



Readings II in Faith & Science

Publication Year: 2003

ID: BK023

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Science and Technology in Cloning From Sheep To Humans: What are the Possibilities of Human Cloning?

ID: BK023-045

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Introduction

The pace and scale of breakthroughs in mammalian reproduction and genetics in 1997 has stunned the world. First Dolly, then Polly and finally Gene demonstrated cloning both by nuclear transfer of cells from an adult animal as well as fusion of embryonic stem cells and fertilized enucleated ova to produce duplicate copies of domestic animals (sheep and cattle). Clearly, the cloning of humans (adult and embryonic) had become a real possibility. A ban on public funding for human cloning was quickly enacted by President Clinton. The Federation of American Societies for Experimental Biology (FASEB) followed with a voluntary 5-year moratorium on human cloning. However, in July, the culture of the first human stem cells was announced providing the evidence that cloning of humans was a step closer. Also, the rapid pace of various genome programs continued with the addition of the one billionth base to the National Genome Database. We are now collecting the genetic sequences of 25,000 different species of animals with no end in sight and the human genome data base is scheduled to finish essentially on schedule around 2005. These events have ignited a worldwide public debate over the scope and control of these technologies. The objective of this paper will be to describe these technologies and their development as well as the forces which have shaped them. The paper which follows discusses the ethical and moral issues raised.

History of Cloning

In biology, the process of cloning is the production of an exact copy of a genetic sequence or the production of an organism containing the same genetic sequence as the founder. Cloning a genetic sequence is a routine component of many molecular biology techniques. Cloning plants has become commonplace in many parts of agriculture. Cloning animals is a recent development but its roots extend back into several fields of agriculture and biology. In the context of agriculture the process of cloning is a logical outcome of the desire to obtain the highest quality plants and animals and to maximize their genetics. This desire is noted in Genesis 30:41-42 where Jacob uses a technique based on superstition to alter the coat color of the offspring from the best males and females but did not use this technique when the weaker males and females mated thereby imposing selection pressure on this uncle's flock. Since the offspring with imperfect coat color belonged to him he quickly built a flock of hardy animals and became wealthy.

It is generally believed that animals were first domesticated around 8000 B.C., but the process of domestication continues today. For example, numerous species of fish are being domesticated every year as populations of these species in the wild decline. Additionally, world population increases demand continued fish production via aquaculture. Domestication of plants and trees also continues. Availability of orchids has grown rapidly since the ability to clone them has developed and many forests are cultivated as a cloned crop rather than a wild assemblage. The process of domestication of some species and the exclusion of others has resulted in some describing nature as a "Social Construct" which is unique to each culture.

Although the process of domestication has been around a long time the use of production records to identify superior plants and animals did not occur until the 1800's. Artificial insemination of cattle was introduced in the 1940's which permitted use of only the best bulls in the national dairy herd. Today a few hundred "proven" bulls supply all of the semen used to inseminate 6 million dairy cows in the U.S. During the period that animal scientists were developing artificial insemination the concept of cloning embryos by transferring the nucleus from

one cell to another (nuclear transfer) was first proposed by Hans Speman, a German embryologist in 1938. In 1953 James Watson and Francis Crick first correctly deduced the double helical structure of DNA and set the stage for the coming biotechnology revolution. About the same time the first cloning attempts using nuclear transfer were taking place using amphibians. It was not until 1970 that these attempts were successful but the resulting tadpoles did not mature properly. By 1984, a live lamb had been produced by nuclear transfer using the cell from an early stage embryo. In 1994, Neal First, of the University of Wisconsin, cloned a mammal from an advanced embryo using nuclear transfer. In 1997, the first clone of an adult was achieved. Thus, in the short span of 30 years mankind had moved from cloning frogs to cloning adult mammals. This was the result of several powerful technologies developing simultaneously.

As animal scientists were refining methods for maximizing the use of the genetics of the male through artificial insemination, reproductive biologists began addressing the issue of maximizing the genetics of the female. Practically, a domestic female animal has only a handful of offspring. Even litter bearing species such as the pig rarely exceed 20 offspring per pregnancy limiting their impact on the genetics of a breed. Rapid genetic progress could be made if two breakthroughs could occur. If genetically superior animals could be identified at the embryonic level the generation interval would be eliminated, increasing the rate of genetic progress and, if those superior embryo's could be cloned to produce thousands of copies, the impact of a single female on a breed or species would be greatly magnified.

Additionally, the several decade effort to find a cure for cancer had resulted in a great increase in the understanding of the cell cycle and how cell growth is regulated. The discovery of enzymes which could cut DNA and repair it resulted in an explosion of research in molecular biology and the production of transgenic organisms. The increase in fertility research in humans increased interest in regulating reproductive function in a wide variety of species which provided models for humans. Finally, the human genome project began the first serious attempt to link human physical traits with underlying genetic differences. All of these forces have converged upon the process of reproduction in general and the embryo in particular.

Reproductive Technologies

Cloning is the outcome of many years of research into the regulation of reproduction. The interest in reproductive processes is directly influenced by both the need to reduce the rate of population growth of some species and improve the fertility of others (endangered species or domestic animals) This process involved understanding the factors governing the production of gametes (sperm and eggs), the culture and freezing of gametes for *in vitro* (outside the body) fertilization, gender (sex) selection, the culture of growing embryos and finally the ability to split and eventually clone embryos in order to multiply superior genetic stock. All of these technologies are evidence of our continued interest in controlling the world we live in.

Gamete Collection

When farmers began collecting records of the productivity of animals, (milk yield, growth rate, disease incidence) they gained the ability to identify superior plants and animals in their crops and animal herds. Subsequently, they needed to multiply the genetic makeup of superior animals or plants by maximizing their breeding. In animals, this is most easily done by maximizing the use of superior males. This is because the male produces sperm far in excess of those required for fertilization of a single or even 20 *ova*. Therefore, artificial insemination was developed in order to collect the sperm of superior males for breeding as many females as possible. This also required development of the ability to freeze sperm for transport around the world. Today, approximately 150 bulls supply the sperm required to mate approximately 6 million dairy cows. However, since most females produce only a small number of offspring in their lifetime there was no way to multiply the genetic makeup of the female except by selecting her male offspring for subsequent breeding. This problem is solved with embryo cloning which permits maximization of the genotype of the female as well as the male.

Early in cloning research, it became clear that widespread application of this technology would be very dependent on the production of low cost *ova* (eggs) for fertilization. In order to fertilize *ova in vitro* (outside the body) and subsequently clone the embryo the process of *ova* culture was developed. This permits the collection of *ova* from unovulated ovaries after removal of the ovary from the animal or by transvaginal needle aspiration. Transvaginal needle aspiration utilizes ultrasound to locate follicles on the ovary inside the animal and subsequent aspiration of the *ova* by inserting a needle through the vaginal wall into the follicle. Using this technique, fresh *ova* can be collected from animals on a weekly or biweekly basis. In order to obtain *ova* on such a short interval the normal cycle of ovulation in the female is accelerated using hormones which stimulate greater than normal number of follicles to develop so more than one *ova* can be collected. The *ova* collected must still undergo maturation *in vitro* to reach the point at which fertilization can occur. Research is still underway on the best method for culturing and maturing *ova in vitro* .

Gender Selection

In many domestic animal species there is a need for gender selection. For instance, in the dairy industry only a few young bulls are required each year for use in artificial insemination. The vast majority are raised as veal or dairy steers which represent much lower profit opportunity since the female can have several lactations, each producing more profit than a male calf or steer going to slaughter. Despite the fact that male calves in the dairy industry have little value to the producer compared to females, half of all calves born are male. Therefore, the ability to select embryos or sperm to produce only females has significant economic value. In the human population, there is interest in using gender selection of the offspring to avoid the occurrence of sex-linked diseases which occur only in one sex.

Gender selection can be accomplished by separating the x and y bearing sperm or by identifying the gender of the embryo and destroying the unwanted gender embryos. In order to identify x and y bearing sperm the semen is processed by flow cytometry which can identify the slight difference in sperm head size due to the x (larger) or y (smaller) chromosome. The y chromosome is smaller because it is missing one arm present in the x chromosome. This process is slow and time consuming and is used sparingly due to high cost. The alternative method is to remove a single cell from a multicellular embryo and determine the sex using genetic probes for the y chromosome. This process can also be used to identify the sex of a clone which need be done only once since all subsequent copies will be the same gender.

Embryo Culture

The successful production of *in vitro* produced (IVP) embryos is pivotal to the successful production of sexed, cloned and transgenic animals. The stages of cattle embryo development after hatching of the blastocyst are not able to occur *in vitro* and must occur *in vivo*. The cattle blastocyst hatches (breaks out of the protective covering called the Zona Pellucida) 8-10 days after fertilization and begins the process of elongation and development of the placenta. Despite several years of research the percentage of IVP embryos developing to the blastocyst stage has not exceeded an average of 26%. Thus the majority of IVT embryos are not viable for reintroduction to a host. Oocyte maturation involves two components, nuclear and cytoplasmic. Nuclear maturation is readily achieved during *in vitro* maturation (IVM) and it is generally regarded that cytoplasmic maturation is suboptimal and largely responsible for the low developmental rates during IVP. Typically *ova* and embryos are cultured in medium containing cells from the follicle or oviduct which secrete factors required for normal maturation. The embryo is undergoing rapid development and has hormonal and nutrient requirements that are typically supplied by the oviduct and uterus as it migrates into the uterus to implant or develop a placenta. If these hormonal and nutrient conditions are not supplied correctly the development of the embryo is affected and embryo death can occur.

Embryo Splitting

Embryo splitting is one way to exploit the superior genetics of animals. An assumption that is inherent in this approach is that one can identify superior animals at the embryonic stage. The procedure for embryo splitting is not complicated but requires delicate instrumentation. Using microdissection tools embryos are separated into two to four pieces. The split embryos are then transferred into the uterus of foster mothers for development. Animals that are produced from the same original embryo are identical twins and should be genetically equivalent. The highest success rate for the production of identical offspring occurs when embryos are split into halves, resulting in identical twins. Splitting embryos into four pieces can yield identical quadruplets, but the probability of pregnancy is much lower. Typically, the survival rate is less than half that of demi-embryos. Embryo splitting has several limitations, of which the most obvious is the low number of offspring resulting from the process. The second limitation is the fact that at the embryonic stage it is presently impossible to identify superior animals. The ability to clone animals that are adults and have proven their superiority is obviously much more desirable. However, until recently it was always believed that adult cells could not be reprogrammed to the embryonic stage.

Embryo Cloning

In 1986 a scientist at Cambridge University in England demonstrated that it was possible to produce clones by fusing a whole nucleated blastomere from a donor sheep embryo with an enucleated recipient oocyte. This process is called “nuclear transfer.” The nucleated recipient oocyte appears to have the ability to reprogram the donor nucleus and tricks it into beginning development as though it were a recently fertilized oocyte. The ability of the enucleated recipient oocyte to reprogram the nucleus from the blastomere is believed to be due to regulatory proteins in the cytoplasm which are taken up by the nucleus of the blastomere. However, the process required a nucleus from a early stage embryo. A nucleus from a later stage embryo would not successfully reprogram and it was generally believed that this was because the nucleus was differentiated and too far along the development pathway to be reprogrammed. Using this example it would appear to be impossible to reprogram a cell from an adult. Ian Wilmut of the Roslin Institute in Scotland proved this was wrong. He took mammary cells from an adult sheep and placed them into culture. He then starved the cells of hormones and nutrients required for growth and forced the cells into a stage of cellular development called G-0. In this stage there is no DNA replication going on since the cells have stopped dividing. When he transferred nuclei of these cells into a recipient fertilized and enucleated oocyte he successfully reprogrammed the nucleus from the adult animal to initiate embryonic development from the very beginning. He then inserted the developing embryo into a recipient animal and Dolly, the first clone of an adult animal became a reality. However, the success came after 277 failures and the process remains difficult.

The process of nuclear transfer now imparts the ability to clone adult animals but the numbers of animals that can be produced still remain relatively low. An immortal cell line could potentially produce unlimited numbers of cells for nuclear transfer as well as genetic manipulation. Recent developments have also occurred in this area as stem cell populations have been identified for several species including humans. Stem cells are undifferentiated immortalized cells which can provide the unlimited supply of nuclei for nuclear transfer. Therefore, the basic tools to produce a cloned population of animals exist. However, similar to the issue with splitting embryos, the stem cell population cannot be classified for its genetic value unless it comes from an adult which has already proven its genetic worth. Presently, stem cell populations are primarily from embryonic sources. These embryos are of unknown genetic value. Since it does no good to clone an animal unless it is superior, the value of the process is doubtful. Cloning adults or embryos that are transgenic and therefore of increased value are more valuable to the animal industry than producing copies of embryos of unknown value.

Genomic Technologies

Genomic technologies produce information relating the genetic makeup of an organism with its physical performance in a given environment. The human genome project is presently mapping the location of all human

genes which will permit identification of genetic bases for human diseases. Similar programs are underway for many domestic plant and animal species in order to better understand the relationship between an animal's genetic makeup and its productivity. Location of the genes which regulate animal growth, milk yield as well as plant production traits will allow improved genetic progress in identifying superior plants and animals. Location of genes will also permit moving certain genes between species in order to confer new properties to a plant or animal. Examples would be improved nitrogen fixation in plants, improved milk composition in cattle, improved food quality and improved pest resistance in plants. These are powerful technologies that go hand in hand with reproductive technologies to manipulate the genome for improved performance.

Chromosome Mapping

Most mammals have about 70,000-100,000 genes arranged along structures known as chromosomes. Each gene codes for a specific protein and each protein has a specific function at some point in the animal's life cycle. At any point in time only a portion of the genes are "expressing" their proteins. The code in each gene is made up of Deoxyribonucleic acid (DNA) which is composed of 4 nucleic acids or nucleotides. The sequence of nucleotides within DNA is called the genotype. The genotype varies across individuals within a species by slight variations in the sequence of nucleotides. This genotypic variation leads to phenotypic (outwardly visible characteristics) variation among animals. Phenotypic variation might be observed as differences between animals in coat color, milk yield, growth rate, behavior and thousands of other observable traits. The variation in sequence of some genes are not economically important. However, genes associated with disease resistance, milk production, growth rate, reproductive performance, to name a few, have great economic value to livestock producers. Therefore, mapping chromosomes to identify the exact location of genes and their sequence of nucleotides holds great promise for agricultural production as well as disease prevention and treatment in humans. However, the task of obtaining the entire genetic sequence of a species (genome) is a monumental task. The average genome of a mammal is about 6 billion nucleotide pairs. Obviously, the size of work involved is too large to obtain the genome map of many species. Therefore, the scientific community has focused on a few major species of interest. The genome with the highest priority is the human genome. Other genomes being studied include cow (bovine), pig (porcine), sheep (ovine) as well as several bacterial genomes. This list is not inclusive and presently partial sequences on 25,000 species exist in the National Genome Database (NGDB). Additionally, since sequences for given genes are highly conserved the human genome data base will provide a template for identification of similar genes in other species.

Generally there are two types of genetic maps; physical and recombination. Physical maps provide the location and order of genes on chromosomes by the physical assignment of genes to chromosomal segments.

Marker Identification

As the process of gene mapping goes forward, large pieces of chromosomes are identified by gene sequences with unknown function. These sequences are referred to as markers and permit the identification of areas of a given chromosome containing gene sequences associated with important traits. An example would be growth rate which is a quantitative trait meaning it can be measured. The location of a gene sequence containing a gene(s) controlling a quantitative trait is called a quantitative trait loci (QTL). As gene mapping progresses the QTL's of important traits are localized. The next task is to identify the specific genes within the QTL's which code for a given quantitative trait.

Mapping Quantitative Traits

Chromosomal locations are assigned to markers after they are identified. This is accomplished by a combination of approaches involving linkage analysis of pedigrees and somatic cell hybrid analysis. The goal of these procedures is to develop a genetic map that has markers at regular intervals throughout the genome. Initial genetic maps contain 400-500 markers. The distance between these markers is about 10 centimorgans or 1 million nucleotide base pairs. After the location of a specific QTL is identified the scientists can concentrate their gene sequencing

efforts on that piece of chromosome and eventually identify the specific genes involved. Once the QTL's are mapped down to specific genes, the pace of genetic progress will increase, since the generation interval will be eliminated and animals will be selected while they are at the embryo level of development.

Transgenesis

The objective in transgenesis is to alter function of the resulting offspring in order to produce novel proteins of significant economic value, to cure genetic diseases, to improve livestock productivity or to produce organs for human transplants. A transgenic animal is an animal that has a modified gene inserted into its DNA. This modified or foreign gene is called a transgene. Transgenesis requires functional knowledge of the gene of interest and the ability to manipulate the embryo in order to insert the transgene and to obtain functional expression of the molecule of interest when needed. This last component has remained a significant challenge, since success in directing the insertion of the transgene and its expression is problematic for several reasons.

Transfer of foreign genes into animals is done at an early stage of embryonic development (one cell to blastocyst stage) prior to implantation or placentation. Embryos at this stage of development can be grown outside the uterus of the mother (in vitro) in specialized medium containing nutrients and growth factors required for their development. For best results, micromanipulation and gene transfer are performed on one-cell embryos because integration of the transgene into the DNA of a one-cell embryo theoretically assures that all cells of the adult animal carry the foreign gene.

Transgenes As Bioreactors

Several species of animals have potential as sources of pharmaceutical proteins for treatment of human disease. In fact, the first cloned animals were produced for this purpose. Creating a transgenic animal is an expensive process and once created it has considerable value. Therefore, cloning an adult transgenic animal has great commercial potential. Recently, PPL Therapeutics, the company responsible for the cloning of Dolly, reported the first successful production of transgenic clones using nuclear transfer of cells from an adult which will produce Human Factor TX for treatment of Hemophilia. The potential for several other types of clones is also apparent. These include cloned cattle producing infant formula with human proteins instead of cattle proteins, pigs with hearts available for human transplantation that would be immunologically acceptable to humans and not cause transplant rejection, milk from cattle and sheep that contain various pharmaceutical peptides to name a few. Transgenes can also be produced to model human diseases such as cystic fibrosis and AIDS in order to develop new treatments.

Summary

The rapid progress in the fields of molecular biology, reproductive technologies and computer technologies have resulted in significant new issues for ethical and moral consideration. Clearly, these technologies are applicable to humans and any other mammal. They are powerful tools capable of redefining any species or to create new species and to clone thousands of copies. The opportunities to improve food production, develop new treatments for disease and to provide new and healthier foods to a growing population are considerable and essential if we are to support a doubling of the planet's population in the next 30 years.

However, these technologies will also provide new issues for society at large. The first public debate over reengineering the human species has already begun. As the human genome project progresses we will learn more about the genetic basis for personality, behavior and mental ability. Will we use this information wisely or will it be used to identify a new elite class and a lower class of people based on genetic makeup. Although there is a Federal ban on human cloning, there is nothing to prevent private money to be used for this purpose. These issues are serious ones which require our full attention as a society.

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Questions

Discuss the "pros" and "cons" of cloning human beings in the future. What are some of the ethical problems associated with cloning human beings?

Is there any real benefit to cloning animals? Discuss the limitations versus the expanded possibilities of cloning certain farm animals, for example. Has the cloning of animals for human consumption helped to stem the tide of hunger around the world?

The author states, "Thus, in the short span of 30 years mankind had moved from cloning frogs to cloning adult mammals. This was the result of several powerful technologies developing simultaneously." Discuss some of those biotechnologies that had developed at the same time, for example, those related to molecular biology and genetics.

The reproductive technologies assist human kind in controlling their "environment" (used loosely). What are some of the dangers associated with technologies that control reproduction in animals and human beings? What are some of the benefits associated with the use of such technologies?

The author states, “As the Human Genome Project progresses we will learn more about the genetic basis for personality, behavior and mental ability” What could that knowledge lead to? Betterment of humankind? Discuss the place of “eugenics” in the expanding role of the biotechnologies within the past 15-20 years. What do we mean by “eugenics”?

What role should the Government have in the regulation of cloning either animals or human beings? Is it ethical to clone a human being? Who should pay for it if it indeed is finally accomplished? Who would benefit from such procedures?

Should insurance companies have access to medical records of the genetic makeup of present or future policy holders? What are some of the ramifications of such access?

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