); and 6 cell lines were derived from 83 blastocysts with grade C ICMs (7.2%) (Table 1 and Figure S1). The ICM grade reflects the size and degree of compactness of ICM; therefore, high-grade ICMs may yield more pluripotent cells and thus favor more efficient derivation. Another factor affecting the derivation efficiency is the developmental stage of the ICM used for derivation (Chen et al., 2009). Chen et al. reported that the ICM and trophectoderm cells are committed to a restricted fate on day 6 postfertilization, and therefore, the derivation efficiency obtained with day 6 or older ICMs is at least 5-fold higher than that obtained with day 5 ICMs (26%–52% versus 5%) (Chen et al., 2009). However, we observed that the derivation efficiency for day 5 blastocysts was comparable to that for day 6 and day 8 blastocysts ($p > 0.05$), and the highest derivation-efficiency value for day 5 blastocysts was 32.0% (Table 1). These differences may be attributed to the influence of the different embryo culture medium on the timing of fate restriction; alternatively, the relatively larger number of day 5 blastocysts (50 in this study compared to 19 blastocysts in the Chen et al. study) may have decreased the bias in sample collection.

Interestingly, although the clinical data from IVF procedures has shown that poor-quality embryos have a high rate (64%–86.6%) of aneuploidy (Findikli et al., 2004; Pellestor, 1995), the rates of abnormal karyotypes in the hESC lines derived from ICMs of different grades were neither high nor significantly different (Table 1). A similar pattern was observed with respect to the zygotic types from which the hESC lines were derived (Figure S2). In our established cell lines, 52 lines were isolated from 137 monopronuclear (1PN) blastocysts; 19 lines

Table 1. Derivation Efficiencies and Karyotypical Abnormality Rates of the 188 hESC Lines

Categories		Number of Blastocyst Used for Derivation	hESC Lines Derived	Derivation Efficiency (%)	Number of hESC Lines with Abnormal Karyotype	Abnormality Rate (%)
ICM Grade						
	A	170	69	40.6[a]	9	13.0
	B	332	99	29.8[b]	17	17.2
	C	83	6	7.2	1	16.7
	Others[c]	107	14	13.1	8	57.1
Day of Derivation Postfertilization						
	D5	50	16	32.0	-	-
	D6	468	138	29.5	-	-
	D7	120	22	18.3	-	-
	D8	10	2	20.0	-	-
	N/D	44	10	22.7	-	-
Pronuclear Type						
	2PN	254	68	25.8	8	10.3
	NPN	62	19	30.6	3	15.8
	1PN	137	52	38.0	1	1.9
	3PN	145	33	22.8	15	42.4[d]
	Others[c]	94	16	17.0	8	50.0
Total		692	188	27.2	35	17.6

The derivation efficiency is expressed as the percentage of cell lines obtained from among the total number of blastocysts used for derivation. The abnormal rate is expressed in terms of the percentage of abnormally karyotypic hESC lines from among the total number of cell lines established.

[a] $p = 4.9 \times 10^{-8}$ comparison of the derivation efficiencies for grades A and C; $p = 0.015524$, comparison of the derivation efficiencies for grades A and B.

[b] $p = 0.000023$, comparison of the derivation efficiencies for grades B and C.

[c] Others include embryos with undetermined ICM grades and pronuclear types and embryos that were diagnosed with chromosomal abnormalities by PGD (see Table S1).

[d] $p = 0.000153$, comparison of the derivation efficiencies for 2PN and 3PN cells; $p = 0.000001$, comparison of the derivation efficiencies for 1PN and 3PN cells; $p = 0.03037$, comparison of the derivation efficiencies for NPN and 3PN cells.

were isolated from 62 nonpronuclear (NPN) blastocysts; and 33 lines were isolated from 145 triple-pronuclear (3PN) blastocysts (Table 1). One cell line isolated from 1PN zygotes showed karyotypic abnormalities, and this finding is consistent with the reports showing that more than 70% of 1PN embryos are actually diploid embryos (Sultan et al., 1995; Staessen and Van Steirteghem, 1997). Surprisingly, half (15 out of the 30 karyotyped cell lines) of the cell lines from 3PN zygotes showed a normal karyotype, and this finding is not consistent with the previously reported finding that 81.9% of 3PN embryos show chromosomal abnormalities (Pellestor, 1995). These results indicate that either ICM development or hESC derivation selects for normal ploidy. Considering the findings of a previous report (Lerou et al., 2008) that showed that poor-quality embryos generate euploid hESC lines, we suggest that the embryos discarded because of various abnormalities are valuable resources for hESC derivation, given that these embryos may be more acceptable on ethical grounds and yield euploid cell lines with high efficiency.

To ensure reliable cryopreservation and to perform the necessary characterization of the cell lines, we cultured all the lines continuously for at least 2 months. Immunostaining and reverse transcription polymerase chain reaction (PCR) were used for identifying the expression of hESC-specific markers on the individual lines, which also exhibited high telomerase activity (Figure S3). Most of the cell lines showed in vitro differentiation into three germ layers through embryoid-body differentiation (Figure S3). In vivo differentiation assays have been performed on 26 cell lines to date, and each line was able to form teratomas with a complex pattern of differentiation to three germ layers (Figure S3). The characteristics of each line are summarized in Table S1 and indicate that each analyzed cell line exhibits properties characteristic of hESCs. More information on these characteristics is available on our stem cell bank website (http://www.hescbank.cn). To facilitate distinction of individual cell lines in the cases of potential cross contamination during routine culture, we analyzed the DNA-fingerprinting profile of each cell line and found that the profiles for each cell line were distinct (Table S2).

To evaluate the HLA-matching capacity of this bank with respect to the regional population, we utilized a relatively low-resolution, serology-level method to determine the genotypes at the HLA-A, HLA-B, and HLA-DRB1 loci in the 188 hESC lines and also determined the ABO blood groups for each case (Table S3). A group of 174 cell lines with diploid genotypes was chosen for HLA-matching estimation. We considered the entire local population to be prospective patients who required stem cell therapy and obtained representative HLA and ABO blood type data from 5236 unrelated individuals (2877 men and 2359 women) from

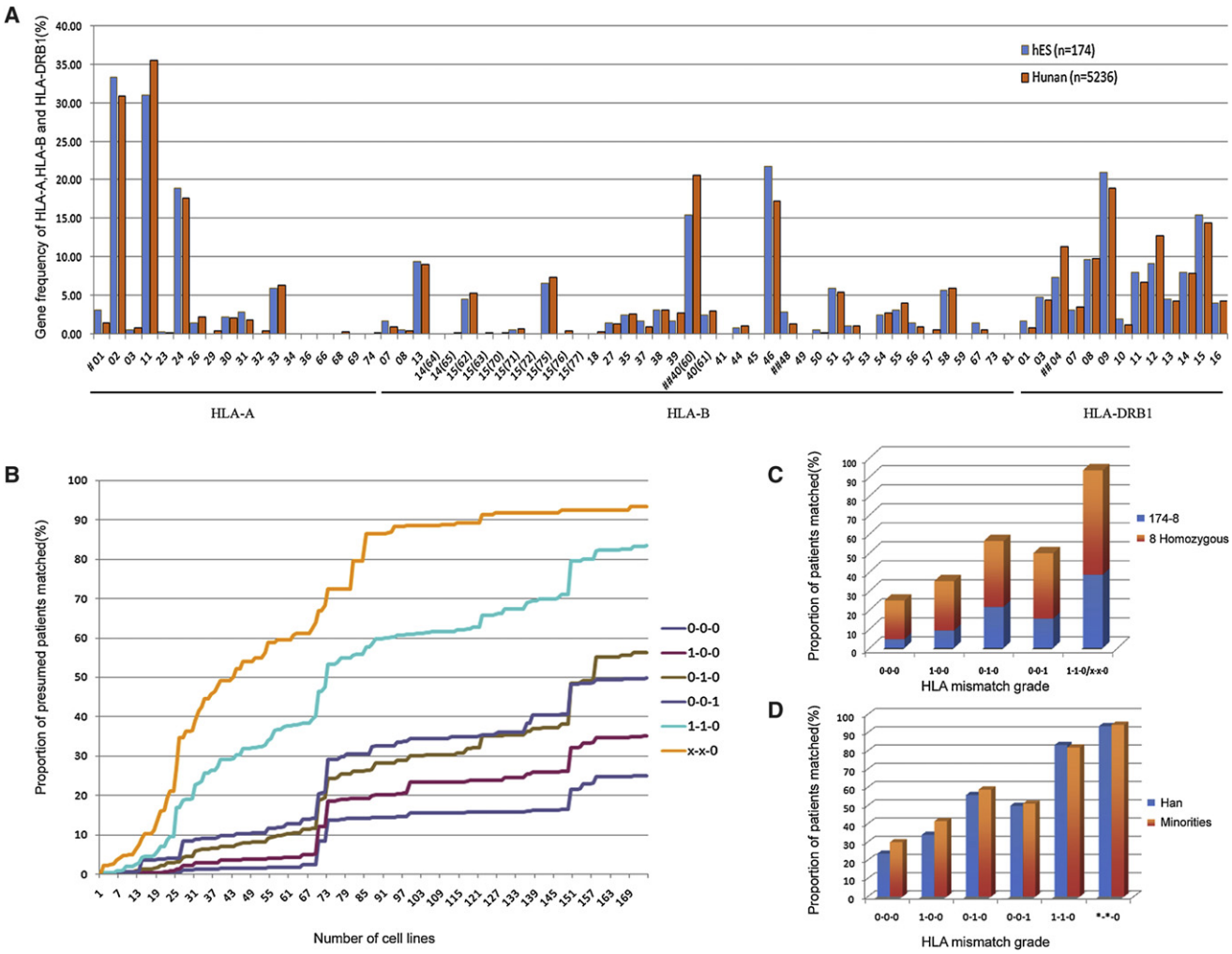

Figure 1. Human Leukocyte Antigen Analysis of 174 hESC Lines and 5236 Individuals from Hunan

(A) Frequency distribution of the human leukocyte antigen (*HLA*) gene in 174 hESC lines and 5236 individuals from Hunan. Most of the major high-frequency alleles showed similar distribution in the 174 hESC lines and the 5236 individuals. Four alleles showed significant difference: #HLA-A*01, $p < 0.01$; *HLA-B*40(60), HLA-B*48, and HLA-DRB1*04, $p < 0.025$.

(B) Cumulative percentages of the presumptive patients (n = 5236) with an HLA-matched donor in the hESC bank (n = 174) at the six mismatch levels: 0-0-0, zero HLA-A, HLA-B, and HLA-DRB1 mismatch; 1-0-0, zero HLA-B, and HLA-DRB1 mismatch with a single HLA-A mismatch or better; 0-1-0, zero HLA-A and HLA-DRB1 mismatch with a single HLA-B mismatch or better; 0-0-1, zero HLA-A and -B mismatch with a single HLA-DRB1 mismatch or better; 1-1-0, zero HLA-DRB1 mismatch with a single HLA-A and a single HLA-B mismatch or better; and *-*-0; zero HLA-DRB1 mismatch.

(C) The proportions of the HLA-matching rates of the eight HLA homozygous hESC lines and the other cell lines in the bank.

(D) HLA-matching proportions of the Han group and the minority group.

the Hunan branch of the Chinese Marrow Donor Program (CMDP). Subsequently, using the 174 lines in our hESC bank as the presumptive-donor group, we calculated the matching rate. We first compared the allele frequencies at the HLA-A, HLA-B, and HLA-DRB1 loci in the cell lines and the population. Although there were differences in the frequencies of the alleles A*01, B*40(60), B*48, and DRB1*04, the major high-frequency alleles in our cell lines and the patients were similar (Figure 1A and Table S4), indicating that there is no significant difference between the HLA distributions in IVF patients and the local population.

To estimate the proportion of the population that would benefit from the hESC bank, we calculated the number of people who would be able to find at least one HLA-matched hESC line with a compatible ABO blood type. We repeated the calculations on a series of cell-line subsets of increasing size, with the maximum size being 174 cell lines. The analysis was repeated for six different levels of HLA matching. We found that an increase in the number of hESC lines used for matching corresponded to an increase in the HLA-matching rate. When all the 174 lines were considered, a fully matched hESC line (0-0-0) was obtained for 24.9% of the population; a single HLA-A mismatch or better (1-0-0) was obtained for 35.1% of the population; a single HLA-B mismatch or better (0-1-0) was obtained for 56.3% of the population; a single HLA-DRB1 mismatch or better (0-0-1) was obtained for 49.8% of the population; single mismatches at 2 loci of HLA-A and HLA-B or better (1-1-0) were obtained for 83.4% of the population;

and HLA-DRB1 match or better (*-*-0) was obtained for 93.5% of the population (Figure 1B). These results, obtained from an established hESC bank and compared with actual population data, are similar to the previously obtained theoretical calculations (Taylor et al., 2005; Nakajima et al., 2007).

Homozygous cell lines for the common HLA haplotypes are regarded as a valuable resource for stem cell banking (Taylor et al., 2005; Nakajima et al., 2007). Among the 174 hESC lines, we identified eight lines that were homozygous at the three loci; these lines showed only five HLA haplotypes (Table S5). Remarkably, these lines contributed to more than 50%–80% of the total matching rates at the different mismatch levels (Figure 1C). Among the five HLA haplotypes, three were common (haplotype frequency > 2%) in the 5236 presumptive patients (Table S4) and contributed to ~90% of the total matching rate of HLA-homozygous cell lines (Table S6). This finding indicates that the collection of homozygous hESC lines with common HLA types will be preferable for clinical hESCs banking. Because of the low incidence (1.5%) of HLA-homozygous individuals in the normal population (Taylor et al., 2005), researchers have suggested that homozygous embryos should be produced through parthenogenesis or IVF of prescreened HLA-homozygous donors (Nakajima et al., 2007; Revazova et al., 2008), or by directly deriving pluripotent cells from prescreened donors by somatic-cell nuclear transfer (SCNT) or induced pluripotent stem cell (iPSC) technology (Taylor et al., 2005; Nakatsuji et al., 2008). Here, we observed that one of the two established parthenogenetic ESC (pESC) lines (*ch*HES-177; Table S1) is HLA homozygous, which is consistent with the previous finding that the MHC loci in some of the pESC lines may be heterozygous as a result of early recombination during meiosis (Kim et al., 2007a, 2007b). Further, the other seven HLA-homozygous cell lines are derived from various abnormal embryos such as 1PN (two cell lines), 3PN (one cell line), and poor-quality 2PN (four cell lines) embryos. To determine whether these seven homozygous cell lines are derived from spontaneous parthenogenesis during IVF, we performed genome-wide single-nucleotide polymorphism (SNP) analysis with the Affymetrix Genome-wide SNP array 5.0. The results (Figure S4) indicate that *ch*HES-32 is a homozygous diploid parthenote because of duplication of a single haploid pronucleus (Lin et al., 2007), unlike the standard pESC line *ch*HES177, which was created by prevention of second-polar-body extrusion, resulting in partial heterozygosity caused by failure to segregate the rearranged chromosomes at meiosis II. In contrast, the other six HLA-homozygous cell lines are highly heterozygous and do not exhibit a pericentromeric homozygosity pattern on the chromosomes, as observed in pESCs (Kim et al., 2007b). The incidence of HLA-homozygous cell lines (7 out of 172, 4.1%) in abnormal embryos is higher than the incidence of HLA-homozygous individuals in a normal population (57 out of 5236, 1.1% in this study [p = 0.001] and 1.5% in Taylor's study [p = 0.013]), and this finding further indicated that the clinically unsuitable embryos are a more feasible source of HLA-homozygous cell lines than parthenogenetically activated human oocytes.

Finally, to determine whether our hESC bank could benefit the ethnic minorities in the local population, we calculated the matched proportions of both the majority Han group (n = 5132) and the minority group (n = 104). The matching rates for the two groups were comparable at each of the six mismatch grades (Figure 1D). This result suggests that the hESC bank can be equally beneficial for both the Han population and other ethnic groups in the local population.

Some HLA alleles are associated with particular diseases (Klein and Sato, 2000), and different transplantation procedures require different levels of HLA matching (Sheldon and Poulton, 2006; Opelz et al., 1999; Petersdorf et al., 1998); therefore, the minimum number of cell lines required in a hESC bank would vary according to the disease being treated. Nevertheless, this study provides evidence that a hESC bank consisting of mainly euploid cell lines can be established from clinically rejected embryos, and this cell bank can serve as a resource for potential cell-replacement therapy in a regional population. Our bank can provide a suitable match (one mismatch only or better) for 16.96–38.26 million people of both Han and other ethnic Chinese groups among the 68 million people in the Hunan population. In addition, this bank can provide new hESC resources for the entire stem cell research community.

SUPPLEMENTAL DATA

Supplemental Data include Supplemental Experimental Procedures, six tables, and four figures and can be found with this article online at http://www.cell.com/cell-stem-cell/supplemental/S1934-5909(09)00520-7.

ACKNOWLEDGMENTS

We thank the IVF team at Reproductive & Genetic Hospital of CITIC-Xiangya. This work was supported by the Ministry of Science and Technology of China (863 program 2006AA02A102), the National Basic Research Program of China (973 program 2007CB948103), National Natural Science Foundation of China (30800659), Bureau Science and Technology Key Project Funds of Hunan Province (No. 03SSY2001), and Chinese Marrow Donor Program (CMDP).

Received: July 12, 2009
Revised: October 12, 2009
Accepted: October 19, 2009
Published: November 5, 2009

REFERENCES

Bradley, J.A., Bolton, E.M., and Pedersen, R.A. (2002). Nat. Rev. Immunol. *2*, 859–871.

Chen, A.E., Egli, D., Niakan, K., Deng, J., Akutsu, H., Yamaki, M., Cowan, C., Fitz-Gerald, C., Zhang, K., Melton, D.A., et al. (2009). Cell Stem Cell *4*, 103–106.

Drukker, M., and Benvenisty, N. (2004). Trends Biotechnol. *22*, 136–141.

Drukker, M., Katchman, H., Katz, G., Even-Tov Friedman, S., Shezen, E., Hornstein, E., Mandelboim, O., Reisner, Y., and Benvenisty, N. (2006). Stem Cells *24*, 221–229.

Findikli, N., Kahraman, S., Kumtepe, Y., Donmez, E., Benkhalifa, M., Biricik, A., Sertyel, S., Berkil, H., and Oncu, N. (2004). Reprod. Biomed. Online *8*, 196–206.

Gardner, D.K., Lane, M., Stevens, J., Schlenker, T., and Schoolcraft, W.B. (2000). Fertil. Steril. *73*, 1155–1158.

Kim, K., Lerou, P., Yabuuchi, A., Lengerke, C., Ng, K., West, J., Kirby, A., Daly, M.J., and Daley, G.Q. (2007a). Science *315*, 482–486.

Kim, K., Ng, K., Rugg-Gunn, P.J., Shieh, J.H., Kirak, O., Jaenisch, R., Wakayama, T., Moore, M.A., Pedersen, R.A., and Daley, G.Q. (2007b). Cell Stem Cell *1*, 346–352.

Klein, J., and Sato, A. (2000). N. Engl. J. Med. *343*, 782–786.

Lerou, P.H., Yabuuchi, A., Huo, H., Takeuchi, A., Shea, J., Cimini, T., Ince, T.A., Ginsburg, E., Racowsky, C., and Daley, G.Q. (2008). Nat. Biotechnol. *26*, 212–214.

Lin, G., OuYang, Q., Zhou, X., Gu, Y., Yuan, D., Li, W., Liu, G., Liu, T., and Lu, G. (2007). Cell Res. *17*, 999–1007.

Nakajima, F., Tokunaga, K., and Nakatsuji, N. (2007). Stem Cells *25*, 983–985.

Nakatsuji, N., Nakajima, F., and Tokunaga, K. (2008). Nat. Biotechnol. *26*, 739–740.

Opelz, G., Wujciak, T., Dohler, B., Scherer, S., and Mytilineos, J. (1999). Rev. Immunogenet. *1*, 334–342.

Pellestor, F. (1995). Hum. Reprod. Update *1*, 581–585.

Petersdorf, E.W., Gooley, T.A., Anasetti, C., Martin, P.J., Smith, A.G., Mickelson, E.M., Woolfrey, A.E., and Hansen, J.A. (1998). Blood *92*, 3515–3520.

Revazova, E.S., Turovets, N.A., Kochetkova, O.D., Agapova, L.S., Sebastian, J.L., Pryzhkova, M.V., Smolnikova, V.I., Kuzmichev, L.N., and Janus, J.D. (2008). Cloning Stem Cells *10*, 11–24.

Sheldon, S., and Poulton, K. (2006). Methods Mol. Biol. *333*, 157–174.

Staessen, C., and Van Steirteghem, A.C. (1997). Hum. Reprod. *12*, 321–327.

Sultan, K.M., Munne, S., Palermo, G.D., Alikani, M., and Cohen, J. (1995). Hum. Reprod. *10*, 132–136.

Taylor, C.J., Bolton, E.M., Pocock, S., Sharples, L.D., Pedersen, R.A., and Bradley, J.A. (2005). Lancet *366*, 2019–2025.