Preface

Human embryonic stem cells are derived from the earliest stages of blastocyst development after the union of human gametes. Prior to fertilization, the oocyte first requires timed completion of meiosis. This vital step does not occur throughout a woman's life; rather, oocytes are arrested at the first meiotic division until puberty when small numbers mature competitively during the reproductive years. Maturation is complete at the one-day event of ovulation that occurs in a regular, approximately monthly cycle. In humans, oocytes can be successfully fertilized only during a short period after ovulation; oocytes that are not fertilized are not retained.

Sperm cells mature from spermatic stem cells through a sequential process that has been well characterized. Spermatic stem cells are in turn generated from primordial germ cells set aside during early embryonic development. Generally, tens of millions of sperm are present in an ejaculate of which only one will successfully fertilize the mature oocyte. Sperm that fail to fertilize are discarded.

Fertilization initiates the process of cell differentiation. Because embryonic transcription is not initiated until later, the earliest developmental events are regulated primarily by maternally inherited mRNA. Once the sperm enters the egg, its DNA-associated proteins are replaced by oocyte histones. The two pronuclei become enveloped with oocyte-derived membranes, which fuse and begin the zygote's mitotic cell cycle. Embryonic development starts with a series of cleavages to produce eight undetermined and essentially equivalent blastomeres. The pattern of cleavage is well coordinated by cytoplasmic factors and in mammalian eggs is holoblastic and rotational. Though genomic DNA is inherited from both parents, mitochondrial DNA is inherited from only the mother. Paternal mitochondria transferred at the time of sperm entry are discarded by a little-understood process.

Human eggs, with a diameter of 100 μm, are generally smaller than eggs of other species. They are normally fertilized within the fallopian tubes and undergo cellular division in a defined milieu as they migrate toward the uterus. Over the first few days, cellular division follows a predictable 12–18-h cycle resulting in 2- to 16-cell pre-embryos. The

sperm centrosome controls the first mitotic divisions until day 4, when genomic activation occurs within the morula stage. The individual blastomeres are initially totipotent until the morula begins compaction and the cells initiate polarization. During compaction, cell boundaries become tightly opposed and cells are no longer equivalent. Cells in the inner cell mass (ICM) contribute to the embryo proper, whereas cells on the outside contribute to the trophoectoderm. The blastocyst forms approx 24 h after the morula stage by the development of an inner fluid-filled cavity, the blastocell.

Implantation is the process through which the compact zonula is thinned and the blastocyst released to implant. The blastocyst must first hatch from the thinning zona pellucida by alternating expansion and contraction; this process of hatching is critical to further development. Implantation of the hatched blastocyst requires several steps, including apposition, attachment, penetration, and trophoblast invasion, and cannot occur until the first cell specification into trophoectoderm has occurred.

As the trophoblast is developing to form the fetal component of the placenta, the endometrial lining of the maternal uterus is undergoing a decidual reaction to generate the maternal component of the placenta. Simultaneously the inner cell mass undergoes gastrulation, defined as a process of complex, orchestrated cell movements that vary widely among species, but include the same basic movements. These include epiboly, invagination, involution, ingression, and delamination.

Thus, several major developmental events have taken place as the fertilized egg migrates from the site of fertilization (fallopian tubes) to the body of the uterus over a period of 4 d. At all stages the egg is shielded from the external environment, initially by the zonula and subsequently by the trophoectoderm, but is accessible to *ex utero* manipulation (*see* Fig. 1). It is important to emphasize that these critical developmental events have occurred prior to implantation and well before blood vessel growth and heart development. The early stage fertilized egg that has not yet been implanted has been termed a pre-embryo to distinguish it from the implanted embryo. Manipulation of the preimplanted embryo has been feasible for the past three decades, and detailed rules governing the use of blastocysts have been developed.

After implantation, the ICM proliferates and undergoes differentiation. Several results suggest that lineage-specific genes are operating in a totipotent blastocyst cell prior to lineage commitment, and strongly support the concept that stem cells express a multilineage transcriptosome. Most genes (including tissue-specific genes) are

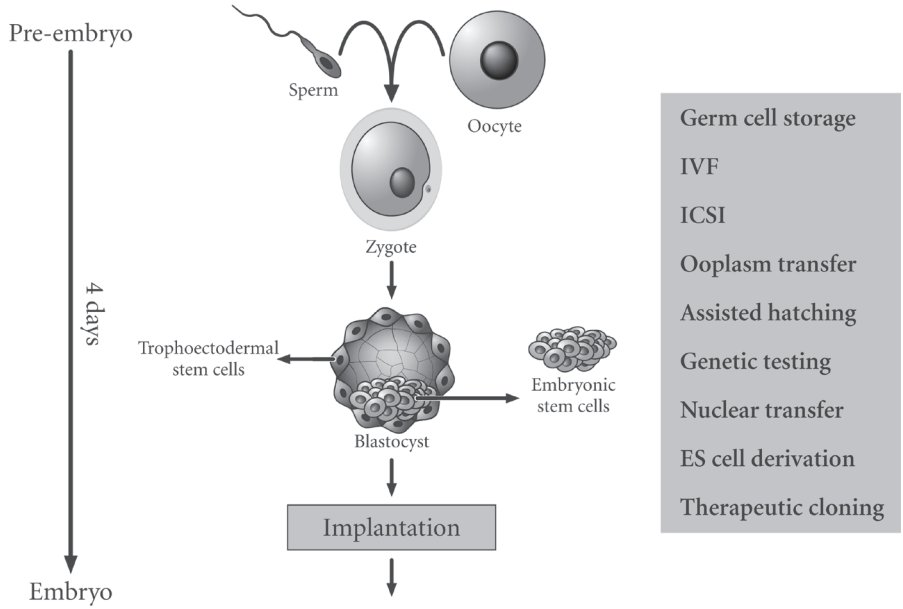

Fig. 1. Many techniques have been devised to manipulate the process of fertilization and maturation prior to implantation (summarized at right). The recent development of techniques to generate embryonic stem cell lines and perform somatic nuclear transfer has increased our ability to understand the process of development and intervene therapeutically. Note that the fertilized egg and early stage zygote are accessible to manipulation prior to implantation.

maintained in an open state with low but detectable levels of transcription with higher levels of specific transcription seen in appropriate cell types. Maintenance of an open transcriptosome in multipotent cells likely requires both the presence of positive factors as well as the absence of negative regulators. Factors that maintain an open transcriptosome include as yet unidentified agents such as demethylases, reprogramming molecules present in blastocyst cytoplasm, and regulators of heterochromatin modeling. Global activators, global repressors, and master regulatory genes play important regulatory roles in switching on or off cassettes of genes, whereas methylation and perhaps small interfering RNA (siRNA) maintain a stable phenotype by specifically regulating the overall transcriptional status of a cell. Allelic inactivation and genome shuffling further sculpt the overall genome profile to generate sex, organ, and cell-type specification.

Few genes have been identified that are required for the maintenance of the epiblast population. *Oct4*-/- embryos die before the egg cylinder stage and embryonic stem (ES) cells cannot be established from *Oct4*-/- cells. Levels of Oct 4 expression are critical to the fate of the cells. Low cell levels lead to differentiation into trophoblast giant cells, whereas high levels cause differentiation into primitive endoderm and mesoderm. FGF4 is required for formation of the egg cylinder and FGF 4-/- embryos fail to develop after implantation and ICM cells do not proliferate in vitro. *Foxd3/Genesis* is another transcription factor that may be required for early embryonic development. TGF-β/SMADs, Wnts, and FGFs are thought to play an important role in the process of gastrulation. BMP4 is essential for the formation of extraembryonic mesoderm and the formation of primordial germ cells. Nodal expression is required for mesoderm expansion, maintenance of the primitive streak, and setting up the anterior–posterior and proximo–distal axis. FGF4 is secreted by epiblast cells and is required for the maintenance of the trophoblast.

As our understanding of early developmental events has increased, our ability to safely manipulate the reproductive process has also increased. In vitro fertilization is now a relatively commonplace procedure that has been performed for more than 20 yr. Today, there are over a thousand established clinics worldwide. Even such technically complex procedures as intracytoplasmic sperm injection (ICSI), ooplasm transfer, assisted hatching, intrauterine genomic analysis, intrauterine surgery, and organ transplants are becoming more commonplace. More then 70 human embryonic cell lines have been established and their ability to differentiate into ectoderm, endoderm, and mesoderm repeatedly demonstrated. Nuclear transfer has become feasible, and the potential of combining ES cell technology with somatic nuclear transfer to clone individuals has caught the attention of people worldwide. At each stage of technological sophistication, profound ethical issues have been raised and publicly debated. Perhaps the most recent technological breakthroughs are the ones that have created the most controversy, primarily because of their potential to be used on a large scale. The ability to generate human ES (hES) cells and the ability to perform somatic nuclear transfer and successfully clone mammalian species raise fears often fueled by limited information.

An additional potential paradigm shift has been the suggestion that pluripotent ES-like cells may exist and indeed may persist into adulthood (*see* Fig. 2). These cells, while differing subtly from ES cells, may be functionally equivalent for therapeutic applications. The possible

existence of such adult cells with ES cell properties has fanned the debate and fueled a drive to assess the properties of all classes of pluripotent cells and to understand their underlying differences.

In *Human Embryonic Stem Cells* we invited leaders in the field to present their work in an unbiased way so that readers can assess the potential of stem cells and the current state of the science. The first section covers issues that regulate the use of human pluripotent cells. Chapters 1–3 begin with a summary of the ethical debate surrounding the derivation of human stem cells, and the current policies governing their use in the United States and abroad. The presidential announcement of August 2001 heralded a change in policy enabling federal support of research with hES cells that meet specific criteria. In Chapters 2 and 3, representatives from the National Institutes of Health (NIH) discuss the rules and conditions regulating federal funding, and issues of intellectual property regarding the use of hES cells. Chapter 2 delves into what constitutes "allowable" research and provides a guide to researchers interested in acquiring funding from US federal agencies such as the NIH for studies in this field.

Part II describes the types of human pluripotent cells that are currently being studied, their sources, methods of derivation, and maintenance. Many tissues are constantly renewed by the activities of resident, multipotent precursor or progenitor cells that have the ability to produce several different mature phenotypes. In the well-characterized hematopoietic system, T and B lymphocytes are derived from the lymphoid stem cell, whereas the myeloid stem cell can generate a host of red and white blood cells, including monocytes, eosinophils, platelets, and erythrocytes. However, both the myeloid and lymphoid stem cells are committed precursors, unable to differentiate along other pathways. There are only a few examples of truly pluripotent stem cells with the developmental capacity to generate cells representing all three germ layers (*see* Fig. 2) . Four types of such pluripotent stem cells are discussed in this section. In Chapter 4, Draper, Moore, and Andrews review the tumorigenic origins of embryonal carcinoma (EC) cells and their developmental counterparts, embryonic germ (EG) cells, present in the germinal ridges of young fetuses. Although there are many claims that pluripotent and highly plastic stem cells reside in adult tissues, the best characterized are those present in bone marrow. Cardozo and Verfaille summarize studies demonstrating their pluripotency in Chapter 5.

The high degree of interest in hES cells arise from two properties: their ability to self-renew essentially indefinitely and to be maintained

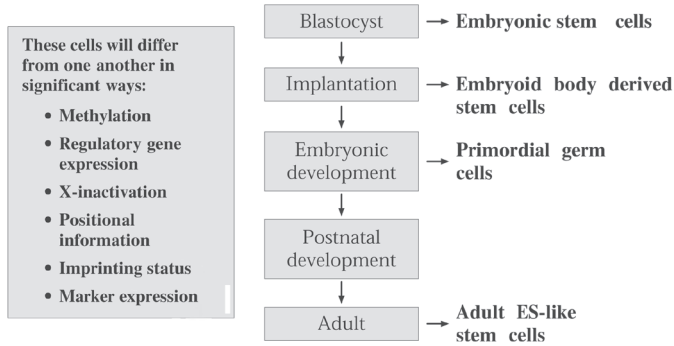

Fig. 2. Many different cell populations have been isolated that appear ES-like in their ability to contribute to chimeras after blastocyst injection, and to differentiate into ectoderm, endoderm, and mesoderm lineages in vitro. These distinct populations, although superficially similar, are likely to differ from each other when examined in more detail.

in an immature state, and their ability to differentiate into a wide range of mature tissues and cells. This enables the same population of cells to be studied under a variety of conditions; their properties, behavior, and fates can be reproduced and predicted. This capacity to standardize, predict, and reproduce results using a particular cell line will in turn greatly enhance the development of treatments and assays. For these reasons, much work is currently focused on methods for the expansion of hES cells and protocols to regulate their differentiation down selective lineages toward defined fates. Procedures for the growth, subcloning, and maintenance of hES cells are presented in Chapters 6 and 7 by three groups that are pioneers in this endeavor.

The following five chapters in Part III focus on specific methods that drive their differentiation into neuroepithelium, pancreatic islet cells, cardiomyocytes, vascular cells, and hematopoietic progenitors. Because much of the groundbreaking work was first conducted on murine ES cells, these initial animal studies are described and compared with the behavior of their human counterparts.

Part IV focuses on the potential uses of human stem cells in a variety of applications. In Chapter 13, Harley and Rao compare the advantages and disadvantages of using hES cells versus stem cells acquired from adult tissues for transplantation therapies. More complex applications to generate cells with the desired genetic composition include genetic manipulation of hES cells (Chapter 14) and somatic cell nuclear transfer (also called therapeutic cloning) (Chapter 15) to produce hES

cells with the patient's genetic composition for autologous transplants. In Chapter 16, Kamb and Rao discuss possible uses of human stem cells as tools for drug and gene discovery in vitro, and as therapeutic agents in vivo. The latter include cell, tissue, and organ replacement and regeneration, as well as the use of cells as peptide manufacturers and as delivery systems. They also explore what is needed to generate a donor cell that is universally accepted.

Most cell transplantation treatments will require the oversight of and approval by the Food and Drug Administration (FDA). In Chapter 17, Fink reviews the regulatory role of the FDA in ensuring the safety, purity, potency, and efficacy of new therapies involving human stem cells. Although the main interest in stem cell research resides in their great potential for cell replacement therapy to treat a long list of diseases that are currently incurable, there are at present no stem cell treatments in use, and only a rare few in clinical studies. In the last chapter, Reier and colleagues review preclinical and clinical studies conducted with neuron-like cells derived from one of the best-studied human EC cell lines, the NT-2 cells. This discussion introduces the complexities involved with in vivo studies, and the behavioral and functional analyses following cell transplantation.

Finally, we include a series of appendices that will provide additional information on useful websites, stem cell patents, and examples of Material Transfer Agreements to facilitate the sharing of cells. We hope that the readers will find the contents of *Human Embryonic Stem Cells* useful, and we welcome comments proposing additions or deletions to what we hope will become the standard reference book in the field of hES cell biology.

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