Milestones in assisted reproduction and related technologies

Part 4: Embryonic stem cell technology with special reference to ART and cloning

Jaffar Ali[1], Nafisa Ali[1,2]

[1]JRBF Online Publications, Shah Alam, Selangor, Malaysia
[2](Previously with: School of Archaeology and Anthropology, College of Arts and Social Sciences, The Australian National University, Canberra, ACT, Australia)

Abstract

This manuscript is intended to provide a historical narrative of the embryonic stem cell technology (ESC) technology. These undifferentiated cells have the capability to mature and differentiate into specialized cells. Stem cells have an unlimited ability to differentiate in vitro under specific laboratory conditions to give rise to specialized cells. Stem cells are very useful to humankind for research, therapeutics and agriculture. The first clue to the "stemness" of certain cells was recognized when scientists noted that red blood cells, white blood cells and platelets all were derived from the same "stem" cell found in the bone marrow. This was in the early 1900's. In 1907 Alexander Maximov postulated during a meeting in Berlin that all blood cells were derived from a single-lymphocyte-like cell. Ernest A. McCulloch, working at the Ontario Cancer Institute, Canada, is credited to be the first to study stem cells in the 1960s together with his colleague James E Till. In 1981 the laboratories of Martin Evans and Kaufman in the UK, and Gail Martin in the USA independently isolated murine ESC. James Thomson and coworkers at the Wisconsin Regional Primate Research Center, University of Wisconsin, reported, for the first time in 1995, the derivation of rhesus monkey ESC followed three years later in 1998, the derivation of human ESC lines from blastocysts for the first time. Shinya Yamanaka and his group in 2006/7 showed that human somatic cells can be reprogrammed to a pluripotent state. The SCT has witnessed very rapid and major groundbreaking developments and achievements in a very short period. It is not unreasonable to speculate that stem cell technology therapeutics will become a routine practice within the next two decades and may be applied to ameliorate or treat with marked efficiency a wide range of disease states and malignancies.

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Keywords: Blastocyst, blood, embryo, embryonic, ESC, history, milestones, stem cells, SCT

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Correspondence: Yousef Alhelou email: jrbf.online@gmail.com cc: alhelou@yahoo.com
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Keywords: Aneuploidy; preimplantation genetic diagnosis, testing, PGD, PGT, single gene disorder, trophectoderm

Chart 8. Milestones in Embryonic Stem Cell (ESC) and CloningTechnology

Year	Milestone/Event	Reference
<n000BCE ?	Hindu mythology alluded to embryo transfer, surrogacy and embryo cloning in the human.	Kane K. Shethepeople. 4 September 2017. Kavita Kane writes about surrogacy in mythology. https://www.shethepeople.tv/news/surrogacy-in-mythology/. Accessed 1 July 2021
<n000BCE	The concept of tissue regeneration was known to the ancient Greeks. In Greek myth, Prometheus was	Harrell DB. Basic science of regenerative biology and application to regenerative medicine: Past, present, and future.

	judged for giving fire to humanity for which Zeus sentenced him to be chained to a rock for 30,000 years during a which an eagle feasted on his liver daily. His liver regenerated each day, and the eagle continued to feast on his liver. Even to this day the liver remains a model for tissue regeneration.	Techniques Regional Anesth Pain Man. 2015;19(1-2):3-9.
-700CE (1986)	Keith L. Moore's work on the translation of the verses of the Qur'an, the holy book of Islam, noted that the narration of some verses on human reproduction and embryology was astonishingly scientifically accurate and are comparable to current knowledge even though these statements originated in the 7th century AD. Moreover, at least one verse in the Qur'an (21:90) states that infertility is curable, suggesting that it is a treatable pathological state.	Moore KL. A Scientist's Interpretation of References to Embryology in the Qur'an. J Islamic Med Assoc North Am. 1986; 18:15-17. https://seekersguidance.org/answers/islamic-belief/were-we-created-from-the-coccyx-bone/ Accessed 28 March 2026
-700CE	Islamic narrations states that individual humans will be resurrected from the cells (stem cells?) in their coccyx bones. Resurrection is mentioned in the Qu'ran, the holy book of Muslims	https://seekersguidance.org/answers/islamic-belief/were-we-created-from-the-coccyx-bone/ Accessed 28 March 2026 (Quran, 50:9-11)
1896 1909	The first clue to the "stemness" of certain cells was recognized when scientists noted that red blood cells, white blood cells and platelets all were derived from the same "stem" cell found in the bone marrow (Pappenheim, A. in 1896). Pappenheim was the first to coin the term "stem cell". Then in the early 1900's (1907/9) Alexander Maximov reiterated during a meeting in Berlin and that all blood cells were derived from a single-lymphocyte-like cell. A paper published in 1909 by Maximov was credited by others to be the first to use the use of word "stem cell".	Pappenheim A. Ueber entwickelung und ausbildung der erythroblasten. Archiv für pathologische Anatomie und Physiologie und für klinische Medicin. 1896 Sep;145(3):587-643. Maximow, A. Der Lymphocytes, especially those that are present in embryonic tissue and post-fetal tissue. Vol. Haematol. 1909, 8, 125–134. In: Vays VB, Vangeli IM, Bakeeva LE, Makievskaya CI, Popkov VA, Zorova LD, Kireev II, Zorov SD, Andrianova NV, Buyan MI, Babenko VA. Identification and Ultrastructural Peculiarities of Nestin-Carrying Progenitor Cells in Kidney. International Journal of Molecular Sciences. 2026;27(4):1695. Maximov, A. 1907. Berlin
1950s	In the 1950s the work of the French oncologist Georges Mathé on bone marrow transplantation in animals demonstrated that transplanted bone marrow cells could survive and propagate in the host if the host animal was first irradiated to neutralize the immune system. In the human however irradiation was not possible due to its potential hazards and it posed ethical dilemmas.	Mathé, G. Cited In: Watts G (2010) Georges Mathé. The Lancet, 376 (9753):1640

1950s-1970s	Edward Donnall Thomas was the first to perform stem cell transplantation using bone marrow-derived stem cells. His work performed at the Fred Hutchinson Cancer Research Center in Seattle, Washington, USA, spanned two decades of research from the 1950s through the 1970s. For his contributions to the advancement of knowledge in transplantation immunology Thomas was awarded a Nobel Prize in Physiology or Medicine in 1990 which many thought should have been shared with Mathé (In: Watts, 2010).	Cited In: Watts G (2010) Georges Mathé. The Lancet. 2010; 376 (9753):1640
1959	An opportunity for such a trial presented itself in 1959 when six Yugoslavian nuclear workers that had damaged their bone marrow due to accidental irradiation sustained about one year previously were treated by Mathé for the first time with bone marrow transplant. All the six physicists survived. Mathé subsequently went on to pioneer the use of bone marrow transplants to treat leukemia in 1963	Mathé, G. Cited In: Watts G (2010) Georges Mathé. The Lancet. 2010; 376 (9753):1640
1950s-1970s	Teratocarcinomas are tumours that develop spontaneously in the gonads in about 1% of inbred mice [F129 strain] (Stevens and Little, 1954) and in a few other strains of mice (Stevens, 1964, 1970a,b). Such tumours are malignant and could be re-transplanted into successive hosts. This work led to the isolation embryonic stem cells. See entries for the 1980s	Stevens LC, Little CC. Spontaneous Testicular Teratomas in an Inbred Strain of Mice. Proc Natl Acad Sci USA.1954; 40(11):1080-1087 Stevens LC. Experimental production of testicular teratomas in mice. Proc Natl Acad Sci U S A. 1964; 52:654-661 Stevens LC. The development of transplantable teratocarcinomas from intratesticular grafts of pre- and postimplantation mouse embryos. Dev Biol.1970a; 21(3):364-82 Stevens LC. Experimental production of testicular teratomas in mice of strains 129, A/He, and their F1 hybrids. J Natl Cancer Inst.1970b; 44(4):923-929
1964	Early work on teratocarcinomas showed that such tumours contained a relatively undifferentiated cell called embryonal carcinoma (EC) that was thought to be the stem cell of all the cell types of the tumour. Kleinsmith and Pierce, (1964) confirmed this by showing that the EC cell could give rise to various types of cells found in the tumour (Kleinsmith and Pierce, 1964).	Kleinsmith LJ, Pierce GB Jr. Multipotentiality of single embryonal carcinoma cells. Cancer Res. 1964;24:1544-1551

1952-1993	EC exhibited a potential to differentiate into an array of cell types reminiscent of early embryonic development but in a grossly disorganized manner (Damjanov 1993; Dixon & Moore 1952;). Chimaeras were obtained when murine ECs were injected into the blastocoelic cavity of mouse blastocysts.	Dixon FJ, Moore RA. Tumors of the testicle. Acta Unio Int Contra Cancrum. 8(2):310-315. Cited In: Andrews PW. (2002) From teratocarcinomas to embryonic stem cells. Phil. Trans. R. Soc. Lond. 1952; B 357: 405–417 Damjanov I (1993) Teratocarcinoma: neoplastic lessons about normal embryogenesis. Int J Dev Biol. 1993;(1):39-46
1960 1961 1963	**Landmark development:** Ernest A. McCulloch, working at the Ontario Cancer Institute, Canada, is credited to be the first to study stem cells in the 1960s together with his colleague James E Till. These workers initiated experiments in which bone marrow cells were injected into irradiated mice. McCulloch and Till observed the appearance of nodules in the spleen of treated mice. The numbers of nodules appeared to be equivalent to the numbers of cells injected. McCulloch and Till called them “spleen colonies”. Subsequently they demonstrated that individual spleen nodules were indeed derived from a single cell (Becker et al., 1963). Becker was a graduate student of McCulloch’s. Together with Lou Siminovitch, a molecular biologist, McCulloch and coworkers sought and obtained the evidence that the bone marrow cells were capable of self-renewal. With this finding these workers finally proved that stem cells indeed exist and that they are self-renewing.	McCulloch EA, Till, JE. The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. Radiation Res. 1960; 13(1):115-125 Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiation Res. 1961;14:213-222 Becker, AJ, McCulloch, EA, Till, JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature.1963;197:452-454 Siminovitch L, McCulloch EA, Till JE. The distribution of colony-forming cells among spleen colonies. J Cell Comp Physiol. 1963; 62:327-336
1981	**Landmark development:** In 1981 the laboratories of Martin Evans and Kaufman in the UK, and Gail Martin in the USA independently isolated murine embryonic stem cells (Evans and Kaufman, 1981 and Martin, 1981) that resulted in the establishment of stable diploid ESC lines that had the capacity to differentiate into over 200 specialized cell types or most if not all cells of the whole animal or organism. They can differentiate into any cell type derived from the three germ layers, which include all tissues and organs of the body, but cannot form extraembryonic tissues like the placenta. The development of ESCs owes its origins to work in	Evans MJ, Kauffman M. Establishment in culture of pluripotent cells from mouse embryos. Nature.1981; 292:154 Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc. Natl Acad. Sci. USA. 1981; 78:7634–7638

	the 1950s and 1970s performed on mouse teratocarcinomas. See entries for the years 1950-70s.	
1984	Cloned cell lines from human teratocarcinomas were isolated that had the potential to differentiate into various cell types including neurons (Andrews, 1984; Thompson et al.1984).	Andrews PW. Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line in vitro. Dev Biol. 1984; 103(2):285-93 Thompson S, Stern PL, Webb M, Walsh FS, Engstrom W, Evans EP, Shi WK, Hopkins B, Graham CF. Cloned human teratoma cells differentiate into neuron-like cells and other cell types in retinoic acid. J Cell Sci.1984 72:37-64
1989	Pera et al. developed cells lines from teratocarcinomas that could differentiate into all three embryonic germ layers however these cells lines proved to be aneuploidic with a limited capacity for spontaneous differentiation into somatic cells but these human EC cells were also found to be phenotypically different to that of mouse ESCs and ECs (see reviews by Andrews, 1988; 1998; Pera et al. 2000).	Pera MF, Cooper S, Mills J, Parrington JM. Isolation and characterization of a multipotent clone of human embryonal carcinomacells. Differentiation. 1989; 42:10-23 Pera MF, Reubinoff B, Trounson A. Human embryonic stem cells. J Cell Sci. 2000 113:5-10 Andrews PW. Teratocarcinomas and human embryology : pluripotent human EC cell lines. APMIS.106:158-167 Andrews PW. Human teratocarcinomas. Biochim Biophys Acta.1988; 948(1):17-36
1995-6	**Landmark development** James Thomson and coworkers (Thomson et al. 1995) at the Wisconsin Regional Primate Research Center, University of Wisconsin, reported, for the first time, the derivation of rhesus monkey ESC followed by marmoset ESC (Thomson et al., 1996) from blastocysts. These primate ESC lines were shown to be diploid and could differentiate into a wide variety of cells that was representative of all the three embryonic germ layers which indicated these cells to be pluripotent.	Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Becker RA, Hearn JP. Isolation of a primate embryonic stem cell line. Proc Natl Acad Sci USA. 1995; 92(17):7844-8 Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Hearn JP. Pluripotent cell lines derived from common marmoset (Callithrix jacchus) blastocysts. Biol Reprod.1996; 55(2):254-259. Cited In: Wobus AM, Boheler KR. Embryonic stem cells: prospects for developmental biology and cell therapy. Physiol reviews. 2005 Apr;85(2):635-78.
1998	**Landmark development** Thomson et al. (1998), were again the first to report the derivation of human ESC lines from blastocysts. Thomson's human ESC lines proved to be pluripotent.	Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science. 1998; 282:1145–1147
1997	**Landmark development** ESC technology spawned other novel technologies such as cloning. Efforts to clone mammalian embryos in Scotland led to cloning of the sheep "Dolly" (Wilmut et al. 1997).	Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. Nature.1997; 385(6619):810-813

2003	Oocytes were derived from mouse embryonic stem cells (Hubner et al., 2003) but were not functional.	Hübner K, Fuhrmann G, Christenson LK, Kehler J, Reinbold R, De La Fuente R, et al. Derivation of oocytes from mouse embryonic stem cells. Science. 2003; 300:1251–1256.
2005	The first feeder-free hESC derivation was reported Klimanskaya and coworkers in 2005 (Klimanskaya et al., 2005). Previously the original methods described for the derivation of hESC required use of mouse feeder cell layers, fetal bovine serum, both of which may contain hazardous animal pathogens and immunogens.	Klimanskaya I, Chung Y, Meisner L, Johnson J, West MD, Lanza R. Human embryonic stem cells derived without feeder cells. Lancet 2005;365:1636–1641.
2006	Ludwig and colleagues formulated the first defined hESC derivation and culture medium, called TeSR1 (Ludwig et al.,2006). They also created an artificial human extracellular matrix to support hESC derivation and long-term culture. These conditions,for the first time, eliminated the use of animal products (Ludwig et al., 2006).	Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, Crandall LJ, Daigh CA, Conard KR, Piekarczyk MS et al. Derivation of human embryonic stem cells in defined conditions. Nat Biotechnol 2006; 24:185–187.
2006-8	**Groundbreaking development** Shinya Yamanaka's group (Takahashi and Yamanaka, 2006; Takahashi et al, 2007; Okita et al., 2007) and others (Yu et al., 2007 and Park et al., 2008) showed that human somatic cells can be reprogrammed to a pluripotent state. It is noteworthy the report from Yu et al above is from James Thomson's group. Yamanaka and colleagues called these cells as induced pluripotent stem (iPS) cells. These cells resemble and behave like ESCs. It can differentiate into every somatic cell of the human body and has the capability for unrestricted replication. Unlike ESCs, iPS cells does not seem to incur ethical debates or controversies. Furthermore it also circumvents the possibility of allogeneic immune rejections. This finding led to a Nobel prize being awarded to Shinya Yamanaka in 2012.	Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126: 663–676 Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. and Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131:861–872 Okita K, Ichisaka T, Yamanaka S. Generation of germline competent induced pluripotent stem cells. Nature. 2007; 448: 313–317 Park I., Zhao R, West J, Yabuuchi A, Huo H, Ince T, Lerou P, Lensch, M, Daley G. (2008) Reprogramming of human somatic cells to pluripotency with defined factors. Nature. 2008 451:141–146
2006-9	**Major development** Human sperm-like cell, supposedly functional, was derived from embryonic stem cells (reported by Nayernia et al.,2006, 2009) that led to birth of fertile offspring.	Nayernia K, Nolte J, Michelmann HW, Lee JH, Rathsack K, Drusenheimer N,et al. In vitro-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. Dev Cell. 2006;11:125–32. 2009;339:b2790In Vitro Derivation of Human Sperm from Embryonic Stem Cells? - Search. Accessed: 07Sep202

	The 2009 original paper published in Stem Cells and Development (8th July 2009) was retracted by the editor due to plagiarism of two paragraphs in the Introduction section of the manuscript, although the editor stated there were no other issues with the scientific content of the manuscript. (See entry for 2021)	
2007	De Coppi and coworkers reported the isolation of amniotic fluid stem cells (AFCs) from both human and murine amniotic fluids that expressed embryonic and adult stem cell markers (De Coppi et al., 2007).	De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, Perin L, Mostoslavsky G, Serre AC, Snyder EY, Yoo JJ, Furth ME, Soker S, Atala A. Isolation of amniotic stem cell lines with potential for therapy. Nature Biotechnology. 2007; 25:100 -106
2008	**Ground breaking development** Macchiarini et al (2008) reported the first successful transplant of a human organ grown from adult stem cells without the use of immunosuppressive drugs. The patient received no immunosuppressive medication because the stem cells used were her own. No immune reaction was noted and the patient recovered sufficiently well to lead a normal life.	Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Rees LE, Cogan TA, Dodson A, Martorell J, Bellini S, Parnigotto PP, Dickinson SC, Hollander AP, Mantero S, Conconi MT, Birchall MA. Clinical transplantation of a tissue-engineered airway. Lancet. 2008; 372(9655): 2023 – 2030 (**Retracted**)
2008	**Major development** The report of the generation of human cloned blastocysts (French et al., 2008) following somatic cell nuclear transfer (SCNT) derived from a differentiated adult somatic cell. This development proved it is now quite possible to clone human blastocysts and therefore human cloning. It is felt that this technology could assist in the generation of stem cells.	French AJ, Adams CA, Anderson LS, Kitchen JR, Hughes MR, Wood SH. Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts. Stem Cells. 2008; 26:485–493
2008	Aasen et al (2008) of the Salk Institute in La Jolla, California, reprogrammed juvenile human primary keratinocytes using retroviral transduction with OCT4, SOX2, KLF4 and c-MYC. The use of keratinocytes is considered far more efficient (almost 100-fold) and two-fold faster than reprogramming of human fibroblasts previously reported by Shinya Yamanaka's group (2006; 2007; see relevant earlier entries). Aasen and coworkers claimed that the keratinocyte-derived iPS cells were similar to human embryonic stem cells in every respect in their cellular behaviour, both in vivo and in vitro (Baker, 2008).	Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R, Bilić J, Pekarik V, Tiscornia G, Edel M, Boué S, Belmonte JCI.(2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nature Biotechnol 26, 1276 – 1284. Baker M. Embryonic-like stem cells from a single human hair Nat Reports Stem Cells .doi:10.1038/stemcells.2008.142, 30 October 2008 (Extracted from abstract)

2009-10	First report of Naïve stem cells. Two distinct states of pluripotent stem cells were proposed, namely the "naïve" and "primed" embryonic stem cells; each with unique characteristics and developmental potential.	Nichols J, Smith A. Naive and primed pluripotent states. Cell Stem Cell. 2009;4(6):487-492. Hanna J, Cheng AW, Saha K, Kim J, Lengner CJ, Soldner F, Cassady JP, Muffat J, Carey BW, Jaenisch R. Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs. Proc Nat Acad Sci. 2010;107(20):9222-9227.
2009	**Major development** Keisuke Kaji and coworkers (Kaji et al., 2009) for the first time obtained embryonic stem cell-like cell from human fibroblast without the use of viral vectors. This overcame the disadvantage in which the use of viral vectors resulted in viral vector integration can now be avoided.	Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature. 2009; 458(7239):771-775
2011	A chemically defined albumin-free medium, medium that supported growth of hESC was described by Chen and colleagues in 2011 (Chen et al.,2011).	Chen G, Gulbranson DR, Hou Z, Bolin JM, Ruotti V, Probasco MD, Smuga-Otto K, Howden SE, Diol NR, Propson NE et al. Chemically defined conditions for human iPSC derivation and culture. Nat Methods 2011;8:424–429.
2011	Hayashi's group (2011) reported the successful derivation mouse primordial germ cells-like cells from ESC/iPS cells	Hayashi K, Ohta H, Kurimoto K, Aramaki S, Saitou M. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells.Cell. 2011;146:519–32.
2012	Hayashi's group subsequently induced female mES/iPS cells to derive primordial germ cell-like cells which later developed to GV stage oocytes which was matured by in vitro maturation, then fertilized ultimately resulting in fertile offspring. This was not an efficient process with success noted only in 3.7% of the attempts. Some of the fertilized eggs demonstrated digynic 3PNs.	Hayashi K, Ogushi S, Kurimoto K, Shimamoto S, Ohta H, Saitou M. Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. Science. 2012;338:971–975. Hayashi K, Saitou M. Generation of eggs from mouse embryonic stem cells and induced pluripotent stem cells. Nat Protoc. 2013;8:1513–1524. (Extracted from abstract)
2013	First report of derivation of human embryonic stem cells using therapeutic cloning by the group of Tachibana (Tachibana et al., 2013)	Tachibana M, Amato P, Sparman M, Gutierrez NM, Tippner-Hedges R, Ma H, Kang E, Fulati A, Lee HS, Sritanaudomchai H, Masterson K. Human embryonic stem cells derived by somatic cell nuclear transfer. Cell. 2013;153(6):1228-1238.
2013	First report of derivation of mouse eggs from embryonic stem cells with the potential for fertilization resulting in fertile offspring (Hayashi et al., 2013).	Hayashi K, Saitou M. Generation of eggs from mouse embryonic stem cells and induced pluripotent stem cells. Nature protocols. 2013;8(8):1513-24. (Extracted from abstract)
2013	Report of limited or negligible immunogenicity of transplanted cells terminally differentiated from iPSCs and embryonic stem cells in the mouse.	Araki R, Uda M, Hoki Y, Sunayama M, Nakamura M, Ando S, Sugiura M, Ideno H, Shimada A, Nifuji A, Abe M. Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. Nature. 2013;494(7435):100-104 (Extracted from abstract)

2013	De novo derivation of naïve human embryonic stem cells (hESCs). Naïve hESCs are capable of robust differentiation to all three germ lineages, with a heightened capabilityof endoderm formation not previously seen in hESCs	Ware CB, Nelson AM, Mecham B, Hesson J, Zhou W, Jonlin EC, Jimenez-Caliani AJ, Deng X, Cavanaugh C, Cook S, Tesar PJ. Derivation of naive human embryonic stem cells. Proc Nat Acad Sci. 2014;111(12):4484-4489.
2013, 2015 & 2019	Derivation of androgenetic embryonic stem cells	Shuai L, Feng C, Zhang H, Gu Q, Jia Y, Wang L, Zhao XY, Liu Z, Zhou Q. Derivation of androgenetic embryonic stem cells from m-carboxycinnamic acid bishydroxamide (CBHA) treated androgenetic embryos. Chinese Sci Bulletin. 2013;58(23):2862-2868. (Extracted from abstract). Ding C, Huang S, Qi Q, et al. Derivation of a Homozygous Human Androgenetic Embryonic Stem Cell Line. Stem Cells and Development. 2015; 24(19): 2307-2316. (Extracted from abstract). Lei L, Hu L, Li T, Shen X, Liang X, Chen Y, Feng X, Yang Z, Shan Z. Establishment of mouse androgenetic embryonic stem cells by double sperm injection and differentiation into beating embryoid body. Zygote. 2019; 27(6):405-412. (Extracted from abstract).
2015	Birth of fertile bimaternal offspring following intracytoplasmic injection of parthenogenetic haploid embryonic stem cells.	Li Z, Wan H, Feng G, Wang L, He Z, Wang Y, Wang XJ, Li W, Zhou QI, Hu B. Birth of fertile bimaternal offspring following intracytoplasmic injection of parthenogenetic haploid embryonic stem cells. Cell research. 2016;26(1):135-8. (Extracted from abstract)
2016	Zhou et al.(2016) demonstrated for the first time the successful maturation of mouse ESCs-derived primordial germs cells-like cells resulting in haploid spermatid, that subsequently resulted in viable and fertile offspring after ICSI.	Zhou Q, Wang M, Yuan Y, Wang X, Fu R, Wan H, et al. Complete meiosis from embryonic stem cell-derived germ cells in vitro. Cell Stem Cell 2016;18:330–340
2016	Hikabe et al. 2016 a reconstitution of oogenesis in vitro using mouse embryonic stem cells and iPS cells to generate oocytes which were further used to derive pluripotent stem cell lines, but their success rate in oogenesis was low (3.5%).	Hikabe O, Hamazaki N, Nagamatsu G, Obata Y, Hirao Y, Hamada N, et al. Reconstitution in vitro of the entire cycle of the mouse female germ line. Nature. 2016;539:299–303. Cited in: Bhartiya D, Anand S, Patel H, Parte S. Making gametes from alternate sources of stem cells: past, present and future. Reprod Biol Endocrinol. 2017;15(1):89.
2020	hESC-derived mesenchymal cells (hESC-MCs) restored ovarian function after chemotherapy in the mouse.	Yoon SY, Yoon JA, Park M, Shin EY, Jung S, Lee JE, et al. Recovery of ovarian function by human embryonic stem cell derived mesenchymal stem cells in cisplatin-induced premature ovarian failure in mice. Stem Cell Res Ther. 2020;11:255. Bahrehbar K, Rezazadeh Valojerdi M, Esfandiari F, Fathi R, Hassani SN, Baharvand H. Human embryonic stem cell-derived

		mesenchymal stem cells improved premature ovarian failure. World J Stem Cells. 2020;12:857–878.
2021	Generation of chimeric mice with spermatozoa fully derived from embryonic stem cells by the group of Miura et al., 2021	Miura K, Matoba S, Hirose M, Ogura A. Generation of chimeric mice with spermatozoa fully derived from embryonic stem cells using a triple-target CRISPR method for Nanos3. Biol Reprod. 2021;104(1):223-233.
2021	Functional spermatids derived from nonhuman primate embryonic stem cells capable of fertilization and blastocyst development.	Khampang S, Cho IK, Punyawai K, Gill B, Langmo JN, Nath S, Greeson KW, Symosko KM, Fowler KL, Tian S, Statz JP, Steves AN, Parnpai R, White MA, Hennebold JD, Orwig KE, Simerly CR, Schatten G, Easley CA 4th. Blastocyst development after fertilization with in vitro spermatids derived from nonhuman primate embryonic stem cells. F S Sci. 2021;2(4):365-375
2022	Wakayama et al. (2022) demonstrated that freeze-dried somatic cells can be utilized to produce healthy, fertile clones,	Wakayama S, Ito D, Hayashi E, Ishiuchi T, Wakayama T. Healthy cloned offspring derived from freeze-dried somatic cells. Nat Commun. 2022;13(1):3666
2024	A survey of therapeutic clinical applications of human embryonic stem cells has been reviewed by Park et al. (2024)	Park SJ, Kim YY, Han JY, Kim SW, Kim H, Ku SY. Advancements in Human Embryonic Stem Cell Res: Clin Appl Ethical Issues. Tissue Eng Regen Med. 2024;21(3):379-394.
2024	Smith and coworkers demonstrated that haploid embryos and embryonic stem cells can be used to produce offspring with predetermined parental genomes in cattle	Smith LC, Paredes LA, Sampaio RV, Nociti RP, Therrien J, Meirelles FV. Haploid embryos and embryonic stem cells to produce offspring with predetermined parental genomes in cattle. Anim Reprod. 2024;21(3):e20240030
2025	Yang et al., (2025) reported the derivation of cow and sheep haploid embryonic stem (haES) cells and their application to generate offspring by intracytoplasmic haES cell injection (iCHI), similar to intracytoplasmic sperm injection.	Yang L, Di A, Song L, Liu X, Wu D, Wang S, Hao Z, Bu L, Bai C, Su G, Wei Z. Generation of modified cows and sheep from spermatid-like haploid embryonic stem cells. Nature Biotechnology. 2025; 7:1-9. (Extracted from the abstract)
2026	Pham and coworkers enriched the G0/G1 population of ESCs by culturing them to overconfluency (>90% cell confluence). This demonstrated that during mouse embryonic stem cell (ESC) cloning via nuclear transfer, overconfluency culture induced cell cycle synchronization in the first cleavage that led to improved developmental potential of ESC-derived cloned embryos to live birth.	Pham DQ, Matoba S, Funaya S, Hirose M, Ike F, Isotani A, Ogonuki N, Ikawa M, VAN Thuan N, Ogura A, Inoue K. Overconfluent culture promotes cell cycle synchronization in mouse embryonic stem cells for efficient cloned embryo production. J Reprod Dev. 2026 Jul 9. doi: 10.1262/jrd.2026-069.
2026	Tsai et al., (2026) obtained artificial oocytes through haploidization of mouse endometrial stromal cells and bone marrow-derived mesenchymal stem cells.	Tsai S, Xie P, Cheung S, Rosenwaks Z, Palermo GD. Artificial oocytes through haploidization of mouse endometrial stromal cells and bone marrow-derived mesenchymal stem cells. F S Sci. 2026;7(2):113-123.
2026	Lari and coworkers (2026) successfully performed neogame-togensis to generate neo-gametes from mouse embryonic stem cells.	Lari E, Ng L, Xie P, Cheung S, Bains S, Rosenwaks Z, Palermo GD. Induction of male neogametogenesis in a three-dimensional microenvironment supporting successful

	They successfully fertilized oocytes and achieved blastulation. Although normal fertilization and blastulation were demonstrated but the efficiency rate was below optimal levels but their method could produce viable offspring in the mouse.	fertilization and proper embryo development. Mol Hum Reprod. 2026;32(3):036. (Extracted from the abstract)