UNIT 1 TEACHER BACKGROUND INFORMATION

Note: Terms that are bolded are defined in Glossary for teachers and students.

EMBRYONIC DEVELOPMENT

Day 0: Fertilization of egg with a sperm
D ~0.5: Genetic bundles called "pronuclei" from mom and dad fuse, forming the zygote
Day 1: ZYGOTE (a single cell) divides into 2 cells (EMBRYO)
Day 2: 4-cell EMBRYO
Day 3: 8-cell MORULA (*morula* refers to the berry-like shape of the early embryo)
Day 4: 16, 32, 64-cell MORULA
Day 5: Formation of hollow BLASTOCYST with embryonic stem cells and TROPHOBLAST
Days 5-14: Uterine implantation of blastocyst
Day 14: Gastrulation: three distinct layers begin to form (no more embryonic stem cells)
Days 14-21: Neurulation: beginning of future nervous system
Days 21-24: Beginning of future face, neck, mouth, and nose
Weeks 3-8: Beginning of organ formation (organogenesis)
Week 8: Now it's called a FETUS (definitions of this time-point vary, up to 10 weeks)

Natural Fertilization

Fertilization refers to the fusion of the egg (ovum) and sperm, and usually takes place in the upper third of the fallopian tubes. Once a month, a woman releases an egg during the process of ovulation, which is part of the menstrual cycle. Ovulation is triggered by the release of two hormones by the pituitary gland (a gland of the endocrine/hormonal system) and usually occurs during day 14 of an average 28-day cycle. In humans, the fertile phase, or time during which a woman can become pregnant, occurs during the few days near ovulation.

Following intercourse during this fertile phase, sperm travel from the vagina to the uterus and into the fallopian tubes. Several thousand sperm will reach the egg, out of the many million released during ejaculation, but only one will fertilize it. Once this sperm fuses with the egg, a series of chemical changes in the egg are instantly initiated that prevent any other sperm from entering the egg. The nuclei of the egg and sperm also fuse creating a "single" cell, now called the zygote (day 0). As the zygote travels down the fallopian tube towards the uterus, it begins a series of cell divisions:

Day 1: ZYGOTE divides into 2 cells (EMBRYO)

Day 2: 4-cell EMBRYO

Day 3: 8-cell MORULA

Day 4: 16, 32, 64-cell MORULA

Day 5: Formation of hollow BLASTOCYST and TROPHOBLAST

Between days 5 and 14: Implantation (the adhering of the blastocyst to the wall of the uterus) occurs. The placenta also begins to form from the trophoblast.

Handout: "Development and Implantation of the Embryo," which covers stages up to implantation.

After Implantation, the embryo continues to develop through the following stages:

Day 14: Gastrulation: three distinct layers begin to form

Days 14-21: Neurulation: beginning of future nervous system

Days 21-24: Beginning of future face, neck, mouth, and nose

Weeks 3-8: Beginning of organ formation (organogenesis)

Week 8: Now it's called a FETUS (although definitions of this time-point vary. Growth to the fetal stage can take up to 10 weeks.)

INFERTILITY AND TREATMENTS

Infertility is a disease defined by the failure to achieve successful pregnancy after 12 or more months of regular unprotected intercourse. There are many reasons why sex may not result in a viable pregnancy. Biological dysfunctions at the genetic, cellular, and systems level within a man, woman, or both can prevent sperm and egg from combining and developing into a healthy baby. A man might have a low sperm count (oligospermia) or produce semen without sperm; his sperm might be irregularly shaped and have motility problems; his sperm might have trouble attaching to the egg; or he might have a blockage in his reproductive tract that prevents ejaculation. A woman might not be producing an egg because she may not be ovulating properly, possibly due to abnormal hormone levels (estrogen, progesterone, or prolactin) crucial for maintaining a menstrual cycle and a pregnancy. Or, the egg might be blocked on its path from the ovaries through the fallopian tubes to the uterus. Blockages can be caused by inflammation in the fallopian tube due to menstrual **cytokines** (small molecules that cause an inflammatory response) or infection. Uterine scarring, cysts, or an abnormally-shaped uterus could prevent an embryo from implanting correctly. Additionally, even if sperm and egg are able to combine, a chromosomal abnormality (**aneuploidy** or **translocation**) in either or both gametes may disrupt cell division and prevent further development.

Environmental factors, notably cigarette smoke, contribute to infertility. The percentage of

women experiencing a 12 month (or more) conception delay was 54% higher in smokers than non-smokers. Chemicals in cigarette smoke have direct mutagenic effects (cause DNA damage) on egg cells. These chemicals have a variety of effects on female reproduction, such as a shorter menstrual cycle, accelerated follicular depletion, and earlier age of menopause. Male smokers experience a 22% average decrease in sperm production as compared to non-smokers. Smoking has adverse effects on sperm function as well. Second-hand smoke contributes to infertility almost to the same degree as first-hand smoke.

In vitro fertilization (IVF) is a procedure that couples may use when one or both of the partners are diagnosed with infertility. For couples in which one or both partners have a genetic disease, regardless of infertility, IVF can increase the chances of having a child without the disease. In the latter, early embryos created by IVF undergo a procedure called **Preimplantation Genetic Diagnosis (PGD)**, in which one **blastomere** (cell) is removed and its DNA tested for identifiable genetic disorders or chromosome abnormalities. "Disease-free" embryos can then be chosen for transfer into the uterus. Alternatives to IVF are adopting an IVF embryo from another couple, paying a sperm or egg donor to achieve in vitro fertilization with one parent's gametes, and adopting or parenting a child.

IN VITRO FERTILIZATION PROCEDURE

In vitro fertilization has several parts. Before the procedure begins, the parents are tested for infertility. Then, they must sign informed consent documents before they move on to the treatment. Egg maturation, egg collection, egg culture, sperm collection, in vitro fertilization, embryo culture, embryo selection, and embryo transfer are all part of the IVF procedure.

If a couple is investigating IVF as a treatment option, basic screening tests are performed to make sure the woman has a sufficient "ovarian reserve." Follicle Stimulating Hormone (FSH) produced by the anterior pituitary acts as a signal to the ovaries to produce the hormone Estrogen and mature follicles. Follicles are sacs of cells and fluid encompassing a single growing ovum. Mature follicles are about 20mm in diameter, the size of a small grape, before the primary follicle bursts, releasing the egg into the fallopian tube. Levels of FSH tend to increase as a woman gets older, as it takes more of this hormone to promote follicle development with age. Therefore, a doctor will test the level of FSH around day 3 (at the beginning) of a woman's natural menstrual cycle. A lower FSH level is better, as a higher level may indicate poor egg quantity and quality.

Another test for a woman's ovarian reserve (her reproductive potential) is the number of **antral** follicles remaining in her ovaries. The number of antral follicles strongly correlates with the number of primordial eggs, which have the potential to mature into ova ready for fertilization. The number of antral follicles can be determined by vaginal ultrasound, and having more follicles is better. Advanced age can contribute to a low ovarian reserve, and

test results indicating this may convince a doctor to discontinue the IVF procedure.

Tests performed on the man would indicate sperm count, motility, and **morphology**. If these factors were low or abnormal, a couple may opt to proceed with a modified IVF procedure called **Intracytoplasmic Sperm Injection (ICSI)**, explained below. Once these tests are complete, both partners must sign consent forms which clearly explain the risks of the medical procedures to them and their future child/children. Risks include Ovarian Hyperstimulation Syndrome, an exaggerated response to **exogenous gonadotropins** that can lead to serious and life-threatening illness; discomfort, bleeding, or injury to organs near the ovaries; pelvic infection which may lead to removal of infected ovaries or tubes; multiple pregnancy which can affect the babies' health; and **ectopic** pregnancy, where the embryo implants outside the uterus often leading to spontaneous abortion. Besides the physical risks, there are psychological risks that come with a failed procedure.

The entire IVF procedure, from the beginning of hormone treatments through embryo transfer, takes approximately 24 days. A regimen of hormone injections is designed to stimulate the woman's ovaries to overproduce mature follicles. These hormones include: agonists and antagonists of Gonadotropin Releasing Hormone (GnRH) that delay the LH surge and ovulation to allow time for many mature follicles to develop; FSH to stimulate the development of multiple follicles; and Human Chorionic Gonadotropin (hCG) to induce final maturation of the eggs. These **subcutaneous** injections are self-administered, totaling up to twice per day for 17 days. In addition estrogen and **progesterone** pills are taken longer-term to develop and maintain a uterine lining necessary for pregnancy.

After this ovarian stimulation, and once the eggs are mature, anesthesia is given and then an ultrasound-guided needle inserted through the vagina aspirates between 10 and 30 eggs from the mature follicles. On the same day, sperm is collected from the man. If his tests showed normal sperm counts, his semen is mixed with the eggs in a petri dish, fertilizing them. If his test results are abnormal, a doctor may use the ICSI procedure, where one robust sperm is sucked up into an injection pipette. While a holding pipette secures one egg in place, this "hand-picked" sperm is injected into the center of the egg. This is repeated for all eggs. ICSI successfully fertilizes 50-80% of eggs, but this procedure may damage the eggs, prevent the fertilized egg from dividing,

The long-term effects of ICSI on the health of children fertilized this way are unknown since the procedure was perfected in 1992. From this point on, fertilized eggs produced by *in vitro* ("within the glass") fertilization develop similarly to those produced by natural fertilization (*in vivo*, or "within the living organism," fertilization). The only difference is the environment supporting development from fertilized egg (**zygote**) to blastocyst. In natural fertilization, this environment is the fallopian tube and the uterus. With IVF, this environment is a petri dish filled with drops of culture medium that mimics the natural environment. The petri dish is placed within an incubator, keeping the developing embryos

at body temperature while they mature.

EMBRYONIC DEVELOPMENT AND TRANSFER

In both natural and in vitro fertilization, the genome of the zygote is a unique combination of the haploid nucleus from the mother's egg cell and the haploid nucleus from the father's sperm cell. On day 1 post-fertilization, the zygote divides (mitosis) into two cells. On day 2, each of those cells divides, making four identical cells. On day three, the cells divide again, making eight cells (and so on). At this stage of embryonic development, the embryo is called a **morula**. The cells in the morula continue to divide, and on day 5, fluid builds up inside the ball of cells causing it to be hollow. At this stage, the embryo is called a **blastocyst**. The outside layer of the blastocyst, called the **trophoblast** (or trophectoderm), is made up of cells that attach to the uterine lining and eventually become the placenta. Inside the hollow ball, a clump of cells called the inner cell mass will eventually become the fetus. These cells are called **embryonic stem cells**, and they are **pluripotent** because they have the potential to specialize into every mature cell type *in the adult*. **Totipotent**—a property of the zygote (day 1) and every cell existing through the morula (day 3)—cells have the potential to become ALL types of cells in the mature adult and in the trophoblast cells that develop into the placenta and extra-embryonic tissues. Thus, a totipotent cell could potentially make an *entire* human being, whereas a pluripotent cell could not because it has already specialized past the point of being able to form a trophoblast.

On day 5, the doctor chooses a few healthy-looking blastocysts out of the batch of 10- 30 blastocysts (from the 10-30 harvested eggs) and transfers them into the woman's uterus. Hopefully one (and preferably only one) will successfully implant into the uterine lining and continue developing. Typically, more than one embryo is implanted to increase the probability of a successful pregnancy and birth. However, there are specific guidelines for the maximum number of embryos to transfer because multiple pregnancies can develop if more than one embryo successfully implants and develops. Multiple pregnancies may cause harm to the fetuses and the mother.

According to the guidelines from the American Society for Reproductive Medicine, as the age of a woman undergoing IVF increases, the allowed number of embryos transferred increases. For example, women under 35 can have a maximum of one blastocyst transferred; women aged 35-40 can have two; and women over 40 can have up to three. Exceptions are if a woman has a poor prognosis—previous IVF attempts have been unsuccessful or the embryos are of poor quality—or if cleavage-stage embryos (up to the 8-cell stage) are used. In these cases more embryos (up to five in age 40+) can be transferred.

After the doctor has transferred the blastocysts, the leftover embryos are frozen at -80°C. In suspended animation, these frozen embryos can later be used by the couple if the pregnancy is unsuccessful or if they want to have more children. They are often discarded.

In a few cases, embryos are donated to another infertile couple for adoption. They can also be donated, with consent, to stem cell research. A scientist would aspirate the inner cell mass containing embryonic stem cells and plate them onto a petri dish to make an embryonic stem cell line. This destroys the embryo because it removes the pluripotent cells that would have developed into the fetus, leaving a shell of trophoblast cells that do not have the developmental capacity to replace the embryonic stem cells.

PREIMPLANTATION GENETIC DIAGNOSIS

Preimplantation genetic diagnosis (PGD) is a procedure that allows a couple with or without infertility to know if their children have a chance of inheriting one or both of the parents' genetic diseases. Huntington's Disease is a **neurodegenerative** disease passed dominantly on to the next generation. For a carrier of Huntington's (Hh, who would manifest the disease), there is a 50% chance this person's child would also have Huntington's. For two unaffected carriers of an **autosomal** recessive disorder, like Cystic Fibrosis, there is a 25% chance their child would manifest the disorder.

Using IVF with PGD, a couple can see if of their embryos are carriers of disease alleles and then choose which of the embryos they want to transfer. In this procedure, one blastomere (or in some cases two) is removed from an 8-cell embryo. This does not damage the totipotent embryo; it continues to divide normally, quickly replacing the missing blastomeres. To genetically test the cells, first Polymerase Chain Reaction would be used to amplify the amount of DNA for further analysis.

One of the following techniques can be used to screen for disease alleles: **heteroduplex analysis** or **Fluorescence In Situ Hybridization (FISH)**. In heteroduplex analysis, specific DNA fragments containing genes responsible for a disease would be amplified from the unknown test sample and a known normal sample. Then the two would be hybridized, or mixed together so they are allowed to bind. A control sample is also made, containing hybridized DNA from two known normal samples. Mutations in the unknown DNA would cause irregularities in the shape of the hybridized DNA. After running **DNA gel electrophoresis** on the test and control samples, a difference in migration down the gel due to the slight shape differences in samples would indicate the presence of disease alleles.

In the FISH technique, DNA probes attached to different-colored fluorescent molecules are allowed to bind to DNA sequences unique to each chromosome. These chromosomes can be examined under a fluorescence microscope for the number of each chromosome and for the presence of **translocations**. Down Syndrome is caused by the presence of three copies (trisomy) of chromosome 21, and can be identified by FISH.

ETHICS OF PREIMPLANTATION GENETIC DIAGNOSIS

PGD gives couples with genetic diseases a chance to decide the genetic fate of their child. However, this decision comes with ethical challenges. For example, although some parents with Down Syndrome may not want their children to inherit this disease, others feel that their condition gives them a unique and valuable perspective on life and thus they disagree with selection against an embryo with Down Syndrome or other genetic variables such as deafness.

Genetic "diseases" that society labels abnormal or that confer a disability can be thought of as natural biological variation, creating people with extraordinary characteristics and communities that support them. Conversely, the widespread ability to select for the genetic makeup of children could lead to a eugenic society (as in the movie Gattaca), filled with inherent inequalities. On the other hand, PGD gives people with genetic disorders a chance to have "biologically related" children who are free of that particular disease, which some might consider a human right. In the future, techniques may be developed to make it easier to screen for multiple disease alleles using the DNA from one or two cells, creating opportunities for parents to lead a preventative lifestyle and help their children to do so. For example, parent(s) with schizophrenia, a disease associated with multiple genes, may be able to screen for all of the genes previously identified to increase vulnerability to schizophrenia, like genes that control stress and neurotransmitter levels. This information could encourage parents to modify the child's lifestyle to decrease the chances of psychosis.

There is no conclusion on whether the PGD procedure damages the embryo. The chance of harm to the developing embryo is very small:: 0.6%; normally, taking a cell from the morula does not disturb development. The diagnosis error of PGD for detecting translocation is approximately 10%. This includes chances of false negative, false positive, no result, and mosaicism. A mosaicism is when cells in the embryo are heterogeneous in chromosomal makeup because, for example, of a translocation during the early cell divisions.

There is a chance that the tested cell does have the defect while the other cells don't (and vice versa). No single test can predict the risk of all gene variations in a child. Many conditions, such as those related to toxic exposure, are environmentally-based and are not detected with genetic testing. Additionally, in PGD there are risks to the mother inherent to the IVF procedure required for egg collection.

REVIEWING THE CHARACTERISTICS OF EMBRYONIC STEM CELLS

Human embryonic stem cells are pluripotent cells derived from the inner cell mass of an IVF blastocyst. They have the ability to divide symmetrically (common in cell culture) as well as asymmetrically. During **asymmetric division**, a stem cell splits into two daughter cells—one remains a stem cell (**self-renews**) and the other changes into a more mature cell (**differentiates**). Depending on outside signals, an embryonic stem cell can differentiate

into any of the 200+ cell types in the human body. This is medically important because scientists can direct the differentiation of these cells, using combinations of chemicals, to create any

In development, the presence of embryonic stem cells persists until **gastrulation**, the formation of three germ layers in a 14-day-old embryo, after which adult stem cells appear. Adult stem cells (explained in Unit 2) can still divide asymmetrically, but differ from embryonic stem cells in that they are lineage-restricted and can only become certain types of cells in the

Umbilical cord stem cells are a type of adult stem cell, but they may have some advantages over adult stem cells found in the tissues of the child and adult, with regard to reduced transplant immune rejection. Adult stem cells have incredible medical potential, as do embryonic stem cells, but come with fewer ethical concerns.

REFERENCES

Brian Vastag. "Merits of Embryo Screening Debated." *JAMA*. 2004;291(8):927-929. http://jama.ama-assn.org/cgi/content/full/291/8/927 **Pay to access.**

David I. Hoffman, M.D., Gail L. Zellman, Ph.D., C. Christine Fair, M.A., Jacob F. Mayer, Ph.D., Joyce G. Zeitz, B.Sc., William E. Gibbons, M.D., and Thomas G. Turner, Jr., M.S. "Cryopreserved embryos in the United States and their availability for research." Fertility and Sterility 79(5) May 2003. 1063-1069. http://www.fertstert.org/article/S0015-0282(03)00172-9/abstract **Free to subscribers.**

Elizabeth, Finkel. "Stem Cells Without Killing Embryos." *Cosmos Magazine* 2007 - No longer available. http://www.cosmosmagazine.com/news/1398/stem-cells-without-killing-embryos

"In Vitro Fertilization IVF Procedures & Process." *IVF – In Vitro Fertilization Procedures Step by Step.* 1996-2010. Advanced Fertility Center of Chicago, Web. 23 Jan 2010. http://www.advancedfertility.com/ivfprocedures.htm **Free.**

"IVF Stimulation Medication Protocols & Follicle Numbers." *Ovarian Stimulation Protocols for IVF.* 1996-2010. Advanced Fertility Center of Chicago, Web. 23 Jan 2010. http://www.advancedfertility.com/ivfstim.htm **Free.**

Klimanskaya, Irina, Young Chung, Sandy Becker, Shi-Jiang Lu, and Robert Lanza. "Human embryonic stem cell lines derived from single blastomeres." *Nature* 444 (November 2006): 481-485. http://www.nature.com/nature/journal/v444/n7118/full/nature05142.html **Pay to access.**

Marian D. Damewood. "Ethical Implications of a New Application of Preimplantation Diagnosis." *JAMA*. 2001;285(24):3143-3144. http://jama.ama-assn.org/cgi/content/full/285/24/3143 **Pay to access.**

Northwest Association for Biomedical Research. "The Science and Ethics of Stem Cell Research." http://www.nwabr.org/teacher-center/stem-cell-research#overview **Free.**

"Ovulation Problems, Fertility, and Infertility." *Infertility due to ovulation problems Anovulation – Egg release or ovulation disorders.* 1996-2010. Advanced Fertility Center of Chicago, Web. 23 Jan 2010. http://www.advancedfertility.com/anovulat.htm **Free.**

"Patient Fact Sheet: Genetic Screening for Genetic Birth Defects." American Society for Reproductive Medicine. 2005. http://www.asrm.org/uploadedFiles/ASRM_Content/Resources/Patient_Resources/Fact_Sheets_and_Info_Booklets/genetic_screening.pdf **Free.**

"Patient Fact Sheet: How Doctors Evaluate Infertility in Women." American Society for Reproductive Medicine. 2008. http://www.asrm.org/uploadedFiles/ASRM_Content/Resources/Patient_Resources/Fact_Sheets_and_Info_Booklets/InfertilityInWomen.pdf **Free.**

"Patient Fact Sheet: Intracytoplasmic Sperm Injection (ISCI)." *American Society for Reproductive Medicine.* Revised 2008. http://www.asrm.org/publications/detail.aspx?id=1389 **Free.**

"Patient Fact Sheet: Risks of In Vitro Fertilization (IVF)." American Society for Reproductive Medicine. 2007. http://www.asrm.org/publications/detail.aspx?id=1477 **Free.**

"Patient Fact Sheet: Smoking and Infertility." American Society for Reproductive Medicine. Nov 2003. http://www.asrm.org/publications/detail.aspx?id=3959 **Free.**

Practice Committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology.
"2008 Guidelines for gamete and embryo donation: A Practice Committee report." Fertil Steril. 2008;90:S30-44.
http://www.asrm.org/uploadedFiles/ASRM_Content/News_and_Publications/Practice_Guidelines/Guidelines_and_Minimum_Standards/2008_Guidelines_for_gamete(1).pdf **Free.**

Practice Committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology. "Blastocyst culture and transfer in clinical-assisted reproduction." Fertil Steril. 2008;90:S174-7.
http://www.asrm.org/uploadedFiles/ASRM_Content/News_and_Publications/Practice_Guidelines/Committee_Opinions/Blastocyst_culture(1).pdf **Free.**

Practice Committee of the American Society for Reproductive Medicine. "Definitions of infertility and recurrent pregnancy loss." Fertil Steril. 2008;90:S60.

http://www.asrm.org/uploadedFiles/ASRM_Content/News_and_Publications/Practice_Guidelines/Committee_Opinions/Definitions_of_infertility_and_recurrent.pdf **Free.**

Practice Committee of the American Society for Reproductive Medicine. "Intracytoplasmic Sperm Injection (ISCI)." Fertil Steril. 2008;90:S187. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6K-4TWWJPV-1B&_user=10&_coverDate=11%2F30%2F2008&_rdoc=1&_fmt=high&_orig=search&_sort=d&_docanchor=&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=364333bc7a6ff85fbcbb65e0c173191f
Pay to access.

Practice Committee of the American Society for Reproductive Medicine. "Ovarian hyperstimulation syndrome." Fertil Steril. 2008;90:S188-93. http://www.asrm.org/uploadedFiles/ASRM_Content/News_and_Publications/Practice_Guidelines/Educational_Bulletins/Ovarian_hyperstimulation_syndrome(1).pdf **Free.**

Practice Committee of the American Society for Reproductive Medicine. "Smoking and Infertility." Fertil Steril. 2008;90:S254-9. http://www.asrm.org/publications/detail.aspx?id=3959 **Free.**

Practice Committee of the Society for Assisted Reproductive Technology and the Practice Committee of the American Society for Reproductive Medicine. "Guidelines on number of embryos transferred." Fertil Steril. 2008;90:S 163-4. http://www.asrm.org/uploadedFiles/ASRM_Content/News_and_Publications/Practice_Guidelines/Guidelines_and_Minimum_Standards/Guidelines_on_number_of_embryos(1).pdf
Free.

Practice Committee of the Society for Assisted Reproductive Technology and the Practice Committee of the American Society for Reproductive Medicine. "Preimplantation Genetic Testing: a Practice Committee Opinion." Fertility and Sterility.90(2008): S136-143. http://www.asrm.org/uploadedFiles/ASRM_Content/News_and_Publications/Practice_Guidelines/Committee_Opinions/Preimplantation_genetic_testing(1).pdf **Free.**

"Pre-implantation Genetic Diagnosis (PGD)." *Bluegrass Fertility Center.* 2008. Bluegrass Fertility Center, Web. 23 Jan 2010. http://bluegrassfertilitycenter.com/pgd.htm **Free.**

"Sample IVF Calendar Schedule." *In Vitro Fertilization: IVF Sample Calendar – Step by Step.* 1996-2010. Advanced Fertility Center of Chicago, Web. 23 Jan 2010.

http://www.advancedfertility.com/sampleivfcalendar.htm **Free.**

Sermon, Karen. "Current Concepts in preimplantation genetic diagnosis (PGD): a molecular biologist's view." 2002. Human Reproduction Update, Vol.8, No.1 pp.11-20. http://humupd.oxfordjournals.org/cgi/content/abstract/8/1/11 **Pay to access.**

Thomson, James A., Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, and Jeffrey M. Jones. "Embryonic Stem Cell Lines Derived from Human Blastocysts." *Science* 282 (November 1998): 1145-1147. http://www.sciencemag.org/cgi/reprint/282/5391/1145.pdf **Pay to access.**