Blastocyst Stem Cell Research Literatures

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Abstract: The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

[Ma H, Young M, Yang Y. **Blastocyst Stem Cell Research Literatures**. Stem Cell. 2015;6(4):26-35] (ISSN 1545-4570). http://www.sciencepub.net/stem. 4. doi:10.7537/marsscj060415.04.

Key words: stem cell; blastocyst; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell.

The following introduces recent reports as references in the related studies.

Baharvand, H., S. K. Ashtiani, et al. "Establishment and in vitro differentiation of a new embryonic stem cell line from human blastocyst." Differentiation. 2004 Jun;72(5):224-9.

Embryonic stem cells have the ability to remain undifferentiated and proliferate indefinitely in vitro while maintaining the potential to differentiate into derivatives of all three embryonic germ layers. These cells have, therefore, potential for in vitro differentiation studies, gene function, and so on. The aim of this study was to produce a human embryonic stem cell line. An inner cell mass of a human blastocyst was separated and cultured on mouse embryonic fibroblasts in embryonic stem cell medium with related additives. The established line was evaluated by morphology; passaging; freezing and thawing; alkaline phosphatase; Oct-4 expression; anti-surface markers including Tra-1-60 and Tra-1-81; and karyotype and spontaneous differentiation. Differentiated cardiomyocytes and neurons were evaluated by transmission electron microscopy and immunocytochemistry. Here, we report the derivation of a new embryonic stem cell line (Royan H1) from a human blastocyst that remains undifferentiated in morphology during continuous passaging for more than 30 passages, maintains a normal XX karyotype, is viable after freezing and thawing, and expresses alkaline phosphatase, Oct-4, Tra-1-60, and Tra-1-81. These cells remain undifferentiated when grown on mouse embryonic fibroblast feeder layers in the presence or absence of recombinant human leukemia inhibitory factor. Royan H1 cells can differentiate in vitro in the absence of feeder cells and can produce embryoid bodies that can further differentiate into beating cardiomyocytes as well as neurons. These results define Royan H1 cells as a new human embryonic stem cell line.

Bongso, A. and S. Tan "Human blastocyst culture and derivation of embryonic stem cell lines." Stem Cell Rev. 2005;1(2):87-98.

Human embryonic stem cell (hESC) biology is expected to revolutionize the future of medicine by the provision of cell-based therapies for the treatment of a variety of deliberatig diseases. The tremendous versatility of hESCs has reinforced this hope. To understand the biology of these mysterious cells and attempt to differentiate them into desirable tissues, bona fide hESCs that maintain their stability with time are required for research and clinical application. This review discusses the various protocols to derive and propagate hESCs from high quality embryos. The nature and properties of hESCs are also described together with unanswered questions that need to be addressed if this science is to be taken to the bedside.

Cheong, S. A., E. Kim, et al. "Improvement in the blastocyst quality and efficiency of putative embryonic stem cell line derivation from porcine embryos produced in vitro using a novel culturing system." Mol Med Rep. 2015 Aug;12(2):2140-8. doi: 10.3892/mmr.2015.3634. Epub 2015 Apr 16.

Porcine embryonic stem cells (pESCs) have great potential for application in translational biomedical research, including xenotransplantation and disease models. Obtaining highquality blastocysts is the most important factor in the isolation and colonization of primary ESCs and the establishment of ESC lines. In pigs, in vitroderived blastocysts have a limited cell number compared to in vivoderived blastocysts and show an indefinite inner cell mass, which may result in failure to establish pESC lines. In the present study, the effects of resveratrol (RES), granulocytemacrophage colony stimulating factor (GMCSF) and betamercaptoethanol (betaME) on the quality of blastocysts and the efficiency of colony derivation were investigated for the establishment of ESCs. A novel culturing system was developed in which 2 microM RES was added to the oocyte in vitro maturation (IVM) medium, and 10 ng/ml pGMCSF and 10 microM betaME were added to embryo in vitro culture (IVC) medium. This novel system showed significantly more parthenogenetic activation (PA) blastocysts (54.5+/-1.8% vs. 43.4+/-1.2%; P<0.05) and in vitro fertilization (IVF) blastocysts (36.9+/-3.3% vs. 26.2+/-2.9%; P<0.06) at day seven as compared with that in the control system. The PA and IVF blastocysts from the novel system showed a significantly greater hatching rate (P<0.05) and greater cell numbers (55.1+/-2.0 vs. 45.6+/-2.0; P<0.05 and 78.9+/-6.8 vs. 58.5+/-7.2; P<0.06, for PA and IVF, respectively) at day seven compared to that in the control system. After seeding on feeder cells, the PA blastocysts produced by the novel system showed a significantly increased rate of attachment (28.8+/-3.9% vs. 17.2+/-2.4%; P<0.062). Finally, two putative pESC lines from PA embryos produced by the novel system and one by the control system were established. In conclusion, the novel system improved blastocyst quality and increased the derivation efficiency of putative pESC lines from porcine PA and IVF embryos produced in vitro.

Cortes, J. L., L. Sanchez, et al. "Whole-blastocyst culture followed by laser drilling technology enhances the efficiency of inner cell mass isolation and embryonic stem cell derivation from good- and poor-quality mouse embryos: new insights for derivation of human embryonic stem cell lines." Stem Cells Dev. 2008 Apr;17(2):255-67. doi: 10.1089/scd.2007.0157.

The optimization of human embryonic stem (hES) cell line derivation methods is challenging because many worldwide laboratories have neither access to spare human embryos nor ethical approval for using supernumerary human embryos for hES cell derivation purposes. Additionally, studies performed directly on human embryos imply a waste of precious human biological material. In this study, we developed a new strategy based on the combination of whole-blastocyst culture followed by laser drilling destruction of the trophoectoderm for improving the efficiency of inner cell mass (ICM) isolation and ES cell derivation using murine embryos. Embryos were divided into good- and poor-quality embryos. We demonstrate that the efficiency of both ICM isolation and ES cell derivation using this strategy is significantly superior to whole-blastocyst culture or laser drilling technology itself. Regardless of the ICM isolation method, the ES cell establishment depends on a feeder cell growth surface. Importantly, this combined methodology can be successfully applied to poor-quality blastocysts that otherwise would not be suitable for laser drilling itself nor immunosurgery in an attempt to derive ES cell lines due to the inability to distinguish the ICM. The ES cell lines derived by this combined method were characterized and shown to maintain a typical morphology, undifferentiated phenotype, and in vitro and in vivo three germ layer differentiation potential. Finally, all ES cell lines established using either technology acquired an aneuploid karyotype after extended culture periods, suggesting that the method used for ES cell derivation does not seem to influence the karyotype of the ES cells after extended culture. This methodology may open up new avenues for further improvements for the derivation of hES cells, the majority of which are derived from frozen, poor-quality human embryos.

Doetschman, T. C., H. Eistetter, et al. "The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium." J Embryol Exp Morphol. 1985 Jun;87:27-45.

The in vitro developmental potential of mouse blastocyst-derived embryonic stem cell lines has been investigated. From 3 to 8 days of suspension culture the cells form complex embryoid bodies with endoderm, basal lamina, mesoderm and ectoderm. Many are morphologically similar to embryos of the 6- to 8-day egg-cylinder stage. From 8 to 10 days of culture about half of the embryoid bodies expand into large cystic structures containing alphafoetoprotein and transferrin, thus being analagous to the visceral yolk sac of the postimplantation embryo. Approximately one third of the cystic embryoid bodies develop myocardium and when cultured in the presence of human cord serum, 30% develop blood islands, thereby exhibiting a high level of organized development at a very high frequency. Furthermore, most embryonic stem cell lines observed exhibit similar characteristics. The in vitro developmental potential of embryonic stem cell lines and the consistency with which the cells express this potential

are presented as aspects which open up new approaches to the investigation of embryogenesis.

Fan, Y., Y. Luo, et al. "A modified culture medium increases blastocyst formation and the efficiency of human embryonic stem cell derivation from poor-quality embryos." J Reprod Dev. 2010 Oct;56(5):533-9. Epub 2010 Jul 20.

Human embryonic stem cells (HESCs) are defined as self-renewing cells that retain their ability to differentiate into all cell types of the body. They have enormous potential in medical applications and as a model for early human development. There is a need for derivation of new HESC lines to meet emerging requirements for their use in cell replacement therapies, disease modeling, and basic research. Here, we describe a modified culture medium containing human recombinant leukemia inhibitory factor and human basic fibroblast growth factor that significantly increases the number of human blastocysts formed and their quality, as well as the efficiency of HESC derivation from poor-quality embryos. Culturing poor-quality embryos in modified medium resulted in a two-fold increase in the blastocyst formation rate and a seven-fold increase over the derivation efficiency in conventional medium. We derived 15 HESC lines from poor-quality embryos cultured in modified culture medium and two HESC lines from quality embryos cultured in conventional culture medium. All cell lines shared typical human pluripotent stem cell features including similar morphology, normal karyotypes, expression of alkaline phosphatase, pluripotency genes, such as Oct4, and cell surface markers (SSEA-4, TRA-1-60, TRA-1-81), the ability to form teratomas in SCID mice, and the ability to differentiate into cells of three embryonic germ layers in vitro. Our data suggest that poor-quality embryos that have reached the blastocyst stage in our modified culture medium are a robust source for normal HESC line derivation.

Goossens, E., V. Frederickx, et al. "Blastocyst development after assisted reproduction using spermatozoa obtained after testicular stem cell transplantation in mice." Hum Reprod. 2006 Jul;21(7):1759-64. Epub 2006 Feb 22.

BACKGROUND: Since its introduction in 1994, testicular stem cell transplantation (TSCT) has been widely used for research. This technique may also become important for preserving fertility in pre-pubertal cancer patients. Therefore, it is necessary to investigate the safety aspects of reproduction using spermatozoa obtained after TSCT. In this study, preimplantation development of mouse embryos, using spermatozoa obtained after TSCT, was examined. METHODS: TSCT-derived spermatozoa were used for IVF and ICSI. Embryos were cultured for five days until they reached blastocyst stage and were evaluated by differential staining. RESULTS: IVF revealed significantly lower fertilization and development rates after TSCT-IVF compared to control-IVF. Blastocysts derived from TSCT-IVF had significantly lower inner cell mass numbers (ICMs) and lower ICM/trophectoderm (TE) ratios compared to control-IVF blastocysts. No differences in fertilization and development rates were observed between TSCT-ICSI and control-ICSI, and blastocyst quality in the transplanted group was similar to that of the control blastocysts. CONCLUSION: Our study showed that after TSCT-IVF, fertilization and preimplantation development were disturbed and blastocysts showed reduced ICM and ICM/TE ratio. However, after TSCT-ICSI, both fertilization and preimplantation development were normal and blastocyst formation was comparable to control-ICSI.

Gossler, A., T. Doetschman, et al. "Transgenesis by means of blastocyst-derived embryonic stem cell lines." Proc Natl Acad Sci U S A. 1986 Dec;83(23):9065-9.

This study demonstrates that blastocyst-derived embryonic stem cells (ES cells) can be used as a vehicle for transgenesis. The method is nearly as efficient as other methods, and the introduced neomycin phosphotransferase (neo) gene is stably transmitted through several generations with no apparent loss in G418 resistance. An important factor contributing to the efficiency of this process is the rigorous selection, before blastocyst injection, of genetically transformed cells for in vitro developmental pluripotency. One of the advantages of the ES cell route to transgenesis is that it provides investigators with the opportunity to screen for the desired genetic alterations before reintroducing the ES cells into the animal.

Greco, B., H. P. Low, et al. "Differentiation prevents assessment of neural stem cell pluripotency after blastocyst injection." Stem Cells. 2004;22(4):600-8.

Earlier studies reported that neural stem (NS) cells injected into blastocysts appeared to be pluripotent, differentiating into cells of all three germ layers. In this study, we followed in vitro green fluorescent protein (GFP)-labeled NS and embryonic stem (ES) cells injected into blastocysts. Forty-eight hours after injection, significantly fewer blastocysts contained GFP-NS cells than GFP-ES cells. By 96 hours, very few GFP-NS cells remained in blastocysts compared with ES cells. Moreover, 48 hours after injection, GFP-NS cells in blastocysts extended long cellular processes, ceased expressing the NS cell marker nestin, and instead expressed the astrocytic

marker glial fibrillary acidic protein. GFP-ES cells in blastocysts remained morphologically undifferentiated, continuing to express the pluripotent marker stage-specific embryonic antigen-1. Selecting cells from the NS cell population that preferentially formed neurospheres for injection into blastocysts resulted in identical results. Consistent with this in vitro behavior, none of almost 80 mice resulting from NS cell-injected blastocysts replaced into recipient mothers were chimeric. These results strongly support the idea that NS cells cannot participate in chimera formation because of their rapid differentiation into glia-like cells. Thus, these results raise doubts concerning the pluripotency properties of NS cells.

Hwang, W. S., Y. J. Ryu, et al. "Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst." Science. 2004 Mar 12;303(5664):1669-74. Epub 2004 Feb 12.

Somatic cell nuclear transfer (SCNT) technology has recently been used to generate animals with a common genetic composition. In this study, we report the derivation of a pluripotent embryonic stem (ES) cell line (SCNT-hES-1) from a cloned human blastocyst. The SCNT-hES-1 cells displayed typical ES cell morphology and cell surface markers and were capable of differentiating into embryoid bodies in vitro and of forming teratomas in vivo containing cell derivatives from all three embryonic germ layers in severe combined immunodeficient mice. After continuous proliferation for more than 70 passages, SCNT-hES-1 cells maintained normal karyotypes and were genetically identical to the somatic nuclear donor cells. Although we cannot completely exclude the possibility that the cells had a parthenogenetic origin, imprinting analyses support a SCNT origin of the derived human ES cells.

Kim, I. W., S. P. Gong, et al. "Derivation of developmentally competent oocytes by the culture of preantral follicles retrieved from adult ovaries: maturation, blastocyst formation, and embryonic stem cell transformation." Fertil Steril. 2009 Nov;92(5):1716-24. doi: 10.1016/j.fertnstert.2008.08.084. Epub 2008 Nov 2.

OBJECTIVE: To determine whether the preantral follicles in adult ovaries can generate developmentally competent oocytes after in vitro culture. DESIGN: Prospective, animal-model study. SETTING: Gamete and Stem Cell Biotechnology Laboratory, Seoul National University, Seoul, Korea. ANIMAL(S): B6CBAF1 mice. INTERVENTION(S): Preantral follicles collected from 8-week-old mice were cultured in vitro. MAIN OUTCOME MEASURE(S): Follicle development, embryogenesis, and embryonic stem cell characterization. RESULT(S): A mean of 50.3 preantral follicles were retrieved from one adult animal, which is significantly less than the number (88.7 follicles) retrieved from a prepubertal female. Extension of the culture period greatly improved oocyte maturation; increased follicular growth to the pseudo-antral (89%-91% vs. 32%) or mature oocyte stage (65%-77% vs. 13%) was observed after 12 or 13 days of culture compared with 9 days of culture. Blastocyst formation after parthenogenesis was detected in only one case; in comparison, the use of IVF yielded a large number of embryos that developed into blastocysts. A mean of 14.7 intrafollicular oocytes per animal were produced after 13 days of culture, and 41% of those developed into blastocysts after IVF. Embryonic stem cell-like colonies were established by subculturing the inner cell mass cells from the blastocysts. CONCLUSION(S): Developmentally competent oocytes can be generated by culturing adult preantral follicles. These results may help increase the feasibility of follicle culture systems.

Kumar, A., A. Lo Nigro, et al. "Reversal of hyperglycemia by insulin-secreting rat bone marrow- and blastocyst-derived hypoblast stem cell-like cells." PLoS One. 2013 May 9;8(5):e63491. doi: 10.1371/journal.pone.0063491. Print 2013.

beta-cell replacement may efficiently cure type 1 diabetic (T1D) patients whose insulin-secreting beta-cells have been selectively destroyed by autoantigen-reactive T cells. To generate insulin-secreting cells we used two cell sources: rat multipotent adult progenitor cells (rMAPC) and the highly similar rat extra-embryonic endoderm precursor (rXEN-P) cells isolated under rMAPC conditions from blastocysts (rHypoSC). rMAPC/rHypoSC were sequentially committed to definitive endoderm, pancreatic endoderm, and beta-cell like cells. On day 21, 20% of rMAPC/rHypoSC progeny expressed Pdx1 and C-peptide. rMAPCr/HypoSC progeny secreted C-peptide under the stimulus of insulin agonist carbachol, and was inhibited by the L-type voltage-dependent calcium channel blocker nifedipine. When rMAPC or rHypoSC differentiated d21 progeny were grafted under the kidney capsule of streptozotocin-induced diabetic nude mice, hyperglycemia reversed after 4 weeks in 6/10 rMAPC- and 5/10 rHypoSC-transplanted mice. Hyperglycemia recurred within 24 hours of graft removal and the histological analysis of the retrieved grafts revealed presence of Pdx1-, Nkx6.1- and C-peptide-positive cells. The ability of both rMAPC and HypoSC to differentiate to functional beta-cell like cells may serve to gain insight into signals that govern beta-cell differentiation and aid in developing culture systems to commit other

(pluripotent) stem cells to clinically useful beta-cells for cell therapy of T1D.

Liao, S. M. "Rescuing human embryonic stem cell research: the Blastocyst Transfer Method." Am J Bioeth. 2005 Nov-Dec;5(6):8-16.

Despite the therapeutic potential of human embryonic stem (HES) cells, many people believe that HES cell research should be banned. The reason is that the present method of extracting HES cells involves the destruction of the embryo, which for many is the beginning of a person. This paper examines a number of compromise solutions such as parthenogenesis, the use of defective embryos, genetically creating a "pseudo embryo" that can never form a placenta, and determining embryo death, and argues that none of these proposals are likely to satisfy embryoists, that is, those who regard the embryo as a person. This paper then proposes a method of extracting HES cells, what might be called the Blastocyst Transfer Method, that meets the ethical requirements of embryoists, and it considers some possible concerns regarding this method. It concludes by encouraging future HES cell research to investigate this method.

Lim, C. Y., W. L. Tam, et al. "Sall4 regulates distinct transcription circuitries in different blastocyst-derived stem cell lineages." Cell Stem Cell. 2008 Nov 6;3(5):543-54. doi: 10.1016/j.stem.2008.08.004. Epub 2008 Sep 18.

Stem cells self-renew or differentiate under the governance of a stem-cell-specific transcriptional program, with each transcription factor orchestrating the activities of a particular set of genes. Here we demonstrate that a single transcription factor is able to regulate distinct core circuitries in two different blastocyst-derived stem cell lines, embryonic stem cells (ESCs) and extraembryonic endoderm (XEN) cells. The transcription factor Sall4 is required for early embryonic development and for ESC pluripotency. Sall4 is also expressed in XEN cells, and depletion of Sall4 disrupts self-renewal and induces differentiation. Genome-wide analysis reveals that Sall4 is regulating different gene sets in ESCs and XEN cells, and depletion of Sall4 targets in the respective cell types induces differentiation. With Oct4, Sox2, and Nanog, Sall4 forms a crucial interconnected autoregulatory network in ESCs. In XEN cells, Sall4 regulates the key XEN lineage-associated genes Gata4, Gata6, Sox7, and Sox17. Our findings demonstrate how Sall4 functions as an essential stemness factor for two different stem cell lines.

Lin, T. C., J. M. Yen, et al. "IGF-1/IGFBP-1 increases blastocyst formation and total blastocyst cell number in mouse embryo culture and facilitates the establishment of a stem-cell line." BMC Cell Biol. 2003 Sep 19;4:14.

BACKGROUND: Apoptosis occurs frequently for blastocysts cultured in vitro, where conditions are suboptimal to those found in the natural environment. Insulin-like growth factor-1 (IGF-1) plays an important role in preventing apoptosis in the early development of the embryo, as well as in the progressive regulation of organ development. We hypothesize that IGF-1 and its dephosphorylated binding protein (IGFBP-1) may be able to improve embryo culture with an associated reduced cell death, and that the resultant increase in the total cell number of the embryo could increase the chances of establishing an embryonic stem-cell line. RESULTS: In vivo fertilized zygotes were cultured in medium containing supplementary IGF-1, or IGFBP-1/IGF-1. The stages of the resultant embryos were evaluated at noon on day five post-hCG injection. The extent of apoptosis and necrosis was evaluated using Annexin V and propidium iodine staining under fluorescent microscopy. The establishment of embryonic stem-cell lines was performed using the hatching blastocysts that were cultured in the presence of IGF-1 or IGFBP-1/IGF-1. The results show that the rate of blastocyst formation in a tissue-culture system in the presence of IGF-1 was 88.7% and IGFBP-1/IGF-1 it was 94.6%, respectively, and that it was significantly greater than the figure for the control group (81.9%). IGFBP-1/IGF-1 also resulted in a higher hatching rate than was the case for the control group (68.8% vs. 48.6% respectively). IGF-1 also increased the number of Annexin V-free and propidium iodine-free blastocysts in culture (86.8% vs. 75.9% respectively). Total cell number of blastocyst in culture was increased by 18.9% for those examples cultured with dephosphorylated IGFBP-1/IGF-1. For subsequent stem-cell culture, the chances of the successful establishment of a stem-cell line was increased for the IGF-1 and IGFBP-1/IGF-1 groups (IGF-1 vs. IGFBP-1/IGF-1 vs. control: 45.8% vs. 59.6% vs. 27.3% respectively). CONCLUSION: IGF-1 or dephosphorylated IGFBP-1/IGF-1 supplement does result in an anti-apoptotic effect for early embryo development in culture, with a subsequent increased total cell number resulting from cell culture. The effect is beneficial for the later establishment of a stem-cell line.

Meng, G., S. Liu, et al. "Derivation of human embryonic stem cell lines after blastocyst microsurgery." Biochem Cell Biol. 2010 Jun;88(3):479-90. doi: 10.1139/o09-188.

Embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of the blastocyst.

Because of their ability to differentiate into a variety of cell types, human embryonic stem cells (hESCs) provide an unlimited source of cells for clinical medicine and have begun to be used in clinical trials. Presently, although several hundred hESC lines are available in the word, only few have been widely used in basic and applied research. More and more hESC lines with differing genetic backgrounds are required for establishing a bank of hESCs. Here, we report the first Canadian hESC lines to be generated from cryopreserved embryos and we discuss how we navigated through the Canadian regulatory process. The cryopreserved human zygotes used in this study were cultured to the blastocyst stage, and used to isolate ICM via microsurgery. Unlike previous microsurgery methods, which use specialized glass or steel needles, our method conveniently uses syringe needles for the isolation of ICM and subsequent hESC lines. ICM were cultured on MEF feeders in medium containing FBS or serum replacer (SR). Resulting outgrowths were isolated, cut into several cell clumps, and transferred onto fresh feeders. After more than 30 passages, the two hESC lines established using this method exhibited normal morphology, karyotype, and growth rate. Moreover, they stained positively for a variety of pluripotency markers and could be differentiated both in vitro and in vivo. Both cell lines could be maintained under a variety of culture conditions, including xeno-free conditions we have previously described. We suggest that this microsurgical approach may be conducive to deriving xeno-free hESC lines when outgrown on xeno-free human foreskin fibroblast feeders.

Mitsunari, M., T. Harada, et al. "The potential role of stem cell factor and its receptor c-kit in the mouse blastocyst implantation." Mol Hum Reprod. 1999 Sep;5(9):874-9.

Embryo implantation is a complex process that requires the interaction of embryo and endometrium. Several growth factors and cytokines appear to be involved in this process. Stem cell factor (SCF) and its receptor c-kit regulate the proliferation and survival of germ cells and play an important role in follicular development. However, little information is available on the role of SCF and c-kit in the process of blastocyst implantation. In the present study, we examined the expression of SCF and c-kit mRNA in mouse embryos and in the stromal and epithelial cells of the uterine endometrium by reverse transcription-polymerase chain reaction (RT-PCR). SCF mRNA was expressed in the spreading blastocysts and endometrial cells, with especially strong expression occurring in the stromal cells. Expression of c-kit mRNA was detected in the blastocysts and spreading blastocysts, as well as in the endometrial cells. By immunocytochemical studies, staining for c-kit protein was observed in the in-vitro spreading trophoblasts. We found that 50-100 ng/ml SCF significantly promoted the expansion of the surface area of the spreading blastocysts ($P < 0.01$). These results are consistent with the hypothesis that SCF derived from endometrial cells and the implanting embryo exerts paracrine and/or autocrine action on the process of implantation by stimulating trophoblast outgrowth through its receptor c-kit.

Pall, E., I. Groza, et al. "Establishment of an embryonic stem cell line from blastocyst stage mouse embryos." Rom J Morphol Embryol. 2011;52(3 Suppl):1005-10.

Embryonic stem cells have the ability to remain undifferentiated and proliferate in vitro while maintaining the potential to differentiate into derivatives of all three embryonic germ layers. The aim of the present study was to establish mouse ES lines from blastocyst stage embryos obtained after CD1/EGFP mice superovulation. We isolated, cultured and determined the characteristics of mouse embryonic stem cells in early passages, which were first described by Evans M and Kaufman M. Therefore, we evaluated the morphological criteria for the approval of ES cells in early expansion stage. Two cell lines were isolated (CDE1 and CDE2) and analyzed. They showed similar characteristics to those reported earlier for blastocyst-derived ES cell lines.

Shimozawa, N., S. Nakamura, et al. "Characterization of a novel embryonic stem cell line from an ICSI-derived blastocyst in the African green monkey." Reproduction. 2010 Mar;139(3):565-73. doi: 10.1530/REP-09-0067. Epub 2009 Dec 2.

Several cell types from the African green monkey (Cercopithecus aethiops), such as red blood cells, primary culture cells from kidney, and the Vero cell line, are valuable sources for biomedical research and testing. Embryonic stem (ES) cells that are established from blastocysts have pluripotency to differentiate into these and other types of cells. We examined an in vitro culture system of zygotes produced by ICSI in African green monkeys and attempted to establish ES cells. Culturing with and without a mouse embryonic fibroblast (MEF) cell monolayer resulted in the development of ICSI-derived zygotes to the blastocyst stage, while culturing with a buffalo rat liver cell monolayer yielded no development (3/14, 21.4% and 6/31, 19.4% vs 0/23, 0% respectively; $P<0.05$). One of the nine blastocysts, which had been one of the zygotes co-cultured with MEF cells, formed flat colonies consisting of cells with large nuclei, similar to other primate ES cell lines. The African green monkey ES (AgMES) cells

expressed pluripotency markers, formed teratomas consisting of three embryonic germ layer tissues, and had a normal chromosome number. Furthermore, expression of the germ cell markers CD9 and DPPA3 (STELLA) was detected in the embryoid bodies, suggesting that AgMES cells might have the potential ability to differentiate into germ cells. The results suggested that MEF cells greatly affected the quality of the inner cell mass of the blastocysts. In addition, AgMES cells would be a precious resource for biomedical research such as other primate ES cell lines.

Sukoyan, M. A., A. N. Golubitsa, et al. "Isolation and cultivation of blastocyst-derived stem cell lines from American mink (Mustela vison)." Mol Reprod Dev. 1992 Dec;33(4):418-31.

Ten embryonic stem (ES) cell lines from mink blastocysts were isolated and characterized. All the lines had a normal diploid karyotype; of the ten lines studied, five had the XX and five had the XY constitution. Testing of the pluripotency of the ES-like cells demonstrated that 1) among four lines of genotype XX, and X was late-replicating in three; both Xs were active in about one-third of cells of line MES8, and analysis of glucose-6-phosphate dehydrogenase revealed no dosage compensation for the X-linked gene; 2) when cultured in suspension, the majority of lines were capable of forming "simple" embryoid bodies (EB), and two only showed the capacity for forming "cystic" multilayer EBs. However, formation of ectoderm or foci of yolk sac hematopoiesis, a feature of mouse ES cells, was not observed in the "cystic" EB; 3) when cultured as a monolayer without feeder, the ES cells differentiated into either vimentin-positive fibroblast-like cells or cytokeratin-positive epithelial-like cells (less frequently); neural cells appeared in two lines; 4) when injected into athymic mice, only one of the four tested lines gave rise to tumors. These were fibrosarcomas composed of fibroblast-like cells, with an admixture of smooth muscular elements and stray islets of epithelial tissue; (5) when the ES cells of line MES1 were injected into 102 blastocyst cavities and subsequently transplanted into foster mothers, we obtained 30 offspring. Analysis of the biochemical markers and coat color did not demonstrate the presence of chimaeras among offspring. Thus the cell lines derived from mink blastocysts are true ES cells. However, their pluripotential capacities are restricted.

Volkenstein, S., D. Brors, et al. "[Investigation of neural stem cell-derived donor contribution in the inner ear following blastocyst injection]." Laryngorhinootologie. 2008 Mar;87(3):168-73. Epub 2007 Nov 23.

BACKGROUND: Utilising the enormous proliferation and multi-lineage differentiation potentials of somatic stem cells represents a possible therapeutical strategy for diseases of non-regenerative tissues like the inner ear. In the current study, the possibility of murine neural stem cells to contribute to the developing inner ear following blastocyst injection was investigated. METHODS: Fetal brain-derived neural stem cells from the embryonic day 14 cortex of male mice were isolated and expanded for four weeks in neurobasal media supplemented with bFGF and EGF. Neural stem cells of male animals were harvested, injected into blastocysts and the blastocysts were transferred into pseudo-pregnant foster animals. Each blastocyst was injected with 5-15 microspheres growing from single cell suspension from neurospheres dissociated the day before. The resulting mice were investigated six months POST PARTUM for the presence of donor cells. Brainstem evoked response audiometry (BERA) was performed in six animals. To visualize donor cells Lac-Z staining was performed on sliced cochleas of two animals. In addition, the cochleas of four female animals were isolated and genomic DNA of the entire cochlea was analyzed for donor contribution by Y-chromosome-specific PCR. RESULTS: All animals had normal thresholds in brainstem evoked response audiometry. The male-specific PCR product indicating the presence of male donor cells were detected in the cochleas of three of the four female animals investigated. In two animals, male donor cells were detected unilateral, in one animal bilateral. CONCLUSION: The results suggest that descendants of neural stem cells are detectable in the inner ear after injection into blastocysts and possess the ability to integrate into the developing inner ear without obvious loss in hearing function.

Wang, F., H. J. Kong, et al. "Analysis of blastocyst culture of discarded embryos and its significance for establishing human embryonic stem cell lines." J Cell Biochem. 2012 Dec;113(12):3835-42. doi: 10.1002/jcb.24297.

In recent years, applications of stem cells have already involved in all domains of life science and biomedicine. People try to establish human embryonic stem cell lines (hESCs) in order to carry out hESC-related studies. In this study, we explored what embryos are conducive to the establishment of hESCs. The discarded embryos from in vitro fertilization-embryo transfer (IVF-ET) cycles were sequentially incubated into blastocysts, and then the inner cell mass (ICM) was isolated and incubated in the mixed feeder layer. The cell lines which underwent serial passage were identified. After a total of 1,725 discarded embryos from 754 patients were incubated,

448 blastocysts were formed with 123 high-quality blastocysts. The blastulation rate was significantly higher in the discarded embryos with non-pronucleus (0PN) or 1PN than in the discarded embryos with 2PN or >/=3PN. The blastulation rate of the D3 embryos with 7-9 blastomeres was higher. Among the originally incubated 389 ICMs, 22 hESCs with normal karyotype were established, and identified to be ESCs. Therefore, in establishing hESCs with discarded embryos, D(3) 0PN or 1PN embryos with 7-9 blastomeres should be first selected, because they can improve high-quality blastulation rate which can increase the efficiency of hESC establishment.

Wang, L., Y. Du, et al. "INO80 facilitates pluripotency gene activation in embryonic stem cell self-renewal, reprogramming, and blastocyst development." Cell Stem Cell. 2014 May 1;14(5):575-91. doi: 10.1016/j.stem.2014.02.013.

The master transcription factors play integral roles in the pluripotency transcription circuitry of embryonic stem cells (ESCs). How they selectively activate expression of the pluripotency network while simultaneously repressing genes involved in differentiation is not fully understood. Here, we define a requirement for the INO80 complex, a SWI/SNF family chromatin remodeler, in ESC self-renewal, somatic cell reprogramming, and blastocyst development. We show that Ino80, the chromatin remodeling ATPase, co-occupies pluripotency gene promoters with the master transcription factors, and its occupancy is dependent on OCT4 and WDR5. At the pluripotency genes, Ino80 maintains an open chromatin architecture and licenses recruitment of Mediator and RNA polymerase II for gene activation. Our data reveal an essential role for INO80 in the expression of the pluripotency network and illustrate the coordination among chromatin remodeler, transcription factor, and histone-modifying enzyme in the regulation of the pluripotent state.

Wang, Z. Q., F. Kiefer, et al. "Generation of completely embryonic stem cell-derived mutant mice using tetraploid blastocyst injection." Mech Dev. 1997 Mar;62(2):137-45.

Embryonic stem (ES) cells provide a unique tool for producing specifically designed mutations in mice. Here, we describe an alternative approach toward the generation of mice which are derived completely from ES cells (ES mice), as judged by glucose phosphate isomerase (GPI) analysis, without prior passage through the germline. By injecting wild-type and mutant ES cells into tetraploid blastocysts, viable and fertile ES mice were generated, suggesting that totipotency of ES cells was not affected by long-term culture and experimental manipulation in vitro. When ES cell clones harboring a lacZ reporter gene introduced by either targeted insertion or a gene-trap approach were used, the expression pattern of the lacZ gene in ES fetuses was identical to that of fetuses that were derived from breeding of chimeric mice. Thus, this technique can be considered as a useful and rapid approach to produce fetuses and mice directly from ES cells carrying predetermined genetic changes and offers many applications for studies in molecular genetics and developmental biology.

Wu, R., C. Xu, et al. "Derivation, characterization and differentiation of a new human embryonic stem cell line from a Chinese hatched blastocyst assisted by a non-contact laser system." Hum Cell. 2010 Aug;23(3):89-102. doi: 10.1111/j.1749-0774.2010.00090.x. Epub 2010 Oct 1.

Currently worldwide attention has focused on the derivation of human embryonic stem cells (hESCs) for future therapeutic medicine. However, the majority of existing hESCs are directly or indirectly exposed to non-human materials during their derivation and/or propagation, which greatly restrict their therapeutic potential. Besides the efforts to improve culture systems, the derivation procedure, especially blastocyst manipulation, needs to be optimized. We adopted a non-contact laser-assisted hatching system in combination with sequential culture process to obtain hatched blastocysts as materials for hESC derivation, and derived a hESC line ZJUhES-1 of a Chinese population without exposure to any non-human materials during blastocyst manipulation. ZJUhES-1 satisfies the criteria of pluripotent hESCs: typically morphological characteristics; the expression of alkaline phosphatase, human telomerase reverse transcriptase and multiple hESC-specific markers including SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, OCT-4, Nanog, Rex-1, Sox-2, UTF-1, Connexins 43 and 45, TERF-1 and TERF-2, Glut-1, BCRP-1/ABCG-2, GDF3, LIN28, FGF4, Thy-1, Cripto1/TDGF1, AC133 as well as SMAD1/2/3/5; extended proliferative capacity; maintenance of a stable male karyotype after long-term cultivation; and robust multiple-lineage developmental potentials both in vivo and in vitro. Moreover, ZJUhES-1 has distinct identity revealed from DNA fingerprinting. Our xeno-free blastocyst manipulation procedure may promote the progression toward clinical-grade hESC derivation.

Zhao, Q., A. Beck, et al. "Rescue of developmental defects by blastocyst stem cell injection: towards elucidation of neomorphic corrective pathways." J Cardiovasc Transl Res. 2010 Feb;3(1):66. doi: 10.1007/s12265-009-9140-7.

Stem cell-based therapy is an exciting area of high potential for regenerative medicine. To study

disease prevention, we inject mouse embryonic stem cells (ESCs) into a variety of mouse blastocysts, most of which harbor mutations. Mice derived from these mutant blastocysts develop human-like diseases, either at developmental stages or in the adult, but blastocyst injection of ESCs prevents disease from occurring. Rather than entirely repopulating the affected organs, with just 20% of chimerism, the ESCs replenish protein levels that are absent in mutant mice, and induce novel or "neomorphic" signals that help circumvent the requirements for the mutations. We also show data indicating that the "neomorphic" mechanisms arise as a result of blastocyst injection of ESCs, regardless of the nature of the host blastocyst (mutant or wild-type). Thus, blastocyst injection of ESCs not only allows the study of disease prevention, but also unveils novel pathways whose activation may aid in the correction of congenital or acquired disease.

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12/15/2015