REPRODUCTIVE CLONING BACKGROUND INFORMATION

"Have you ever wished you could have a clone of yourself to do homework while you hit the skate park or went out with your friends?

Imagine if you could really do that. Where would you start?"

What exactly is cloning?

There are really three different types of cloning - DNA cloning or genetic engineering, reproductive cloning, and therapeutic cloning. We will spend a great deal of the semester discussing genetic engineering and we will discuss therapeutic cloning next week.

"Reproductive cloning is the creation of an organism that is an exact genetic copy of another. This means that every single bit of DNA is the same between the two!

You might not believe it, but there are human clones among us right now. They weren't made in a lab, though: they're identical twins, created naturally. Below, we'll see how natural identical twins relate to modern cloning technologies.

How is cloning done?

You may have first heard of cloning when Dolly the Sheep showed up on the scene in 1997. Cloning technologies have been around for much longer than Dolly, though.

How does one go about making an exact genetic copy of an organism? There are a couple of ways to do this: artificial embryo twinning and somatic cell nuclear transfer. How do these processes differ?
1. Artificial Embryo Twinning

Artificial embryo twinning is the relatively low-tech version of cloning. As the name suggests, this technology mimics the natural process of creating identical twins.

In nature, twins occur just after fertilization of an egg cell by a sperm cell. In rare cases, when the resulting fertilized egg, called a zygote, tries to divide into a two-celled embryo, the two cells separate. Each cell continues dividing on its own, ultimately developing into a separate individual within the mother. Since the two cells came from the same zygote, the resulting individuals are genetically identical.

Artificial embryo twinning uses the same approach, but it occurs in a Petri dish instead of in the mother’s body. This is accomplished by manually separating a very early embryo into individual cells, and then allowing each cell to divide and develop on its own. The resulting embryos are placed into a surrogate mother, where they are carried to term and delivered. Again, since all the embryos came from the same zygote, they are genetically identical.

2. Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer, (SCNT) uses a different approach than artificial embryo twinning, but it produces the same result: an exact clone, or genetic copy, of an individual. This was the method used to create Dolly the Sheep.

What does SCNT mean? Let’s take it apart:

Somatic cell: A somatic cell is any cell in the body other than the two types of reproductive cells, sperm and egg. These are also called germ cells. In mammals, every somatic cell has two complete sets of chromosomes, whereas the germ cells only have one complete set.

Nuclear: The nucleus is like the cell’s brain. It’s an enclosed compartment that contains all the information that cells need to form an organism. This information comes in the form of DNA. It’s the differences in our DNA that make each of us unique.
Transfer: Moving an object from one place to another.

To make Dolly, researchers isolated a somatic cell from an adult female sheep. Next, they transferred the nucleus from that cell to an egg cell from which the nucleus had been removed. After a couple of chemical tweaks, the egg cell, with its new nucleus, was behaving just like a freshly fertilized zygote. It developed into an embryo, which was implanted into a surrogate mother and carried to term.

The lamb, Dolly, was an exact genetic replica of the adult female sheep that donated the somatic cell nucleus to the egg. She was the first-ever mammal to be cloned from an adult somatic cell.

How can cloning technologies be used?

"If the low success rates can be improved (Dolly was only one success out of 276 tries), reproductive cloning can be used to develop efficient ways to reliably reproduce animals with special qualities. For example, drug-producing animals or animals that have been genetically altered to serve as models for studying human disease could be mass-produced.

Reproductive cloning also could be used to repopulate endangered animals or animals that are difficult to breed. In 2001, the first clone of an endangered wild animal was born, a wild ox called a gaur. The young gaur died from an infection about 48 hours after its birth. In 2001, scientists in Italy reported the successful cloning of a healthy baby mouflon, an endangered wild sheep. The cloned mouflon is living at a wildlife center in Sardinia. Other endangered
species that are potential candidates for cloning include the African bongo antelope, the Sumatran tiger, and the giant panda. Cloning extinct animals presents a much greater challenge to scientists because the egg and the surrogate needed to create the cloned embryo would be of a species different from the clone.

Another potential application of reproductive cloning is the creation of genetically modified pigs from which organs suitable for human transplants could be harvested. The transplant of organs and tissues from animals to humans is called xenotransplantation.

“For many years now scientists have been trying to genetically modify pigs so that they could grow organs suitable for transplant into humans - the procedure known as xenotransplantation.

There is currently a great shortage of organs from human donors, which could be solved by having a reliable supply from pigs.

Many people who need a kidney transplant have to spend years on dialysis waiting for a suitable organ to become available.

However the human immune system swiftly rejects tissue from ordinary pigs, because pigs produce a special sugar called alpha 1,3 galactose, which immune cells target.

The key to preventing this rejection is to remove from the pigs’ DNA a crucial gene which makes an enzyme that transfers this sugar to the surface of cells.

In 2002, two biotechnology firms, PPL Therapeutics in Scotland and Immerge BioTherapeutics from the United States, announced the creation of pigs missing one copy of the gene.

Now PPL has gone a step further by producing pigs lacking both copies. This is an important step because it allows for these pigs to reproduce with similarly modified pigs and produce offspring that have the potentially human-safe organs."
What animals have been cloned?

“Scientists have been cloning animals for many years. In 1952, the first animal, a tadpole, was cloned. Before the creation of Dolly, the first mammal cloned from the cell of an adult animal, clones were created from embryonic cells. Since Dolly, researchers have cloned a number of large and small animals including sheep, goats, cows, mice, pigs, cats, rabbits, and a gaur.

Hundreds of cloned animals exist today, but the number of different species is limited. Attempts at cloning certain species such as monkeys, chickens, horses, and dogs, have been unsuccessful. Some species may be more resistant to somatic cell nuclear transfer than others. The process of stripping the nucleus from an egg cell and replacing it with the nucleus of a donor cell is a traumatic one, and improvements in cloning technologies may be needed before many species can be cloned successfully.”

What are the Risks of Cloning?

“When we hear of cloning successes, we learn about only the few attempts that worked. What we don’t see are the many, many cloning experiments that failed! And even in the successful clones, problems tend to arise later, during the animal’s development to adulthood.

Cloning animals shows us what might happen if we try to clone humans. What have these animals taught us about the risks of cloning?

1. High failure rate

Cloning animals through somatic cell nuclear transfer is simply inefficient. The success rate ranges from 0.1 percent to 3 percent, which means that for every 1000 tries, only one to 30 clones are made. Or you can look at it as 970 to 999 failures in 1000 tries. That's a lot of effort with only a speck of a return!

Why is this? Here are some reasons:
• The enucleated egg and the transferred nucleus may not be compatible

• An egg with a newly transferred nucleus may not begin to divide or develop properly

• Implantation of the embryo into the surrogate mother might fail

• The pregnancy itself might fail

2. Problems during later development

Cloned animals that do survive tend to be much bigger at birth than their natural counterparts. Scientists call this "Large Offspring Syndrome" (LOS). Clones with LOS have abnormally large organs. This can lead to breathing, blood flow and other problems.

Because LOS doesn’t always occur, scientists cannot reliably predict whether it will happen in any given clone. Also, some clones without LOS have developed kidney or brain malformations and impaired immune systems, which can cause problems later in life.

3. Abnormal gene expression patterns

Are the surviving clones really clones? The clones look like the originals, and their DNA sequences are identical. But will the clone express the right genes at the right time?

One challenge is to re-program the transferred nucleus to behave as though it belongs in a very early embryonic cell. This mimics natural development, which starts when a sperm fertilizes an egg.

In a naturally-created embryo, the DNA is programmed to express a certain set of genes. Later on, as the embryonic cells begin to differentiate, the program changes. For every type of differentiated cell - skin, blood, bone or nerve, for example - this program is different.

In cloning, the transferred nucleus doesn’t have the same program as a natural embryo. It is up to the scientist to reprogram the nucleus, like teaching an old
dog new tricks. Complete reprogramming is needed for normal or near-normal development. Incomplete programming will cause the embryo to develop abnormally or fail.

4. Telomeric differences

As cells divide, their chromosomes get shorter. This is because the DNA sequences at both ends of a chromosome, called telomeres, shrink in length every time the DNA is copied. The older the animal is, the shorter its telomeres will be, because the cells have divided many, many times. This is a natural part of aging.

So, what happens to the clone if its transferred nucleus is already pretty old? Will the shortened telomeres affect its development or lifespan?

When scientists looked at the telomere lengths of cloned animals, they found no clear answers. Chromosomes from cloned cattle or mice had longer telomeres than normal. These cells showed other signs of youth and seemed to have an extended lifespan compared with cells from a naturally conceived cow. On the other hand, Dolly the sheep’s chromosomes had shorter telomere lengths than normal. This means that Dolly’s cells were aging faster than the cells from a normal sheep.

To date, scientists aren’t sure why cloned animals show differences in telomere length.

Should humans be cloned?

“Physicians from the American Medical Association and scientists with the American Association for the Advancement of Science have issued formal public statements advising against human reproductive cloning. Currently, the U.S. Congress is considering the passage of legislation that could ban human cloning.

Due to the inefficiency of animal cloning (only about 1 or 2 viable offspring for every 100 experiments) and the lack of understanding about reproductive cloning, many scientists and physicians strongly believe that it would be unethical to attempt to clone humans. Not only do most attempts to clone mammals fail,
about 30% of clones born alive are affected with "large offspring syndrome" and other debilitating conditions. Several cloned animals have died prematurely from infections and other complications. The same problems would be expected in human cloning. In addition, scientists do not know how cloning could impact mental development. While factors such as intellect and mood may not be as important for a cow or a mouse, they are crucial for the development of healthy humans. With so many unknowns concerning reproductive cloning, the attempt to clone humans at this time is considered potentially dangerous and ethically irresponsible.”

1. http://learn.genetics.utah.edu/units/cloning/whatiscloning/
Reproductive Cloning Questions – notes and reading
(10 pts)
1. Starting with frogs in 1952, many animals have been cloned using the embryo splitting method.
   a. In the space below, sketch how embryo splitting works.

b. When was Dolly cloned?

c. Dolly was cloned by SCNT. What does SCNT stand for?

d. Why is SCNT preferred over embryo splitting (hint: think about what is being cloned – the parents or the kids. Will you really know what you are getting?)

e. In the space below, sketch how SCNT works.

f. If other animals had been cloned prior to Dolly, why was Dolly such big news? (Hint: definitely only in reading, not notes)
2. Every year, thousands of people go on waiting list in hopes of obtaining a desperately needed organ transplant. Most of those people end up dying long before an organ becomes available. In order to provide more organs, cloning has been used to try to improve the process of xenotransplantation.
   a. What is xenotransplantation and why can’t these organs be used “as is”?

   b. Which organisms are often used in xenotransplantation? Why?

   c. These organisms needed to be modified by removing a gene from their bodies.
      i. Which gene and why?

      ii. Thinking back to last unit, what’s one possible method for removing this gene from the DNA sequence?

3. Reproductive cloning isn’t easy. In fact, Dolly was the successful result of almost 300 attempts.
   a. Besides low success rate, list and briefly explain three other risks of cloning:

      Risk #1 -

      Risk #2 -

      Risk #3 -
b. It’s no wonder with all of these potential problems that the scientists involved in developing the pigs for xenotransplantation were ecstatic to develop pigs with both genes removed. (Remember, each gene has two copies – one from mom and one from dad).
   i. What would happen if pigs with only one copy of that gene removed were allowed to sexually reproduce - would they produce offspring that had organs suitable for xenotransplantation? Why or why not?

   ii. Why was it such a big deal for that company to develop pigs with both copies of that gene removed?