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STEM CELLS

An Interactive Qualifying Project Report

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ABSTRACT

This project investigates several different aspects of the complex, and in a way existential, topic of stem cells, documenting various types of stem cells, their potencies, their uses, their controversial ethical dilemmas, their legal aspects, and their overall effects on society. We support the efforts to re-establish U.S. federal funding for embryinic stem (ES) cell research. We also support the use of alternatives to ES cells, such as iES cells. Adult stem cells do not require the destruction of an embryo, but are harder to identify and grow than ES cells, and have less potency.

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PROJECT OBJECTIVES

The objective of this IQP was to examine the topic of stem cells, and to document the effect of this controversial new technology on society. The purpose of chapter-1 was to explain the difference between various types of stem cells, distinguishing their different potencies, and delineating which ones require the destruction of an embryo while harvesting. The purpose of chapter-2 was to document the present state of medical applications for each type of stem cell. The purpose of chapter-3 was to examine the ethics surrounding this controversial topic, focusing mostly on embryonic stem cells and their less controversial alternative options. Finally a conclusion is made by the authors regarding their recommendations.

Chapter 1: Stem Cell Types and Sources

Christopher Smith

The human body is comprised of many different cell types, which range from ordinary skin cells, to nerve cells in the brain. The functions of these specialized cells normally does not change; over the course of their lives, they perform a predefined set of operations and functions specific to their cell type. Stem cells on the other hand are the master cells of the body. They are long-lived, and they have the ability to differentiate or change into other cell types. They also have the capability to replicate, allowing pools or lines of stem cells to produce many more generations. (Figure-1). Because of these properties, stem cells can regenerate tissues, and so have applications in medicine. The purpose of this chapter is to document the various kinds of stem cells, as a prelude to subsequent chapters on their applications and ethics.

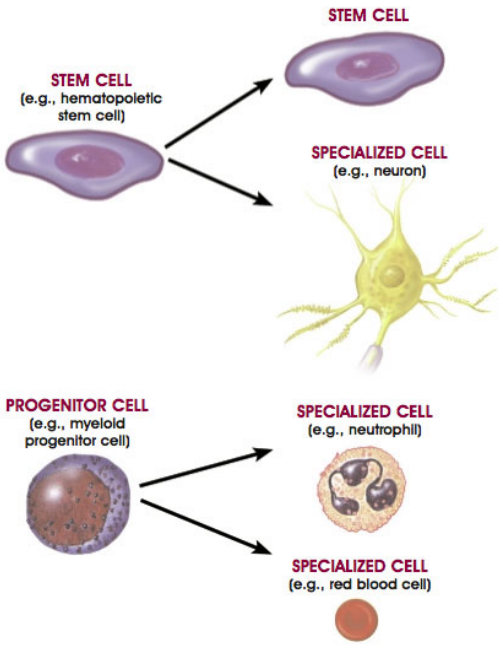

Figure 1 – Diagram of Stem Cell Differentiation. This diagram shows how stem cells are capable of forming either more stem cells, or more specialized tissues, while progenitor cells can only form more specialized tissues. http://stemcells.nih.gov/info/scireport/chapter4**.asp**

Stem Cell Categories

There are two main categories of stem cells, namely adult stem cells (ASCs) and embryonic stem (ES) cells. ASCs are found within the grown human body, and unlike ES cells, can be isolated from either adult tissue or from umbilical cords. ASCs are mainly used to maintain tissue homeostasis and to replace dead or dying cells, and are found only in specific areas of the human body. Unfortunately, ASCs are also very rare, so they are hard to isolate. Furthermore, they are only found within specific regions of the body, and they will have different functions depending on their location. Because of this, not all ASCs are the same, nor do they all have the same capacity to differentiate into all types of cells. Among the different types of adult stem cells, are hematopoietic stem cells (HSCs), neural stem cells (NSCs), mesenchymal cells (MSCs), epithelial stem cells, and cardiac cells, among others (National Institutes of Health 4).

Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) represent the stem cells responsible for forming blood and immune cells. This type of stem cell is the most well known and researched. "They are ultimately responsible for the constant renewal of blood—the production of billions of new blood cells each day. Physicians and basic researchers have known and capitalized on this fact for more than 50 years in treating many diseases with bone marrow transplants. The first evidence of blood-forming stem cells came from studies of people exposed to lethal doses of radiation in 1945" (National Institutes of Health 5).

A hematopoietic stem cell is a cell that has been taken from the blood or from the bone marrow with the ability to regenerate, and which can differentiate into a variety of specialized

cells. It has been estimated that approximately one in every 10,000 to 15,000 bone marrow cells are stem cells, and this number falls even more to one in every 100,000 peripheral blood cells. This means that it can be very difficult to harvest or even find HSCs within the body.

Recent studies have suggested that two kinds of HSCs exist: long-term stem cells with self-renewal capabilities, and short-term 'progenitor' or 'precursor' cells (Figure 1). "Progenitor or precursor cells are relatively immature cells that are precursors to a fully differentiated cell of the same tissue type. They are capable of proliferating, but they have a limited capacity to differentiate into more than one cell type as HSCs do. For example, a blood progenitor cell may only be able to make a red blood cell" (National Institutes of Health 5) (Figure-2).

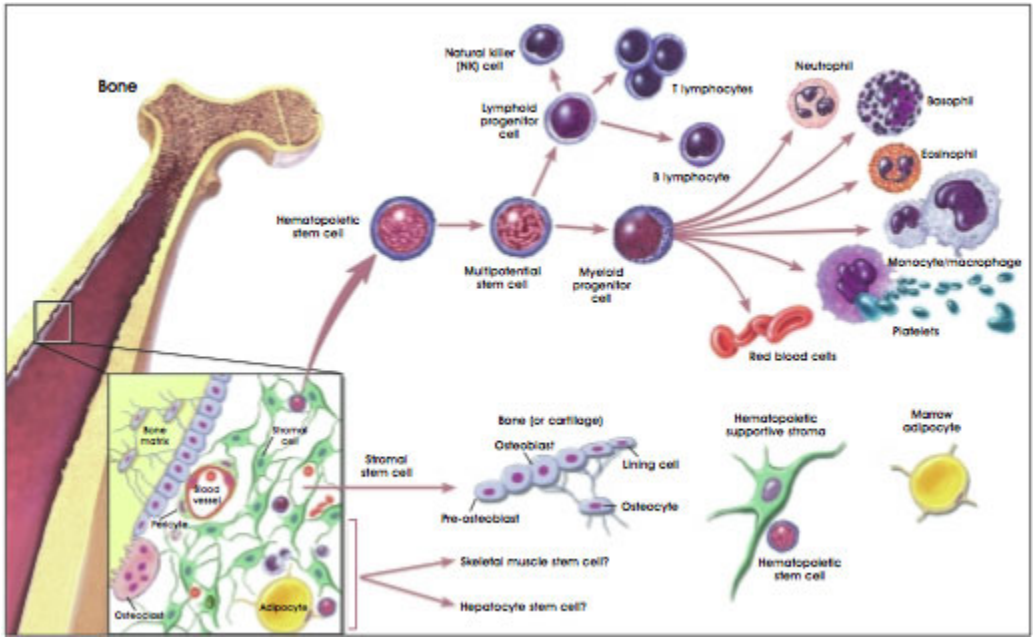

Figure 2 – Diagram of Hematopoietic Stem Cell Differentiation. Hematopoietic stem cells taken from bone marrow (left side of diagram) can differentiate into myeloid or lymphoid blood cells (diagram upper center). http://stemcells.nih.gov/info/scireport/chapter5.asp

The primary source of HSCs is bone marrow. For the past forty years, doctors have performed transplants of bone marrow, but since then, the source for HSCs has slowly shifted from bone marrow retrieval to retrieval from the circulating blood. "Doctors now prefer to harvest donor cells from peripheral, circulating blood. It has been known for decades that a small number of stem and progenitor cells circulate in the bloodstream" (National Institutes of Health 5), especially when the donor is pre-treated with hormones to stimulate HSCs release from the marrow into the peripheral blood.

Perhaps one of the most important HSC discoveries in the late eighties to early nineties is that blood from the human umbilical cord blood and placenta is a rich source of HSCs. This has sparked new fields of research based upon the collection of umbilical cord blood for research. Furthermore, because umbilical cords carry stem cells that are naïve, procedures using those HSCs can bypass problems of physical rejection.

In addition, researchers are now beginning to measure differences among the different hematopoietic sources. It has been found that "[hematopoietic stem cells] taken from tissues at earlier developmental stages have a greater ability to self-replicate … and are less likely to be rejected by the immune system—making them potentially more useful for therapeutic transplantation" (National Institutes of Health 5).

Neural Stem Cells

Neural stem cells (NSCs) are stem cells found within the brain, and can differentiate into the tissues found within the brain. (Figure 3). "The existence of stem cells in the adult brain has been postulated following the discovery that the process of neurogenesis, birth of new neurons, continues into adulthood in rats" (National Institutes of Health 8).

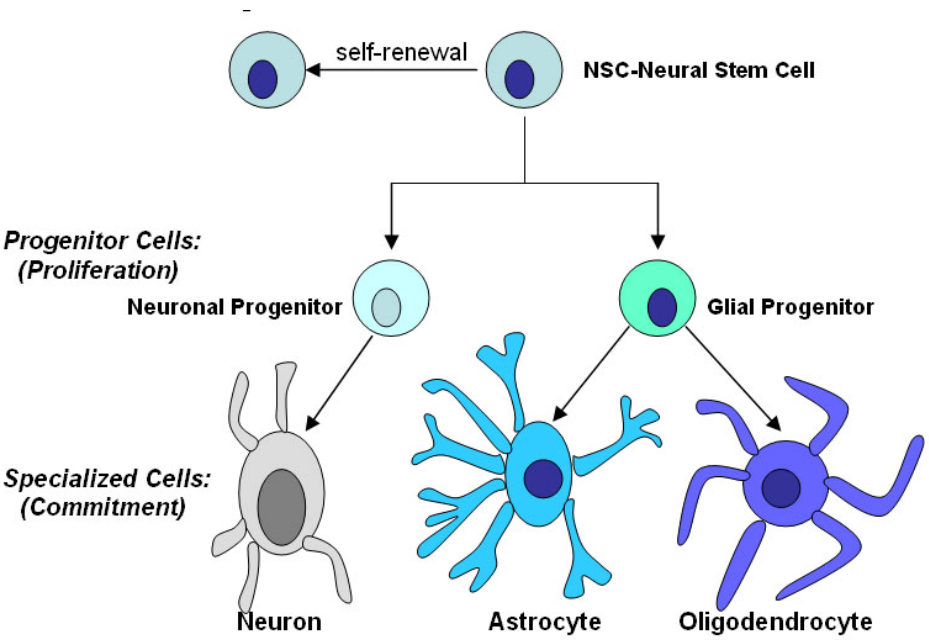

Figure 3 – Diagram of Neural Stem Cell Differentiation. Neural stem cells can either self renew (upper left) or can differendiate into different types of nerve cells (lower). http://www.umdnj.edu/gsbsnweb/stemcell/scofthemonth/scofthemonth2/braincancerstemcellsci.htm

"Stem cells in the adult primate brain occur in two locations. One, the subventricular zone, is an area located under fluid-filled spaces called ventricles. The other is the dentate gyrus of the hippocampus. In primates, very few new neurons normally appear in either place, which is why the phenomenon escaped notice until recently" (National Institutes of Health 8). In the mid 1990s, researchers were able to demonstrate that stem cells in the brain were able to repair damage to injured areas of the brain. Researchers are now trying to discover how extensively stem cells are able to repair damaged tissue, and how effective such treatments might be for curing other nervous tissue.

Furthermore, because of some recent success with restoring motor control to paralyzed rats, scientists are now conducting research into the possibility of utilizing neural stem cells to repair damaged nerve cells and to treat and potentially cure Parkinson's disease.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are multi-potential, and can generally differentiate into bone, cartilage, muscle, and fat cells (Figure 4). They have also been known to differentiate into neuronal cells in certain situations (Engler, Shamik and Sweeney). MSCs are found within the bone marrow and are used in a variety of transplant treatments, including bone marrow transplants.

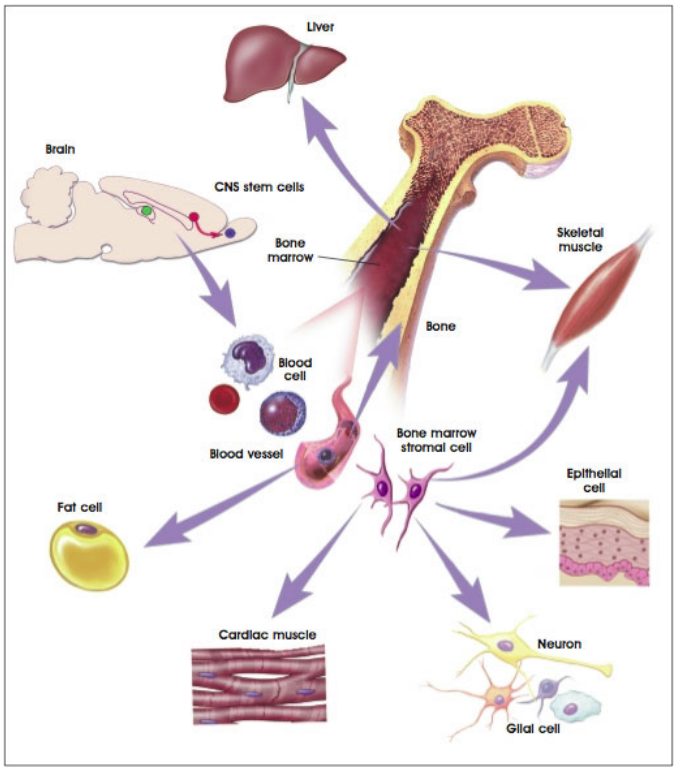

Figure 4 – Diagram of Mesenchymal Stem Cell Differentiation.
Diagram shows the multi-potent nature of MSCs.
http://stemcells.nih.gov/info/scireport/chapter4.asp

Epithelial Stem Cells

Epithelial stem cells, or induced pleuripotent stem cells are not typical adult stem cells. Instead of naturally occurring within the adult body, epithelial stem cells are instead reprogrammed epithelial cells, or skin cells, that gain the ability to differentiate into other cells. The term 'induced pleuripotent stem cells' is used here to indicate that these cells are not found, but are instead 'induced' to become stem cells (Figure 5). In the case of epithelial stem cells, scientists have discovered that through the ingenious use of genetically reprogrammed transcription factors, "pluripotent stem cells equivalent to embryonic stem cells have been derived from human skin tissue" (The Economist).

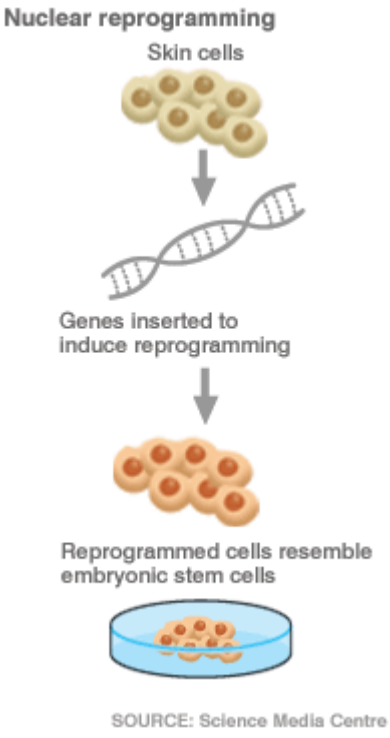

Figure 5 - Induced Pluripotent Stem Cells. These cells represent fibroblast skin cells that are reprogrammed by transfecting 2-4 specific genes into the cells to de-differentiate them to a ES like state. http://news.bbc.co.uk/2/hi/health/7101834.stm

Cardiac Stem Cells

Cardiac stem cells, found in small quantities within the heart, have the ability to regenerate cardiac muscle, and hence repair damage to the heart (Figure 6).

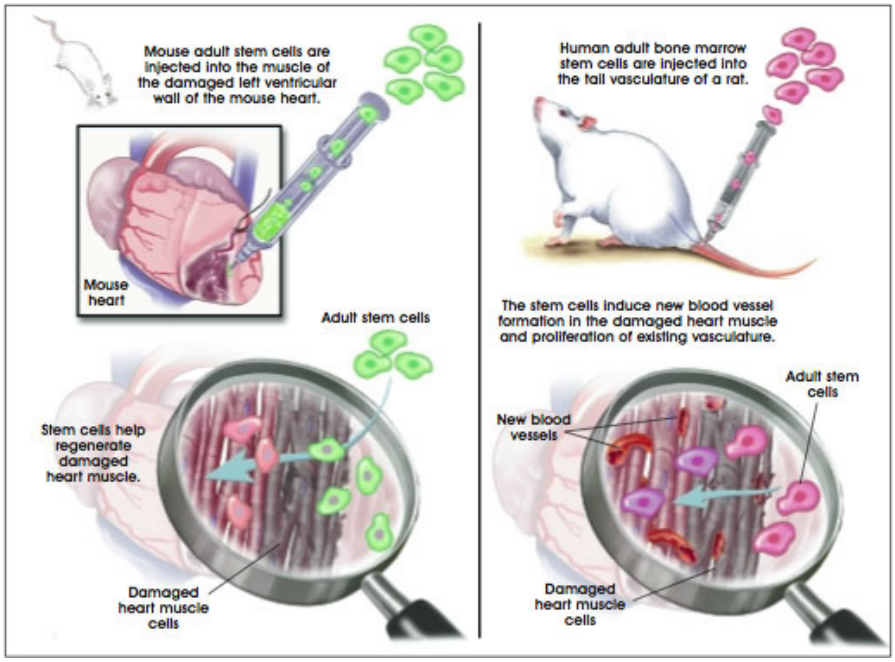

Figure 6 – Heart Muscle Repair with Cardiac Stem Cells.
http://stemcells.nih.gov/info/scireport/chapter9.asp

"Researchers have isolated cardiac stem cells from rats, and showed that when these cells were injected into rat hearts that had been damaged, they reconstituted the injured tissue. The same group has also detected similar cells in human hearts" (Touchette).

Embryonic Stem Cells

An embryonic stem (ES) cell is perhaps the most versatile of all stem cells. It has the potential to replicate itself, and to differentiate into all known types of tissue except the placenta. However, as the National Institute of Health says, "Scientists have found it necessary to develop specific criteria that help them better define the [Embryonic Stem] cell. Austin Smith … has offered a list of essential characteristics that define ES cells". The following list taken from the NIH represents the defining properties of an embryonic stem cell:

- Derived from the inner cell mass/epiblast of the blastocyst.
- Capable of undergoing an unlimited number of symmetrical divisions without differentiating (long-term self-renewal).
- Exhibit and maintain a stable, full (diploid), normal complement of chromosomes (karyotype).
- Pleuripotent ES cells can give rise to differentiated cell types that are derived from all three primary germ layers of the embryo (endoderm, mesoderm, and ectoderm).
- Clonogenic: that is a single ES cell can give rise to a colony of genetically identical cells, or clones, which have the same properties as the original cell.
- Expresses the transcription factor Oct-4, which then activates or inhibits a host of target genes and maintains ES cells in a proliferative, non-differentiating state.
- Can be induced to continue proliferating or to differentiate.
- Lacks the G1 checkpoint in the cell cycle. ES cells spend most of their time in the S phase of the cell cycle, during which they synthesize DNA. Unlike differentiated [adult] cells, ES cells do not require any external stimulus to initiate DNA replication.
- Do not show X inactivation. In every [adult] cell of a female mammal, one of the two X chromosomes becomes permanently inactivated. X inactivation does not occur in undifferentiated ES cells.

(National Institutes of Health 2)

While ES cells offer the most potential in areas of medicine and research because of their ability to differentiate into all different types of tissues except the placenta, they are also the most surrounded in controversy, because to harvest ES cells, scientists usually use embryos in a process that kills the embryo (Figure 7).

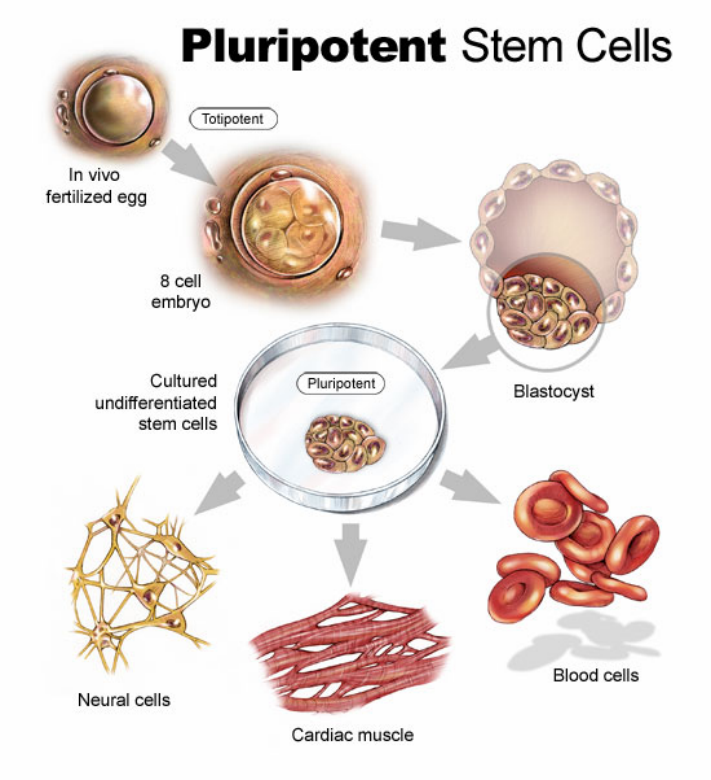

Figure 7 – Isolation and Differentiation of Embryonic Stem Cells. A donated egg and sperm are united by *in vitro* fertilization (upper left) and the embryo is grown to the blastocyst stage (upper right) from which ES cells are obtained (center) that can differentiate into a variety of cell types. http://www.csa.com/discoveryguides/stemcell/overview.php

Stem Cell Potencies

Stem cells have a variety of different *potencies*, which means that different stem cells have different abilities when it comes to differentiating into specific types of cells. There are four potencies that stem cells are categorized under: totipotent, pleuripotent, multipotent, and unipotent. *Totipotent stem cells* are cells capable of making an entire viable embryo, including the placenta. While embryonic stem cells are sometimes mistakenly categorized as totipotent stem cells, the only true totipotent cell is a newly fertilized egg. In turn, totipotent stem cells can differentiate into ES cells. *Pleuripotent stem cells* can differentiate into any type of tissue,

except for the placenta. The type of cell differentiation depends upon how the pleuripotent stem cells are cultured. Embryonic stem cells are a prime example of pleuripotent stem cells. They are usually harvested from the inner mass of a blastocyst embryo, and are direct descendants of the totipotent cell. Among all of the types of stem cells, embryonic pleuripotent stem cells have the greatest potential in the fields of therapy and medicine because of their ability to differentiate into a wide variety of tissue types. *Multipotent stem cells* can differentiate into closely related cells only, such as the hematopoietic stem cell which can differentiate into red blood cells, white blood cells and so forth. Neuronal stem cells are another example of multipotent stem cells, capable of producing neurons and lial cells. *Unipotent stem cells* are the least potent of stem cell types. They are capable of only differentiating into one cell type, but they have the added ability of being able to regenerate through a process of self-renewal. Skin stem cells are a prime example of unipotent stem cells, capable of producing only new skin cells.

Chapter Conclusion

There are many different types of stem cells, with different capabilities, and different ethics apply to each type. Embryonic stem cells currently offer the greatest potential for the future of medical research, but they are embroiled in controversy because of the stigma associated with killing an embryo to harvest the cells. Adult stem cells (ASCs), on the other hand, are very promising and in no way destroy embryos, and though they may not currently have as great a potential as ES cells, ASC research seems very promising.

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Chapter 2: Stem Cell Applications

S. Emre Gazioglu

Although the world we live in may never be cleansed from widespread poverty and violence, diseases like Parkinson's, diabetes, spinal cord injuries, and various types of cancer can be a problem of the past considering the new field of regenerative medicine using stem cells. Even though the media likes to focus on stem cell failures, giving the layperson the idea that no lives have ever been saved with stem cells, there have been some remarkable accomplishments using these cells. The purpose of this chapter is to describe some of the disease treatments using stem cells. Although we have a long ways to go, great improvements have been made in the treatment of Parkinson's, diabetes, and cancer with animals and humans using adult and embryonic stem cells.

Treatment of Diabetes Using Stem Cells

Diabetes is the fifth leading cause of death in America, with an estimated 7% of the population suffering from the disease. Type 1 diabetes generally results from autoimmune destruction of pancreatic islet ß-cells, causing a lack of production of insulin. The sufferers must rely on external sources of insulin injected several times a day, and must do multiple blood tests to monitor glucose. Patients must be aware of their blood glucose levels, and conscious of how the daily activities affect their disease.

Unfortunately, there is also a relative paucity of donations for pancreas or islet allograft transplantation, and this has fueled the search for alternative sources for ß-cell replacement therapies. In a recent study, Assady et al. (2005) used pluripotent undifferentiated human embryonic stem (hES) cells as a model system for lineage-specific (pancreatic) differentiation.

Figure-1 shows the production β-cell-specific mRNAs, while Figure-2 shows the production of insulin from the differentiated hES cells. The authors concluded that hES cells could indeed be induced to differentiate into insulin producing cells. The Assady study indicates that hES cells can differentiate down a pancreatic lineage, even though normal development of the pancreas is the result of several complex interrelated mechanisms involving growth factors, mesynchymal interactions, and extracellular matrix.

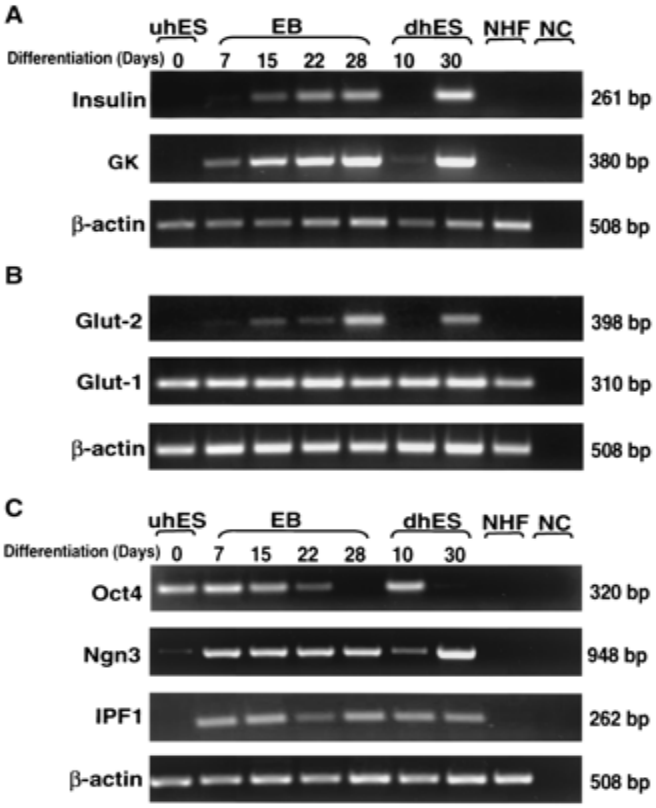

FIGURE-1. Expression of ß-Cell–Related Genes in Differentiated hES Cells. Total RNA was extracted from undifferentiated hES (uhES) cells, from differentiated hES growing either as EBs or as high-density adherent cell cultures (dhES) at various stages of differentiation, and from normal human fibroblasts (NHF). cDNA was synthesized from 7 µg total RNA and oligo dT primer. Aliquots of cDNA were diluted 1:2 for insulin and islet-specific GK or 1:5 for GLUT1, GLUT2, Oct4, Ngn3, and IPF1/PDX1 before PCR. ß-actin served as an internal standard. NC indicates no cDNA. For insulin, GK, GLUT2, GLUT1, Oct4, Ngn3, IPF1/PDX1, and ß-actin, 36, 38, 40, 31, 37, 35, 35, and 28 cycles were applied, respectively, for insulin, GK, and ß-actin (*A*); for GLUT1, GLUT2, and ß-actin (*B*); and Oct4, Ngn3, IPF1/PDX1, and ß-actin (*C*).

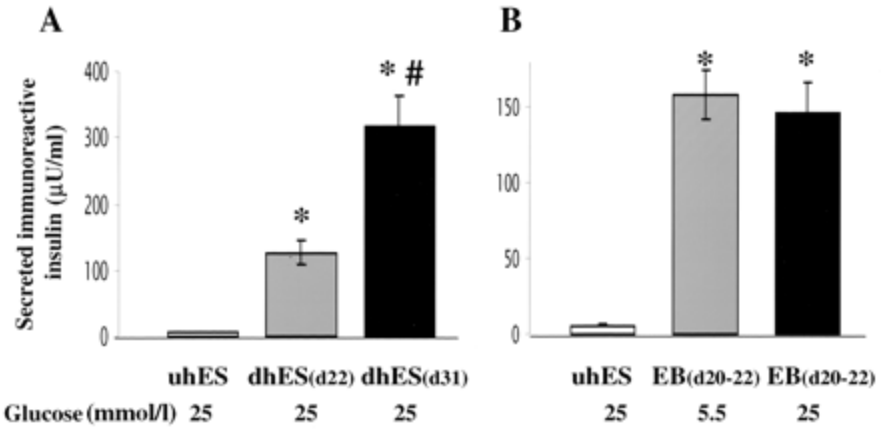

FIGURE- 2. Insulin Secretion from Differentated hES Cells at Various Glucose Concentrations and Growth Conditions. *A*: Undifferentiated hES (uhES) cells were cultured in knockout medium ($n = 6$) or were allowed to differentiate in high-density adherent conditions (dhES) for 22 ($n = 12$) and 31 days ($n = 7$). *B*: hES grown in suspension as EBs (60–70 EBs per dish) for 20–22 days ($n = 6$). Cultures were exposed to 3 ml of serum-free medium for 2 h containing either 25 or 5.5 mmol/l glucose, as indicated. The media were harvested after incubation, and insulin concentration was measured using enzyme immunoassay as described in RESEARCH DESIGN AND METHODS. Data are means ± SE. *$P < 0.0001$ vs. uhES; #$P = 0.0004$ vs. dhES-d22.

NovoCell, Inc. is one of the few companies heavily invested in the use of human ES cells for the treatment of type I diabetes. Their treatment involves in the creation of insulin producing islet cells that are derived from human ES cells that can later be transplanted into type I diabetic patients (D'Amour, 2006). NovoCell combined this ES-based treatment with their cell encapsulation technique which used polyethylene glycol (PEG) to cover the ES-derived islet cells and protect them from the host's immune system. The limitations of islet cell donors restrict the availability of these types of transplantation even though quite a few studies showed improvement in patients using islet stem cell transplantation for type I diabetes. NovoCell's ES cell therapies require less cell injections, and are not limited by islet cell donors because they have procedures to counter immune rejection.

Treatment of Damaged Heart Muscle With Stem Cells

The disease that kills the most Americans is heart disease. In practice now are mostly preventative treatments like diet regulations to prevent the disease from spreading, but there was no solution toward fixing damaged portions of the heart. When a person suffers an heart attack, generally the main cause is poor blood circulation caused by some types of blockage, usually plaque and cholesterol build up. When the blood flow is not in circulation, oxygen, nutrients and waste products can't be transferred which causes the cells to die, eventually lowering the functionality of the organ. At the moment following an heart attack drugs are given to the patient to remove blockages and prevent further blood clotting. Also surgical methods are used to open the arteries. Although this methods reduce the fatalities significantly it lacks the ability to restore the heart's full functionality. Stem cells promise a great possibility that the heart may return back to almost its full functionality.

Researchers at the Mayo Clinic in Rochester, Minnesota, have shown that heart restoration can occur with the use of ES cells (Terzic, 2004). Researchers are aware that the has the capability of regenerating dead cells, but the rate it restores the cells is insufficient, so they are considering using the restorative property of stem cells for the treatment of congenital heart disease. Researchers at the Mayo Clinic injected some of the rats with drugs to induce acute myocardial infraction, and some received murine ES cells which had a high potential of turning into cardiomyocytes. After three weeks of therapy, both myocardial stressed and control groups underwent tests to determine blood pumping out put. The stem cell-treated rats had a considerably higher rate of blood pumping output compared to the control group. Researchers noticed that myocardial necrosis was reduced in the stem cell group, and the cardiac muscle was

rebuilt while the control group continued to have cardiac muscle decay. The biggest concern the researchers had was the migration of cells from the heart to different parts of the body. Using fluorescent antibodies to cell surface proteins that were only expressed by the injected stem cells, the researchers were also able to track the movements of the injected stem cells. The researchers were pleased to see that neither tumor formation nor migration occurred, and the stem cells were limited to proliferating only in the diseased region of the heart (Terzic, 2004).

The first ever human clinical trial was performed in March 2003, and marked a milestone for turning the stem-cell debate from being "Pie in the Sky" to "miraculous innovation" (Philipkoski, 2003).

> "Sixteen-year-old Dimitri Bonnville was accidentally shot in the heart with a nail gun and suffered a massive heart attack; doctors suggested that he needed a heart transplant. The doctors did offer an alternative as well: Bonville could become the first human to receive experimental stem-cell therapy to revive his damaged heart tissue. Doctors at William Beaumont Hospital in Royal Oak, Michigan, used hematopoietic stem cells (HSCs) in his blood.
>
> The teenager's therapy began Feb. 17 with a four-day regimen of a drug that stimulated the production of HSCs in his peripheral blood. On Feb. 21, doctors harvested Bonnville's stem cells. Using a heart catheter, they transplanted the stem cells into the artery that supplies blood to the front of the heart. He was discharged about a week later and recuperated at home. His doctors say they have never seen a recovery like his." [Philipkoski, 2003]

His doctor explained that the entire front wall of his heart was dead before the procedure, and now his heart has almost returned to full functionality. This would have been impossible before the use of stem cells, or unthinkable 5-10 years ago.

Spinal Cord Injuries and Stem Cells

The spinal cord carries information from the brain to other organs. The spine is able to this because it is made from the same neurons and glial cells that make up the brain. The glial

cells allow the production of myelin, which insulates the cells so that the electrical signal remains fast. This insulation of neurons by myelin increases the travel speed of the electrical signal a hundred times. The loss of myelin can result in sensory and motor deficiencies, and in some cases causes paralysis.

In 2005, a study made at University of California, Irvine (UCI) by Henry Kiersten (UCI, 2005) emphasized the importance of the myelin loss in a spinal cord injury, and attempted a new approach to treat myelin loss. His study indicates that a treatment derived from human embryonic stem cells (hESs) improves mobility in rats with spinal cord injuries, providing evidence that use of these cells can help restore motor skills lost from acute spinal cord injuries.

Acute spinal cord damage occurs during the first few weeks of the injury, in turn followed by a chronic period that extends a few months. It is anticipated that the stem cell treatment in humans will be most effective when applied during the acute phase, at the same time when rods and ties are surgically placed in the spinal column to destabilize it after injury. Currently, drug treatments are given during the acute phase to help stabilize the injury site, but they provide only a very mild benefit, and they do not foster regeneration of insulation tissue (UC Irvine, 2005).

For the UCI study, they used a new technique to entice hES cells to differentiate into early-stage oligondendrite cells that produce myelin, the biological insulation for nerve fibers. The researchers injected the hES cells into a group of rats that suffered injury to the spinal cord a week previously, and into another group that suffered the same injury ten months previously. In both groups, hES cells formed into full-grown oligodendrocyte cells, and migrated to appropriate neuronal sites within the spinal cord. In the group that suffered the injury a week prior to treatment, myelin tissue formed as the oligodendrocyte cells wrapped around damaged neurons

in the spinal cord. Within two months, the rats began to show significant improvements in walking ability in comparison to the control group. In the group that suffered the injury 10-months prior to treatment, the motor skills did not return, and the myelin did not form. Because there was scar tissue around the space surrounding neuron cells, the myelin could not form.

In previous studies, Keirstead and his colleagues (Kirsten, 2005) identified how the body's immune system attacks and destroys myelin during spinal cord injury, and that myelin is capable of regenerating when treated with antibodies to block the immune system response. This ultimately restores sensory and motor activity. Hans Kiersten impression of the studies were:

> "We're very excited with these results. They underscore the great potential that stem cells have for treating human disease and injury. This study suggests one approach to treating people who have just suffered spinal cord injury, although there is still much work to do before we can engage in human clinical tests". (UC Irvine, 2005)

Treatment of Parkinson's Disease with Stem Cells

In the United States 500,000 people are known to be suffering from Parkinson's Disease and this is a conservative estimate, and 50,000 new cases are diagnosed each year. It is hard to get an accurate number because many people mistake their symptoms with normal ageing at the early stages of the disease and do not seek evaluation. In general diagnosing a patient with Parkinson's disease is difficult because there are no definitive diagnostic test, and other conditions may produce similar symptoms [Parkinson's Disease Foundation, 2008)]. Parkinson's disease is second only to Alzheimer's disease as the most common neurodegenerative disorder [Parkinson's Disease Foundation, 2008)].

Society pays a great price for Parkinson's disease. The total cost of Parkinson's disease to the United States is estimated to exceed $6 billion annually ("Rebuilding the Nervous System

with Stem Cells", 2005 NIH). As the life span of human beings increases, the financial and public health impact of this disease on society will increase substantially. As of 2008, there is still no cure for Parkinson's disease by conventional medical techniques. Traditional treatment has consisted primarily of drug therapy, sometimes assisted with neurosurgery. The symptoms of the disease, neuromusculature non-coordination, restrain patients from many luxuries we take for granted. The first symptom that gets mistaken with ageing at early stages of the disease is trembling of the hands, arms, legs, jaw or head. Other symptoms are stiffness of the limbs and trunk, slowness of movement, and postural instability or impaired balance.

Parkinson's disease is a disorder of the central nervous system in which the dopamine-producing cells in the substantia nigra region of the brain degenerate. Since dopamine is responsible for transmitting the electrical signals that are required for normal physical motion, a lack of the cells which produce this chemical result in the abnormal movement associated with this disease. Parkinson's disease is both chronic and progressive, meaning that, once diagnosed, it lasts throughout one's lifetime and worsens over time.

Scientists are working on generating the dopamine-producing neurons from mouse induced pluripotent stem cells (iPSC's) ("Rebuilding the Nervous System with Stem Cells" , 2005). These are fibroblast cells induced to a ES-like state by transfecting them with DNA encoding 3-4 key transcription factors that maintain a de-differentiated state. They tested the function of their iES-derived dopamine-producing neurons by injecting them into the brains of rats used as a model for Parkinson's. The treated group showed great improvement in symptoms versus the control group. The results demonstrate that animal induced

pluripotent stem cells (iPSCs) are capable of replacing lost cells that are suppose do the dopamine production.

Overall, the use of stem cells in humans is just beginning, and shows some promise, but human treatments are complicated by the hardships of producing clinical-grade stem cells known to be free from contaminants such like viruses, or free from animal proteins that could induce an immune response in humans. Also there must be lengthy clinical trials to ensure there are no side-effects, like tumor formation [Ryan, 2004). The goal is to one day reach a level that human iES cells made from patient-specific fibroblast cells can enable therapy without immunorejection.

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Chapter 3: Stem Cell Ethics

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In our earlier chapters, we discussed the various types of stem cells, and their medical potential, how such cells can improve chronically ill patients that current medicines can't cure. However, such cells are also highly controversial. Most of the ethical dilemmas rise from embryonic stem (ES) cell research and *in vitro* fertilized (IVF) embryos with metaphorical parallels to abortion. The main debates focus on when life begins, and such views are typically answered by the world's main religions. The purpose of this chapter is to discuss some of the main ethical concerns of stem cells.

In my view there is a great sense of hypocrisy in the stance of the Catholic Church condemning stem cell research as the moral authority, when science leaves off to inhumanitarian tracks, they tend to be the moral compass. But where was the Catholic Church condemning the Iraq War. A war only to keep control of the oil in the Middle East, 1.5 million dead, and about 4 million refugees fled Iraq (the ones who had the opportunity). Were these existing Middle Eastern lives less important than the cellular Christian lives? This is a dilemma to be thought about.

Adult stem cells (ASCs) and embryonic stem (ES) cells are similar in the sense that both cells are capable of renewing and can differentiate into more specialized cell types, but ES cells are pleuripotent (can create any cell in the body except placenta), so they are the most promising and the most intriguing for scientists, they are also the easiest to grow in culture. But ES cells also have the most controversy since embryos are destroyed to obtain them.

Adult Stem Cell (ASC) Ethics

Most of the ethical dilemmas surrounding ES cells do not apply to ASCs because they are obtained from adult tissues and other sources that do not destroy an embryo. Even the Catholic Church, the major institution against ES cell research supports ASCs. Islam, Buddhism and Jewish laws do not prohibit adult stem cell use for therapeutic use. ASC research has received the most federal funding because of the lack of ethical dilemmas, but the limited quantity of ASC that can be obtained from organs and tissues and their difficulty to grow in culture, makes it difficult to produce ASCs in large numbers for practical use. And ASC's have less potential than ES cells.

Embryonic Stem Cell Research (ESCR) Ethics

All religions in the world value human life well before stem cells became a major ethical issue. Other topics involving the destruction of a human life in its early phases have been widely discussed for decades for abortion and euthanasia. Even though the ethical debate over ES cells seems very recent, a highly related debate dates back to the 1970's when *in vitro* fertilization (IVF) was first used to derive human embryos for reproductive purposes. Today excess IVF embryos not used by the parents for reproductive purposes are the main source for embryos used to derive ES cell lines. These excess embryos are usually discarded after the couple is done having children, and with their consent. In the U.S., parental consent is required to use these excess embryos, and no donor can receive money as payment.

Among all these widely debated topics, ES cell research is the most controversial one since it involves human embryo destruction, so we must discuss where the human life begins. If life is considered to begin at conception, the destruction of an embryo to obtain ES cells is

considered murder. But if life begins at day 40 or during implantation into the uterine wall, the use of day-5 blastocysts would be allowed. Perhaps instead of being discarded, the excess IVF embryos could be used to save lives. Because these excess embryos are not enough for ESCR to be available for the public, scientists are working on ways to obtain more with less ethical controversy.

Catholic Stance on ESCR

On July 23, 2001, Pope John Paul II gave President Bush his stance on the controversy of stem cell research, saying that "experience is already showing how a tragic coarsening of conscience accompanies the assault on innocent human life in the womb, leading to accommodation and acquiescence in the face of other related evils such as euthanasia, infanticide and, most recently, proposals for the creation for research purposes of human embryos, destined to be destroyed in the process" (Pope John Paul II, 2001). This made clear the stance of the Catholic church, as the supreme Catholic leader announced his opinion that the Catholic church is against ESCR. Because the Catholic church believes that a human being needs to be respected and treated as a person from the moment of conception, fertilized human embryos have the same rights as a regular person, and no authority can force a person to take his own life. Thus making any possible external scientific interventions to modify the growth of an embryo a violation of its right.

Islamic Stance on ESCR

A general survey of the Islamic literature indicates no significant ethical or moral stances against ES cell research. The Islamic religion is based upon the divine and immutable revelation

known as the Koran. For Muslims, the Shari'ah interpreted from the Koran, dictates their life, thoughts, and actions. The Koran indicates that God creates a human life after a certain degree of biological development has been completed (Weckerly, 2002). It takes a period of time for an embryo to become a human life. The word "ensoulment" is used to explain the early stage of development at which life begins. It states that a soul enters a fertilized embryo to form a human being after pregnancy. This period of time has been defined as the fortieth to hundred twentieth days. This explanation from the Koran points out that an embryo is not considered a person until at least the fortieth day of pregnancy, which is well past the age of a 5-day blastocyst from which ES cells would be obtained. Another important factor is the difference between actual life and *potential* life distinguished in the Islamic world. Although each embryo has the potential to grow into a human being, it is not called a human being until its growth is completed (Kutty and Siddiqi, 2007). This implements that Islam is not opposed to ESCR in general. The clear cut difference from Christianity comes from the understanding of when life begins, and until then it is considered *potential* life when it's an embryo. This difference is the reason that ESCR has been allowed in Iran and Egypt.

Buddhists' Stance on ESCR

A main concept in a Buddhists' belief is the existence of reincarnation and the Beyond. According to Buddhism, three factors are necessary for the "rebirth" of a human being. The first condition is the need of a woman's egg, which is required for the formation of an embryo in the womb. The second condition is the need of a man's sperm. The last condition is the consciousness of "being ready for rebirth", led by karma-energy which is usually explained as "the soul" (Mahathera, 1994). Under the light of this information it can be seen that Buddhists

are mostly against *in vitro* fertilization, because of its unnatural way to obtain human embryos, thus they are clearly against ESCR.

In Buddhism, medical research for mankind's good health is perfectly accepted. But every action associated with "harm" tends to be morally opposed no matter the purpose of that action. Therefore, for the case of ES cell research in Buddhism, an embryo must be as respected as a regular adult should be (Chamany, 2004). In theory, the embryo might be the rebirth of an old soul, a new individual, so it must be respected as an adult. Destroying the embryo would be considered murdering a human.

The Parthenote Proposal

In an attempt to cool the stem cell debate, some researchers have offered imaginative new ways to obtain ES cells without the necessary step of destroying living human embryos. Four proposals have been floated in recent years, and each will be discussed. One of the earliest of these new proposals is the idea to create a parthenote—an egg that develops into an embryo without contribution by the sperm, and creates ES cells. New research has shown that a chemical trigger such as strontium chloride, can cause an egg to begin dividing and organizing—even eggs that have failed to be fertilized by a sperm. Reproductive clinics throw away thousands of eggs that have failed to be fertilized through multiple IVF attempts. Because mammalian parthenotes cannot develop very far due to the lack of paternal DNA, many researchers do not consider them embryos but "embryo-like" entities.

Method – Since eggs have only half of the requisite DNA, they would have to be obtained from women before the final maturation process (before ovulation) when the egg still has a full DNA

complement, or the eggs would have to copy their own 23 chromosomes to produce 46 chromosomes when exposed to a chemical trigger. Such a trigger or an electrical shock tricks the egg into believing that it has been fertilized, and calcium ions flood into the egg to stimulate it. Upon reaching the blastocyst stage, the parthenote would be broken apart and its ES cells harvested.

Technical Challenges – Because of the faulty genetic structure of parthenotes, there are questions about whether stem cells derived from them could be used for treatments. The impact of seriously genetically flawed stem cells is unknown. Incidents of cancer could be higher than the 25% typical when using other ES cells. In addition, the available pool of genotypes for research would be limited since only fertile females can be used.

Ethical Issues – The largest ethical issue with parthenotes is the question of whether a parthenote is an embryo, and there is little consensus. Some argue that it is not an embryo because it can never develop, while others hold that it should be treated like an embryo unless it can be proven otherwise. Currently, several labs have achieved success deriving ES lines from primate parthenote embryos, but only a few non-reproduced studies claim human parthenogenesis. The largest ethical dilemma of Parthenote is on the conception of whether parthenote is a embryo or not. This may be less ethically controversial because we can even use eggs that have failed to fertilize in vitro fertilization. These eggs are usually simply discarded, and would have had no mathematical chance to grow or become useful.

Some scientists claim that producing human embryos by this method can avoid the ethical problems that people have to regular ES cells (Latkovic, 2006). Because the embryo-like entity has no chance to survive, some ethicists agree that using parthenotes does not constitute murder. Buddhism and some fundamental (conservative) Christians continue to be against this method. Buddhism is against it because of its unnatural chemical stimulatino process. Conservative Christians oppose the idea because they accuse the scientists of tricking the naïve cells with evil chemicals to think they are fertilized.

The Morula Proposal

Reproductive Genetics Institute (RGI) in Chicago is one of the world's leading experts in pre-implantation genetic diagnosis (PGD)—a procedure where a cell is removed from an early developing embryo and analyzed. Some use this procedure to identify whether a developing embryo has a genetic disorder such as Tay-Sacs or Huntington's disease. Only those embryos passing the genetic test are implanted. The others are destroyed. Scientists at RGI are claiming a new distinction—a way around of the current objection to pursuing human ES cell research. Instead of destroying living human embryos, RGI scientists think they can use the same principles of obtaining early cells for PGD to develop ES cell lines.

Method – Scientists would take an early-embryo by IVF that has developed to about the 8-cell stage (called a morula), and remove a single cell. They would then attempt to coax that cell to replicate into an ES cell line. The embryo (less the one cell) could then be transferred to a womb, so it would not be destroyed.

Technical Challenges – The largest technical challenge to this proposal is getting a single early cell to replicate sufficiently to turn into an ES cell line. Currently, scientists wait until the blastocyst stage where the embryo has developed into several hundred cells, break the embryo apart to obtain the cells, and use all the available cells to create an ES cell line. Even with hundreds of cells, scientists have a difficult time creating cell lines. Doing so requires dozens if not hundreds of embryos. Robert Lanza at Advanced Cell Technology (ACT) in Massachusetts has said that he believes this single cell process can produce stem cell lines, but procedures do not yet exist (McConchie, 2005).

Ethical Issues – There are two primary ethical issues with this proposal. First, it requires a method that is potentially harmful to the embryo. While hundreds of children have been born using PGD, we do not yet know the long term consequence of taking a cell from the very early embryo. Second, at the morula stage, twinning is still possible; it is possible that the obtained cell could be an embryo itself—the single cell may be able to develop if implanted into a womb.

The Organ Transplant Proposal

50 years after the first successful organ transplant, Donald Landry and Howard Zucker of Columbia University in New York think that the same principles used today to harvest organs from those at the edge of death can be used to find a way out of the current ES cell morass. Modern organ transplant rules follow the following general principle: a person's body does not have to be totally dead for it to be "dead enough" to ethically remove vital tissues for transplant. Because the line between life and death is not precise, this principle has been accepted and is

used to allow a definition of death other than complete death of every cell in the body. This allows the transplantation of living tissue from an otherwise "dead" person.

In this proposal, scientists argue that embryos exist that are, in essence, dead just like those who are brain dead with functioning organs. The term "arrested development" is often used to denote embryos that are believed will never develop further. Landry and Zucker estimate that 60% of human IVF embryos in cryopreservation are in a state of "arrested development."

Method – Scientists hope to identify arrested development embryos created by IVF whose stem cells are functional, obtain the ES cells (using the standard method of breaking embryos apart), and develop stem cell lines for research and possible future treatment.

Technical Challenges – No test currently exists to determine whether an embryo that is not developing is truly dead. Landry and Zucker are working to develop tools to measure the chemical and genetic signatures of embryos after 24 hours of non-development. There is also a question about whether the ES cells obtained from such embryos would be as useful as ES cells derived from fertilized embryos. It is possible that failure to create stem cell lines from "surplus" IVF embryos is due to the failure of the cell from "dead" embryos to replicate.

Ethical Issues – Is it possible to identify a "death" criterion for embryos? This is uncertain, as there are no brain wave functions to assay. There simply is no test similar to that which determines human brain death. Chemical and genetic signatures would measure seemingly arbitrary criterion, particularly since we know so little about embryology (and especially compared to current understandings of a fully-developed nervous system that governs the brain

death criterion).

The Alternate Nuclear Transfer (ANT) Proposal

Suggested by Stanford physician and ethicist William Hurlbut, alternate nuclear transfer (ANT) is similar to cloning. Using the SCNT cloning method, scientists would create an embryo or "embryo-like entity" that lacks a key developmental gene. The created embryo would be similar to those that generally develop into a cancerous tumor—an entity that most scientists and ethicists consider never to have been an embryo.

Method – A developmental gene is turned off in the nucleus about to be transferred. Using the normal cloning process, the changed nucleus is then inserted into an enucleated egg, stimulated to divide, and stem cells are harvested when the resulting embryo or entity reaches the blastocyst stage.

Technical Challenges – Currently, the proposed method would be difficult and expensive; the difficulties of cloning are compounded by the difficulties of genetic alteration. It likely would be a number of years before this method was successful, and, due to the technical hurdles of genetic manipulation, cloning technology, and stem cell cultivation, even longer before it is reasonable.

Ethical Issues – The core question for most ethicists is whether the entity created is a non-embryo or a disabled embryo. Hurlbut suggests that because the entity lacks a developmental / organizational gene and could never develop, it is never an embryo, thus no embryo is destroyed. Others, such as Richard Doerflinger of the US Conference of Catholic Bishops, argue that if the

knocked out gene offers several days of development, the entity is an embryo for that period of time, and only later ceases to be such (McConchie, 2005). The debate as to of whether an embryo-like entity that cannot develop is an embryo is similar for both parthenotes and ANT (McConchie, 2005).

Chapter Conclusion

At the moment, Catholic Christians, Hindus and Buddhists oppose ESCR, but Muslim's do not. But due to the ES cell controversy, scientists are working hard to devise new ways to obtain ES cells that would not destroy an embryo so might be acceptable by Christians in order to make progress in ESCR and get viable federal funding. The hope is that one day ES cells will be able to improve the lives of many suffering individuals in our society.

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CONCLUSIONS

Based on the research performed for this IQP, the authors conclude that adult stem cells (ASCs) should be used whenever possible for medical applications in place of embryonic stem (ES) cells since ASCs do not destroy any embryos to obtain them. ASC research remains a very fruitful field of research, especially with the recent discovery of induced pleuripotent stem (iPS) cells whose potential appears so far to be almost as potent as ES cells. Further research in that area should most definitely be pursued, especially with federally subsidized research.

The authors also conclude that ES cell research, although highly controversial in nature, should also be pursued. There is still a lot that we do not know about stem cells that may be lost without further study. Furthermore, federal funding should not be limited to the few remaining ES cell lines available in accordance with our existing federal laws. At the very least, discarded excess IVF embryos should be made freely available for research with donor consent, and used to derive more ES cell lines. Lastly, we conclude that current U.S. federal stem laws are too strict, and should be expanded to include ES cells and research seeking alternative sources of pleuripotent cells.