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Advances in Animal Biotechnology

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Glossary

Clone A cell, group of cells, or organism that is produced asexually from and is genetically identical to a single ancestor.
Genetic marker Gene or other identifiable portion of deoxyribonucleic acid (DNA) whose inheritance can be followed.
Marker-assisted selection (MAS) The use of DNA markers to improve response to selection in a population. The markers will be closely linked to one or more target loci, which may often be QTL.
Nuclear transfer A laboratory procedure in which a cell's nucleus is removed and placed into an oocyte with its own nucleus removed so the genetic information from the donor nucleus controls the resulting cell. Such cells can be induced to form embryos.
Quantitative trait loci (QTL) Stretches of DNA containing or linked to the genes that underlie a quantitative trait.
Quantitative traits Traits that vary continuously and are affected by multiple genes or loci. Examples include height and weight.
Recombinant DNA A m1olecule formed by joining DNA of interest to vector DNA.
Single nucleotide polymorphism (SNP) A DNA sequence variation at only one base pair between synonymous pieces of DNA.
Transgenics Organisms that have foreign DNA stably integrated into their genome.
Xenotransplantation The act of transplanting or grafting tissue or organs from an individual of one species into an organism of another species, genus, or family. A common example is the use of pig heart valves in humans.

What is Animal Biotechnology?

Animal biotechnology is any technological application that utilizes animals to make or modify products. The practice of animal biotechnology began more than 8000 years ago when humans began domesticating and selectively breeding animals. The modern era of animal biotechnology arrived following the discovery of the genetic code in the mid 1950s. Today new tools including increased computing power, genomic sequencing, cloning, regenerative medicine and direct gene insertion, and manipulation have given people the potential to dramatically alter animals for a broad range of purposes, including food production, medical, and scientific research. Modern biotechnology represents the intersection of man's manipulation of the environment and the emergence of molecular and computing technologies. These advances, as well as the US Supreme Court ruling that designed life could be patented, have spawned new ways of expediting the use of animals in serving society.

Earliest Animal Biotechnology

Prehistoric humans were originally hunter-gatherers who nourished themselves by following the migration of animals and ripening of foods such as wild fruits and berries. Hunter-gatherer communities could not support high population densities in part, because food resources were not steady or predictable. It is believed that the end of the ice age approximately 10 000 BC created conditions suitable for the transition from a hunter-gatherer lifestyle to farming communities. This transition, known as the Neolithic Revolution, marked the beginning of early agriculture. The Neolithic Revolution is believed to have occurred independently in seven to nine major centers, including Mesopotamia, China, Mesoamerica, and East and West Africa (Von Baeyer, 2010). Most accounts identify Mesopotamia, also known as the Fertile Crescent, as the origin of early agriculture. Owing to its impact on civilization, the transition from hunter-gatherer to farmer has been described as the most important technological development

Table 1 Domestication centers of the world

Center of domestication	*Dates (years ago)*
Near East/'Fertile Crescent'	11 000
Northern China	9 000
Southern China	8 000
Central Mexico	5 750
Peruvian Andes	5 250
Papau New Guinea	6 000–9 000
West Africa	4 500
Eastern North America	4 000

Source: Origins of agriculture by Lewis Foote on Prezi. Available at: prezi.com/uczfibsijcj7/origins-of-agriculture/ (accessed 06.05.13).

ever to occur in human history. By becoming farmers, humans were able to gather in greater numbers, have better and more consistent nutrition and develop technology. **Table 1** shows the major centers of domestication in the world.

Scientists believe crop domestication preceded the earliest domesticated animals by approximately 1000 years. In East Asia, rice, millet, and soy were domesticated; in sub-Saharan Africa, millet, sorghum, and African rice were domesticated; and in the Americas potato, sweet potato, corn, squash, and beans were domesticated (Floros *et al.*, 2010). The domestication of cattle, sheep, and goats took place 8000, 11 000, and 10 000 years ago, respectively, whereas buffalos, horses, asses, and camels were domesticated approximately 5000 years ago (Hirata, 2004). These animals were considered suitable for domestication because of their docile temperament, willingness to be dominated, and ability to live in large groups.

As humans became sedentary, they began caring for and controlling wild animals and plants for food production, transportation, protection, production of valuable commodities (cotton, silk, or wool), warfare, and companionship. Domesticated animals that we commonly use today, including dogs, cats, sheep, geese, camels, cattle, pigs, and horses started as wild animals but were changed over time through domestication practices (Zeder *et al.*, 2006; Andersson, 2011).

Domestication is not an instantaneous event. It is a cumulative process characterized by changes in which partner populations become interdependent over time (Zeder *et al.*, 2006). This process is also shaped by the particular environmental, biological, and behavioral profiles of the target species, as well as the cultural context of the human societies involved. The typical changes caused by the domestication process can be external or internal morphological changes, such as modifications in body size, decreased brain size, physiological changes, developmental changes, and behavioral changes, such as reduced fear (Jensen, 2006).

Although domestication initially had a small influence on the economies of human societies, which were originally based on hunting and gathering, it enabled these societies to grow in size and to expand into new and more-challenging environments. For example, the domestication of plants and animals enabled human population to grow by providing a food surplus. Moreover, domesticated dogs and sheep enabled human societies to become pastoral. Farming and raising livestock permitted the creation of permanent communities in place of the temporary ones prevalent in migratory hunter-gatherer groups, and the building of permanent shelters to house livestock and store harvested crops. In addition, new farming tools and technologies were developed once people started to grow their own crops (Zeder *et al.*, 2006; Jensen, 2006).

Fewer than 20 animal species have been successfully domesticated (Diamond, 1997), only 7 of which (cats, dogs, cattle, sheep, goats, pigs, and horses) are found worldwide. As pointed out by Hale (1969) and Diamond (1997), animals that have been successfully domesticated and farmed share and exhibit a unique combination of characteristics. They are relatively docile, flexible in their dietary habits, grow, and reach maturity quickly on an herbivorous diet, and breed readily in captivity. They also have hierarchical social structures that permit humans to establish dominance over them and are adapted to living in large groups. They do not include species that generally have a tendency to be fearful of humans or disturbed by sudden changes in the environment. Our ancestors no doubt based their selection methods for improving their herds and flocks on how easy the animals were to farm, as well as on potential agricultural value. In turn, the animals adapted to thrive in a domesticated environment.

Dogs (*Canis lupus familiaris*) were the first animal species to be domesticated, probably in East Africa and Asia. According to archeological evidence, dogs first began to show differences in appearance compared to wolves approximately 15 000 BCE. Many researchers believe that dogs essentially domesticated themselves by scavenging near human camps. Humans then bred them to bark in warning and for reduced aggression compared to wolves (Gray *et al.*, 2009; Skoglund *et al.*, 2011).

Sheep (*Ovis aries*) were probably first domesticated approximately 15 000–11 000 BCE. Their remains have been found at a wide range of sites of early human habitation in the Middle East, Europe, and Central Asia (Chen *et al.*, 2006; Chessa *et al.*, 2009). According to deoxyribonucleic acid (DNA) and mitochondrial DNA (mtDNA) studies of European, African, and Asian domestic sheep, it is believed that they descended from at least three different subspecies of the wild mouflon (*Ovis gmelini* spp.) and that there are three major and distinct lineages: Type A or Asian, Type B or European, and Type C, which has been identified in modern sheep from Turkey and China. Initially sheep were domesticated mainly for meat production. Later, these animals were also used to provide milk, wool, and leather. Nowadays, sheep continue to be important agricultural animals, as well as model organisms for scientific research (Chen *et al.*, 2006; Chessa *et al.*, 2009; Pedrosa *et al.*, 2005).

Goats (*Capra hircus*) were domesticated for their milk and meat, as well as materials for clothing and building (hair, bone, skin, and sinew). Their dung was also used for fuel. They are thought to have been domesticated in Iran and neighboring countries approximately 10 000–11 000 BCE. Recent mtDNA research has shown that all modern goats probably descended from a wide range of animals and may have been domesticated in a variety of different places (Fernández *et al.*, 2006; Luikart *et al.*, 2001).

Pigs (*Sus scrofa domesticus*) have mostly been domesticated for meat production; however their bones, hide, and hair are also used for items such as weapons and brushes. Domestic pigs, especially pot-bellied pigs, are also kept as pets. Archeological studies have shown that the domestic pig was domesticated from wild boars approximately 13 000 BCE in

the Tigris basin. However, remains of domesticated pigs have been found in southeast Anatolia dated to earlier than 13 000 years BCE (Vigne *et al.*, 2009). According to DNA evidence from Neolithic pigs, domesticated pigs were brought west to Europe. Zooarchaeological evidence suggests the domesticated pig was also brought east to China from the Near East, in addition to a separate domestication in China that took place approximately 10 000 years ago. These findings have led to the conclusion that pig domestication occurred independently in several places across Eurasia (Larson *et al.*, 2007; Chen *et al.*, 2007).

Cattle (*Bos primigenius*) have been domesticated since at least the early Neolithic for their meat, milk, leather, dung for manure or fuel, and for use as load-bearers and to pull plows. According to archeological records and modern genetics research for the domestication of wild forms of cattle, the process occurred independently from as few as 80 aurochs (the now-extinct predecessor of cattle) in Mesopotamia approximately 10 500 years ago near the villages of Çayönü Tepesi in southeastern Turkey and Djade al-Mughara in northern Iraq (Allan and Smith, 2008; Ajmone-Marsan *et al.*, 2010; Beja-Pereira *et al.*, 2006).

In addition, several other animal species also went through a process of domestication, such as farmed fowl (chickens, geese, and turkeys), horses, aquatic animals, and some insects. All of them, like those mentioned above, are of great importance to humans, providing products and inputs used routinely.

Assisted Reproductive Technology

Since animals were first domesticated, many technologies have been developed to select for desirable qualities, make breeding easier, and make animals produce more offspring. Many of those technologies, including artificial insemination, *in vitro* fertilization (IVF), embryo flushing, and cloning, involve the manipulation of animal reproduction.

Artificial Insemination

History of artificial insemination

Artificial insemination refers to the introduction of semen and viable sperm into the female reproductive tract via artificial means. Lazzaro Spallanzani, a French physiologist, was the first person to successfully demonstrate artificial insemination in animals, when he artificially impregnated a dog in 1784. However, the use of artificial insemination for commercial purposes began in 1937, when the first artificial insemination cooperative was established in the US. Artificial insemination is still widely practiced today; approximately 60% of dairy cows in the US are bred by artificial insemination.

Spermatozoa extraction and storage

Although many different animals require different methods of artificial insemination, the basic premises remain the same. First semen must be extracted from the male. There are a variety of extraction techniques; however, most often a mechanical breeding mount containing an artificial vagina is used. In the case of dairy cattle, the bull is allowed to first mount a live cow, which is known as the teaser animal. The bull is allowed to repeatedly mount the teaser animal without ejaculating. After a few live mounts, the bull is now directed to an artificial vagina and ejaculation is allowed to take place. The teaser animal serves to increase the amount of viable sperm per ejaculation. After ejaculation, the sperm is collected and sperm sorting may be applied. The sperm is sorted into a male and female population by a flow cytometer, and is typically 90–98% accurate for most breeding species. Sperm sorting is primarily reserved for industries where one sex is more valuable, such as the dairy industry where females are required for milk production. Because one ejaculation contains exponentially more sperm than is necessary for fertilization, an extender solution is added to the semen for dilution and freezing purposes. Depending on the fate of the semen, the extender can be composed of a variety of ingredients. Extenders typically contain milk or egg yolk to protect against cold shock, cryoprotectants such as glycerol, buffers to protect against pH changes, and energy sources for the sperm such as glucose. It is also common for extenders to contain antibiotics to protect against contamination. Once the extender is added to the semen, the semen is frozen down in multiple plastic tubes known as straws. The straws are stored in liquid nitrogen at −196 °C until they are needed for insemination. These advances in cryopreservation of semen have greatly advanced the practice and prevalence of artificial insemination.

Insemination procedure

The female's estrous cycle must be continuously monitored to detect when the animal reaches her estrus phase and thus is ready for the insemination procedure. This estrus phase is also referred to as the 'heat phase' of the female because she is sexually receptive to males. Many behavioral and physical signs indicate the animal is in estrus. The most prominent sign is the standing position the animal assumes, which is referred to as 'standing heat.' This is a natural position the female assumes to be mounted by the male. Other physical indicators of estrus are swelling and reddening of the vulva, discharge of mucous from the vagina, and increased affectionate behavior toward other animals. Because ovulation occurs at the end of the estrus phase, the most efficient and effective time for sperm deposition is 12–26 h after the onset of estrus. This ensures that the sperm are viable in the uterus before ovulation occurs, which leads to a higher conception rate. To make breeding more efficient and simple, many cattle farmers practice estrus synchronization. Estrus synchronization is the practice of synching a female population's estrus cycles through the injection of natural and artificially synthesized hormones. Once the animal is ready for insemination the sperm must be properly thawed and loaded into the insemination catheter or gun. For most species, the sperm should be thawed to 36.7 °C for optimal results. It is also crucial that the sperm not be thawed for more than 10 min, as exceeding this threshold leads to infertile sperm. There are a variety of insemination techniques, and the ideal location for sperm deposition varies between species. Generally, depositions in the uterus lead to a higher conception rate versus deposition in the vagina and cervix (Dalton, 1999). Transcervical insemination is a common technique used among many animals and is

preferred because it does not require surgery. Transcervical insemination utilizes an endoscope to locate the cervix and then a catheter is passed through the cervix into the uterus for sperm deposition.

Advantages of artificial insemination

Artificial insemination has opened numerous doors for animal biotechnology in the past 80 years. Perhaps the greatest advantage conferred by artificial insemination is the ability to quickly pass desirable traits to many offspring. Artificial insemination is also extremely cost-efficient, as sperm can be collected and shipped all across the world. This reduces the need for many breeding grounds to house and maintain male animals. Not only does this reduce costs, but it also provides a safer environment because males can be aggressive and become a safety threat. Other advantages include higher conception rates, the elimination of many genital diseases, and more comprehensive records of animals.

In Vitro Fertilization

History of in vitro fertilization

IVF refers to the fertilization of an ovum by a spermatozoon outside of the body. Research on the possibilities of animal IVF began in the late 1800s, whereas the first attempts at animal IVF began in the early 1930s (Bavister, 2002). The first attempts used rabbit oocytes and spermatozoa, but were unsuccessful. In 1951 the discovery of sperm capacitation by researchers Austin and Chang explained why those initial experiments failed: Spermatozoa need to develop and undergo changes in the female reproductive tract before fertilization can occur (Bavister, 2002). This discovery enabled Chang to successfully fertilize rabbit oocytes *in vitro* using sperm that was capacitated *in vivo*. This led to the first mammalian IVF birth, a rabbit born in 1959 (Brackett, 2001). However, it was approximately 20 years later when the first successful mammalian IVF was performed using spermatozoa capacitated *in vitro*. Today, IVF is still being heavily researched and the full extent of its promises for animal biotechnology has not been reached. However, it has already greatly increased researchers' knowledge of animal reproductive mechanisms. Commercially, the bovine industry has seen the greatest impact from IVF. Hundreds and thousands of bovine embryos created via IVF are sold and exchanged worldwide each year.

Oocyte extraction and fertilization

Like every assisted reproductive technique, the actual mechanics will vary among species. Regardless of which technique is employed, five basic steps define IVF. They are (1) superovulation of the oocyte donor, (2) immature oocyte collection, (3) oocyte maturation *in vitro*, (4) mature oocyte fertilization, and (5) embryo development and growth *in vitro*. Superovulation is achieved in the donor animal through the injection of gonadotropins. Immature oocytes are typically collected in the form of cumulus–oocyte complexes (COCs) either from a live animal or from the ovaries at a slaughterhouse. If the immature oocytes come from a live donor animal, this is most often performed by a procedure called transvaginal oocyte recovery. Transvaginal oocyte recovery is a nonsurgical technique that employs an ultrasound probe, a vacuum pump, and a needle aspiration system to collect the COCs. Once the COCs are collected, they are placed into an oocyte maturation medium, which can contain numerous hormones and other reagents. This medium mimics the *in vivo* environment that induces meiosis of the oocytes, thus arresting them in metaphase II and preparing them for fertilization. Once the oocytes have matured, they are ready for fertilization. The oocytes must be washed before fertilization to ensure that all hormones and unwanted reagents from the maturation medium are removed. Likewise, the spermatozoa must also be purified from the extender and cryopreservation reagents if it came from a frozen straw. Finally, the spermatozoa are screened to ensure they are motile, and a capacitance-inducing medium is added to the spermatozoa. The prepared spermatozoa can be added to the mature COCs to initiate fertilization.

Culture of embryos

Once fertilization occurs, there are a number of different methods to culture the fertilized oocytes. Some species require that the 2–8-cell stage embryos be transferred to the oviduct of a live animal (Havlicek *et al.*, 2005). For *in vitro* culturing, there are numerous protocols that can be used. One method frequently used is a coculture in which the medium contains oviduct cells to replicate *in vivo* conditions. A sequential medium method is also commonly used, where the components of the media are changed depending on the cell stage of the embryos, mimicking the different chemical environments embryos experience as they mature *in vivo*. Many factors affect the development of embryos, including temperature, pH, and gas concentrations. Depending on the researchers' needs the embryos may be used for embryo transfer, the implantation of embryos into viable females, or cryopreserved for shipping or future use.

Embryo transfer in vitro fertilization

After being cultured for 7 days the embryos reach the blastocyst stage and are ready to be transferred to a recipient animal. It is crucial that the recipient animal's estrous cycle be synchronized to the current stage of the embryo. That is, if the embryo is 7 days old, the recipient animal must be close to her 7th day of estrous (Senger, 2003). This ensures that the environment of the uterus is suitable for attachment of the embryo and that proper embryonic development occurs. Embryos can be transferred into the recipient by surgical or nonsurgical means. Nonsurgical methods are preferred because they are quicker and less expensive. The most common form of nonsurgical embryo transfer utilizes a transfer pipette or loading gun to insert the embryo into the uterine horn. When transferring into a cow, the technician often reaches through the rectum to grasp the cervix to help guide the loading gun through. Epidural anesthesia is often used to relax the reproductive tract and make the embryo insertion easier. If the embryo successfully attaches to the endometrium, the recipient will become pregnant.

Advantages of in vitro fertilization and embryo transfer

Like artificial insemination, IVF and embryo transfer allow for the mass production of genetically superior progeny by

allowing females to produce more offspring. These technologies have allowed desirable female donors to produce up to 20 offspring each year. In addition, embryo transfer has allowed for genetically inferior females to be utilized for their birthing capabilities, serving as the recipient female. Embryo transfer also allows for the diversification of species within geographical regions, as embryos can be easily shipped across the globe. This is also a much more cost-efficient and bio-secure method compared to transporting live animals (Senger, 2003).

Embryo Flushing

Instead of fertilizing oocytes and culturing the embryos *in vitro* (as in IVF), embryos are often produced *in vivo* and then 'flushed' out of the uterus. In fact, embryo flushing is much more prevalent and cost-efficient than IVF for the production of embryos. Although the first successful embryo flush and transfer was performed in rabbits in 1890, the procedure is primarily done with cattle today. More cattle undergo embryo flushing each year than all other species combined. Embryo flushing is primarily accomplished by artificially inseminating a superovulated female donor with spermatozoa from a genetically superior male. The embryos are collected from the donor after fertilization occurs, typically within 6–8 days. Bovine embryo collection typically employs a Foley catheter, flushing medium, and a collection vessel. The Foley catheter is inserted into the uterus and the flushing medium is passed through the catheter. The catheter typically contains a small balloon that seals off the uterus and prevents the backflow of the flushing medium. The flushing medium is allowed to flow back out of the catheter and is collected in a vessel. Depending on the success of superovulation and fertilization, the flushing medium may contain 1–30 embryos. The typical yield for cattle that undergo superovulation and artificial insemination is 5–7 viable embryos (Senger, 2003). The embryos can be examined by a microscope for viability and transferred to a recipient or cryopreserved using the same methods previously discussed for IVF.

Advantages of in vitro *fertilization embryo production over embryo flushing*

Although embryo flushing is more cost-efficient than IVF, there are a handful of instances when IVF is preferred or necessary. IVF can generate embryos after the death of an animal by surgical extraction of the oocytes. Oocytes can remain viable for 9–12 h after the death of most species. IVF also must be employed when the female is infertile but still has functional ovaries, often the result of infectious diseases. Another cause of infertility in livestock species is the continuous injection of hormones into females. Many female animals may receive hormonal injections throughout their lifetime. This leads to the inability to generate embryos, but oocytes can still be collected.

An in vitro *fertilization technique: Intracytoplasmic sperm injection*

Intracytoplasmic sperm injection (ICSI) is a form of IVF that utilizes only one spermatozoon and one oocyte. Oocytes are first extracted by the transvaginal oocyte recovery procedure. Under high-powered magnification, the oocyte is held by a micropipette while the spermatozoon is injected into it. Once fertilization occurs, the zygote is allowed to mature *in vitro*. Although ICSI has only been around for the past 25 years, many domesticated animals have been reproduced through the procedure, including cattle, pigs, horses, and sheep (Horiuchi and Numabe, 1999). However, the procedure requires more expensive technology and labor than other assisted reproductive techniques, and therefore it is mainly used for research purposes. There are situations where ICSI is used for reproductive purposes, although it is primarily after standard IVF has not been effective. Standard IVF is typically ineffective when the spermatozoa are defective, either because they are nonmotile or cannot complete the acrosome reaction. ICSI remains an inefficient breeding technology and is not as reliable as standard IVF in most domesticated animals even though recent advances have made ICSI more successful. One such advance has been the use of piezo-actuated micromanipulation during ICSI. Piezo-actuated micromanipulation entails rapid and precise insertion of the spermatozoon in response to an externally applied voltage. Piezo-actuated micromanipulation has been shown to improve ICSI fertility in numerous species, including mice and cattle.

Cloning

History of cloning

Although recent advances have opened bountiful opportunities and discussions on animal cloning, cloning experiments have been taking place for more than 100 years. An animal clone is broadly defined as an animal that originates from another animal, and both animals share identical chromosomal DNA. Hans Dreisch created the first animal clones in the late 1800s. He created sea urchin clones by splitting a two-cell embryo and allowing both cells to independently develop into sea urchins. These embryo-splitting experiments continued into the 1900, led by the Nobel Prize winning Hans Spemman's work on salamander embryos. The next major advance came in 1952 when Robert Briggs cloned a frog using a new technique; he used nuclear transfer to transplant the nucleus of a blastomere from a frog embryo into an enucleated egg. Although Briggs showed embryonic nuclear transfer could produce clones, not many believed that adult somatic cells could be used as donors. However, in 1996 the largest breakthrough in animal cloning came in the form of a sheep named Dolly. Dolly became the first animal to be cloned using the nucleus of a differentiated adult cell as a donor. Dolly opened the door to cloning via somatic cell nuclear transfer (SCNT), and many other species have been cloned in the last few decades.

Somatic Cell Nuclear Transfer Procedure

Along Came Dolly

Dolly, the world's most famous sheep, became a sensation in 1996. Dolly was famous because she was the first animal clone that originated from an adult somatic cell. Scientists Ian Wilmut, Keith Campbell, and

their colleagues at the Roslin Institute in Scotland created Dolly from an udder cell of a 6-year-old Finn Dorset white sheep. The udder cell was inserted into an enucleated oocyte of a Scottish Blackface ewe. Once it is was confirmed that the embryo was undergoing normal development at day 6, it was inserted into a different Scottish Blackface ewe. In a sense you could say Dolly had three mothers. On 5 July 1996, 148 days after embryo transfer, Dolly was born as 'a normal vigorous lamb and was standing and sucking unaided within minutes.' When asked how Dolly got her name, Dr. Wilmut responded, 'Dolly is derived from a mammary gland cell and we couldn't think of a more impressive pair of glands than Dolly Parton's.' Out of 277 cell fusions, 29 embryos produced, and 13 surrogate mothers, Dolly was the only live offspring born from Wilmut's experiment. Dolly lived for 6 1/2 years and was euthanized on 14 February 2003. She suffered from an incurable disease known as sheep pulmonary adenomatosis (SPA). SPA is caused by a virus that induces lungs tumors in affected sheep. Dolly's death was not directly caused by her being a clone, as other sheep in Dolly's vicinity died from the same virus. In Dolly's 6 1/2 years she gave birth to 6 offspring, battled arthritis, and was found to have shortened telomeres. Dolly shattered the theory that differentiated cells lose their ability to develop into other cell types. Dolly proved that cell differentiation is not simply a one-way process, and most somatic adult cells are capable of being reprogrammed into any other cell type.

SCNT is now the primary method used in animal cloning. The procedure first begins by extracting oocytes from a female donor and allowing the oocytes to mature *in vitro*. Once an oocyte has matured the nucleus can be removed using a needle aspiration system. The enucleated oocyte is now ready to accept the donor cell. There remains healthy debate concerning whether the donor cell should undergo a serum starvation treatment before being inserted into the oocyte, as well as the significance of the type and age of the cell used. The serum starvation treatment arrests the donor cell in the G0 phase, stopping further division. Once a donor cell is selected it can be inserted under the zona pellucida of the oocyte. The two cells are fused together by a brief electrical stimulus, which is referred to as electrofusion. The developmental and directing factors of the ooplasm reprogram the somatic nucleus to develop into an embryo and eventually a blastocyst, after which it can be transferred into a recipient.

Current State of Animal Cloning

Cloning on the Wild Side

Some scientists are investigating the use of cloning technology as an option to save endangered species and even resurrect extinct ones. Russian and South Korean scientists have been working together to try to clone a woolly mammoth using cells recovered from 10 000 years old frozen remains of a baby woolly mammoth. The scientists plan to clone the mammoth by extracting nuclei from the frozen mammoth cells, transferring them to elephant eggs and stimulating the cells to start dividing. The resulting embryos would be implanted into elephant wombs for gestation.

Brazilian scientists also aim to clone endangered animals. A project designed by scientists from the agricultural research agency Embrapa, together with the Brasília Zoological Garden, will attempt to clone and hybridize jaguars, collared anteaters, maned wolves, and other endangered species. Somatic cells and spermatozoa from eight threatened species have already been collected. The researchers must now receive permission from the government to conduct experiments on the 420 samples already collected. Several environmentalists are concerned about this project because these cloned, hybridized, and captive-bred animals, if mixed with wild populations, could result in potential environmental risks. However, the project was specifically designed to supply zoos rather than replenishing wild populations.

The cloning of endangered species has raised several issues between conservationists and environmentalists, who say that instead of cloning to save these species, more efforts to protect and recover their natural habitats should be made. They believe that conserving the natural habitat where these animals live would have a greater impact on the preservation of these species.

The potential advantages of cloning are innumerable for many industries including agriculture and biomedical research. However, the field is still relatively new and needs extensive research to make animal cloning more efficient. Cloning efficiency is defined as the number of live offspring per embryos transferred. Currently, the efficiency rate for cattle is 6–15% and 6% for pigs. However, for some animals the efficiency is as low as 1–2%, whereas others still have not been successfully cloned (Fiester, 2005). Although cloning efficiency has improved in the past 10 years, these proportions are still substantially lower than other reproductive techniques. In addition, some clones are born with phenotypic abnormalities. The most common abnormality is an unusually increased birth weight, known as large offspring syndrome (LOS). LOS causes difficulties in the birthing process, as well as other health risks for the animal, such as organ defects and diabetes. These abnormal phenotypes are not transmitted to the clone's offspring, which suggest *in vitro* conditions alter the epigenetic patterns of the cloned embryo, as these patterns are reprogrammed during gametogenesis (Prather *et al.*, 2004). These *in vitro* conditions are being studied to help improve efficiency and reduce abnormalities in animal cloning, as a number of biological factors are known to influence the reprogramming of the nucleus.

Production of Transgenic Animals

Inserting human genes into an animal's genome allows animals to produce important human proteins, such as the blood clotting agent factor IX. However, the methods for producing transgenic animals are not very efficient. Incorporation rates of the new gene into their genome are low and occur at random sites which often do not allow the gene to be expressed. Also, the insertion can cause disruption in the expression of another gene. Researchers at the Roslin Institute sought to use cloning as an efficient way to produce transgenic animals. In theory, once a cell line successfully incorporates and expresses a transgene, that cell line can be used as a donor cell for cloning. The clones produced will have the transgene incorporated into their genome and can successfully pass it to their offspring through traditional breeding methods. This could lead to entire herds of transgenic animals expressing important genes for medical and agricultural purposes. Transgenics and cloning also hold enormous potential for

producing organs in animals for human transplants, or xenotransplantation. If animals can be modified to produce viable organs for humans, cloning could drastically increase the human organ supply.

Genome Editing

Precision Editing Opens a World of Possibilities for Transgenics

The precision of nuclease-based genomic editing can lead to custom designed animals with improved traits and modifications to better serve as human disease models. Cow's milk allergy (CMA) is an immunologically mediated allergic reaction to certain proteins in cow's milk. The CMA-inducing protein beta-lactoglubulin causes diarrhea and vomiting in children. It is estimated that the prevalence of CMA varies between 0.25% and 4.9% and is higher in children than in adults. For the last few decades researchers have been trying to create transgenic cows that produce beta-lactoglobulin-free milk, but have been unsuccessful because of a lack of precision associated with gene editing. Recently researchers found a gene encoding microribonucleic acid (miRNA) in mice that targets beta-lactoglubulin mRNA and silences its production. This technology is known as RNA interference (RNAi), and it allows for the elimination of beta-lactoglobulin protein without needing to alter the gene itself. After successfully inserting the miRNA gene into the genome of cow embryos, one calf was born that produced beta-lactoglobulin-free milk.

Although RNAi is effective at silencing genes, it cannot eliminate the protein completely. Transcription activator-like effector nucleases (TALENs) are artificial restriction enzymes created by the fusing of a DNA binding domain with a DNA cleavage molecule. TALENs work by cutting DNA at specific sequences, introducing double-stranded breaks into a gene of interest. When the cells repair the breaks they introduce mutations into the gene that can render the gene nonfunctional. In pigs, TALENs have been used to disrupt genes encoding low-density lipoprotein (LDL) receptors. Pigs lacking these receptors are unable to remove LDL from the bloodstream, causing them to develop atherosclerotic arteries. Such pigs can be used as disease models to aid biomedical research in human atherosclerosis.

Many transgenic technologies are inefficient because they involve nonspecific integration of the transgene into the target genome. In contrast, nuclease-mediated genome editing results in a specific integration. The method relies on the use of artificial proteins made up of customizable, sequence-specific DNA-binding domains fused to a nuclease that cuts DNA in a nonsequence specific way (Joung and Sander, 2013). Zinc-finger nucleases (ZFNs) and recently, TALENS are employed in performing targeted genome editing. ZFNs and TALENS can be described as molecular scissors that cleave double-stranded DNA at a specific site in a predetermined sequence of the genome. The cleavage triggers DNA repair that can be exploited to modify the genome either by targeted introduction of insertions and deletions (gene disruption), base substitution specified by a homologous donor DNA construct (gene correction), or the transfer of entire transgenes into a native genomic locus (Urnov *et al.*, 2010). This new technique has the potential to be used in many applications including therapeutic approaches to treat genetic disease, production of model organisms, and generation of new agriculturally relevant varieties.

Transgenic Technologies for Food and Other Products

Transgenic Animals Can Provide New and Improved Products

Farmers have been using selective breeding to increase desirable traits in agricultural animals since the dawn of domestication. However, the increased production potential possible from traditional selective breeding practices is limited. Advances in molecular biology have made it possible to develop traits in animals quicker and with more precision, allowing farmers an alternative means to increase yields, improve the nutritional value of food products, make animals resistant to diseases, and produce human pharmaceuticals in the milk of transgenic cows, goats, pigs, or rabbits. Some transgenic animals already have been approved by the Food and Drug Administration (FDA) for production of nonfood products, and the AquAdvantage® salmon is close to becoming the first transgenic animal approved by the FDA for human consumption (at the time of publication).

Less smelly pig

From Egg to Plate in Half the Time: Speedy Salmon Promise Profit

AquAdvantage® Salmon are in line to become the first genetically engineered (GE) nonplant food source approved for human consumption by the US FDA. This accomplishment is the culmination of more than 20 years of work, which began during a coffee break. One winter day in the 1980s, when Dr. Choy L. Hew, who studied an antifreeze protein that allows fish to survive subzero temperatures, was chatting with his colleague, Dr. Garth Fletcher. Fletcher was frustrated because he just returned from a fish farm where all the salmon had frozen to death and he challenged Hew to use his molecular biology to do something about this problem. Having limited success enhancing the cold tolerance of the salmon, they altered the plan to increase the growth rate so that they could be harvested before the onset of winter cold. They GE the salmon by attaching the antifreeze protein promoter to the growth hormone gene, causing growth hormone to be produced during the winter months, allowing the salmon to grow year round. They saw the first fast-growing fish in the summer of 1990, and received a patent in 1996. In the same year they met Elliot Entis, who was running his father's wholesale seafood business, at an academic conference. Entis showed a great interest in the fast-growing salmon. He licensed their technology and started A/F Protein. The company, now called AquaBounty Technologies, brought the AquaAdvantge Salmon to a marketable stage. These fish outgrow any wild or farm-raised salmon, and can grow from the egg stage to market weight in 16–18 months, as opposed to 3 years for traditional salmon. The AquAdvantage® salmon are waiting on final approval of the FDA while environmental issues are studied.

One goal of current research is to create transgenic farm animals that are more environmentally friendly. At the University of Guelph in Canada, for example, transgenic pigs have been developed with the issue of manure-related environmental pollution in mind. Referred to as the EnviroPig, this transgenic pig is capable of digesting phosphorus in plants more efficiently than conventional pigs. The EnviroPig contains a bacterial phytase gene controlled by a salivary-gland-specific promoter, which limits the production of phytase to the saliva. Phytase is an enzyme that releases

phosphate from phytate, which accounts for up to 80% of phosphorus content in most feeds. The ability to digest plant phosphorus limits the need for costly feed supplements such as phosphate minerals or commercially produced phytase. In addition, the EnviroPig excretes 30–70% less phosphorus in its waste than conventional pigs. This is environmentally important, as excess phosphate from manure alters the local water environment, causing increased algae growth, production of greenhouse gases, and the death of fish and aquatic animals. The lower levels of phosphorus in pig feces reduce water pollution. The hope for this project was to be able to market the EnviroPig as a more environmentally friendly option with reduced feed costs. The university has applications with Health Canada and the US FDA for the EnviroPig to be approved for human consumption. According to the company website, in 2012, Ontario Pork decided to redirect its research dollars, ending its funding of the EnviroPig program, and the remaining EnviroPigs were euthanized. Ontario Pork's Director of Communications and Consumer Marketing Keith Robbins said of the decision to stop funding of the EnviroPig, "we sort of felt that we weren't getting the kind of return that was originally looked at in concept of that product." The decision ultimately was because of the lack of public demand for genetically modified animals in the food system.

GloFish: GM Pets That Brighten Homes and Hearts

In 2003, Yorktown Technologies created a genetically modified pet. The GloFish®, a fluorescent red zebrafish, has become the first transgenic animal commercially available in the US and a really popular aquarium item. The GloFish is available in five fluorescent colors with the exciting names – Starfire Red®, Electric Green®, Sunburst Orange®, Cosmic Blue®, and Galactic Purple®.

The original zebrafish (*Danio rerio*), from which the GloFish was developed, is native to India and Bangladesh. Because the GloFish was developed from a tropical fish and cannot survive in the colder US waters, it is believed that these GE zebrafish pose no risk to the environment. Because GloFish pose no risk to the environment or entering the food supply, the FDA decided not to regulate these transgenic animals. However, although the GloFish is permitted in the US, the sale of this GM pet is not allowed in the state of California.

The fluorescent zebrafish was primarily developed with the aim to detect pollution by selectively fluorescing when in the presence of environmental toxins. This first fluorescent fish was created in 1999, when Dr. Zhiyuan Gong and his team at the National University of Singapore (NUS) were working with the green fluorescent protein (GFP) gene extracted from jellyfish, which naturally produced bright green fluorescence. This gene was then inserted into a *Danio rerio* embryo and integrated into the zebrafish's genome, allowing the fish to be fluorescent under natural white light and ultraviolet light. Sometime later, these scientists made a deal with Yorktown Technologies to develop the GloFish®.

Although GloFish® pose no risk to the environment, a new variety of the GloFish introduced in February 2012 has raised some concerns. The Electric Green Tetra, a GE black tetra fish, includes genetic material from a fluorescent coral that makes it neon-bright and makes the fish fluorescent when placed under a black light. Some environmentalists and experts are concerned about the new black tetra. If released, this South American fish would be able to survive in the waters of South Florida and Latin America, where they could pose potential environmental risks and have an undesirable influence on natural biodiversity.

More – and better – meat on their bones

The ability to produce transgenic pigs and cattle with enhanced muscle growth is an area of increasing interest. Researchers have been studying the effects of targeting myostatin, the only secreted protein known to negatively affect muscle mass *in vivo*, as well as genes for growth-related hormones and lean muscle mass (Long *et al.*, 2009). Transgenic myostatin knockout cows have been produced in the US; however, there are concerns regarding the increased neonatal morbidity that arises from giving birth to larger calves with increased fetal muscle mass (Tessanne *et al.*, 2012). Currently no myostatin knockout pigs have been developed; however, transgenic pigs for growth-related hormones have been produced. Although they show improvement in growth rate, feed conversion and body fat/muscle ratios, they also showed signs of fatigue, gastric ulcers, and low libido. Transgenic pigs containing insulin-like growth factor-1 and a desaturase gene from spinach have been shown to have increased growth rates and increased levels of polyunsaturated fatty acids, respectively. These new developments come without the negative side effects of previous transgenic pigs. In addition, researchers at the University of Illinois have produced transgenic pigs expressing bovine α-lactalbumin, which leads to an increase in milk production (Wheeler *et al.*, 2001). This increase in milk production was shown to increase the weight gain of piglets suckling from the transgenic gilts compared to control gilts. Gilts are female pigs that have had no more than one litter. When pigs give birth to a second litter, they are referred to as sows. These technologies allow for the decreased use of less effective techniques, such as growth hormones whose residues can be found in the final animal product. Despite these advances, none of these transgenic animals have been approved for human consumption.

Omega-3 fatty acids are found mainly in fish oils and largely considered beneficial to human health. Conventional meat products contain large amounts of omega-6 fatty acids, and low levels of omega-3 fatty acids. Diets with a high omega-6/omega-3 fatty acid ratio are correlated with coronary artery disease, cancer, diabetes, arthritis, and depression. To try and create a healthier balance, researchers have recently developed transgenic pigs and cows containing high levels of omega-3 fatty acids in both their tissue and milk (Lai *et al.*, 2006; Wu *et al.*, 2012). This was done by inserting a gene encoding for an omega-3 fatty acid desaturase into the genome of the pig and cow. Omega-3 fatty acid desaturases are enzymes required for the conversion of omega-6 fatty acids to omega-3 fatty acids. The end result is an increase in omega-3 fatty acids and a decrease in omega-6 fatty acids, thus creating the potential for meat and dairy products with a healthier omega-6/omega-3 ratio.

Not your mother's milk

A team of scientists at AgResearch and the University of Waikato in New Zealand has successfully produced a transgenic cow lacking β-lactoglobulin (BLG) (Jabed *et al.*, 2012). This whey protein is believed to be the main cause of milk allergies in humans, and knocking out this gene could allow for the production of hypoallergenic dairy products. The researchers use miRNA technology to silence the expression of BLG in the milk, making it potentially less allergenic. In addition, high casein levels were reported in the BLG-deficient milk. Casein

makes up 80% of milk protein in conventional cows and is an extremely valuable component of milk because of its nutritional value and processing properties. The increased casein levels associated with this BLG knockout cow could provide increased calcium levels and higher cheese yields. In addition, another group in New Zealand has produced transgenic cows containing additional β- and κ-casein genes (Brophy *et al.*, 2003). These cows have been shown to produce milk with a twofold increase in κ-casein, and up to 20% increase in β-casein levels. The increase in κ-casein has been associated with improved heat stability and cheese-making properties, whereas increased β-casein has been associated with increased milk calcium levels and whey expulsion.

In addition to cows, there is much interest in producing transgenic goats to create healthier milk for human consumption. For instance, changes in the fatty acid composition of milk produced by goats containing a transgene encoding a stearoyl-CoA desaturase (SCD) enzyme has been reported (Reh *et al.*, 2004). SCD works by converting saturated fatty acids into monounsaturated fatty acids. Because one-third of saturated fatty acids in American diets come from dairy products, and saturated fatty acids can lead to increased blood cholesterol levels, leading to increased risk of atherosclerosis and coronary heart disease, the decreased level of saturated fatty acids in milk is an important heath concern. The SCD transgenic goats were shown to have increased levels of monounsaturated fatty acids as well as decreased levels of saturated fatty acids, which could prove to have increased health benefits compared to milk from conventional animals.

Baa baa, transgenic sheep, have you any wool?

Green Pigs Light the Way for Biomedical Research

Dr. Randy Prather is a professor of reproductive biotechnology at the University of Missouri. He has been involved in the push for transgenic pigs in biomedical research, having produced both GFP and yellow fluorescent protein (YFP) transgenic pigs. These pigs contain a GFP gene that comes from a jellyfish, and is commonly used as a molecular marker because it is easily visible under ultraviolet light. Dr. Prather summarizes the importance of this work, saying 'these animals prove that we can make genetic modifications to express desired traits. For xenotransplantation, this is a large step because it means it's possible to change the genetic makeup of the cells to prevent the body's rejection of transplanted organs. However, not everyone is excited about these technological advancements. Kathy Guillermo, a spokeswoman for people for the ethical treatment of animals, believes that transgenic animal experiments like these are unethical and of little use. "On one level, we're just opposed to it period because it's the treatment of animals like objects," Guillermo said. "On another stance, we see it as bad science." Regardless, Dr. Prather and other researchers around the world are hoping transgenic animal models like the GFP and YFP pig will result in advances in the fields of agriculture and biotechnology. "Application of this technology can help feed an ever-growing population, and it can have tremendous potential to alleviate human suffering," Prather said.

In addition to products for human consumption, there are a number of other new, as well as improved products transgenic animals could be utilized for. Increased wool growth in transgenic sheep has been achieved in New Zealand by introducing an insulin-like growth factor-1 gene associated with a keratin promoter (Damak *et al.*, 1996). The keratin promoter allows production of the transgene in the skin and results in an increase in the production of clean fleece weight to conventional sheep. Although no health issues were observed in the transgenic sheep, the staple strength of the wool produced by the male transgenic sheep was lower than that of female transgenic and nontransgenic animals. Further research could result in herds of transgenic sheep capable of higher wool yields than conventional sheep, potentially lowering costs for farmers.

Silk in milk

Transgenic goats are also being produced for dragline silk in their milk. Dragline silk is made by orb spiders and is the strongest known material by weight. Because of its strength as well as its elasticity, there is much interest in large-scale production of dragline silk for use in military uniforms, medical sutures, and tennis racket strings. After failing to produce the material in bacteria and mammalian cell culture, scientists in Canada have successfully inserted the spider silk genes into goat embryos. When the transgenic goats matured, the spider genes were expressed in the mammary glands of females, which began to secrete tiny strands of spider silk in their milk. Once protocols are in place for the purification and spinning, the resulting thread could be used for a number of commercial as well as medical applications.

Biopharming: Transgenic Animal Advances in Medicine and Research

Transgenic animals not only have potential to improve agriculture, but could also lead to significant breakthroughs in biomedical research. For decades proteins such as insulin and human growth hormone have been produced in bacteria and yeast cultures. However, proteins such as blood clotting factors and monoclonal antibodies require complex folding patterns and additional sugar molecules to become biologically active. These sophisticated modifications require the proteins be produced in mammalian cells to be carried out properly, thus showing the limitations of *in vitro* bacterial culture techniques to be able to produce complex proteins. Some examples of transgenic animal systems that are currently being researched include milk, blood, and egg whites.

Complex Protein Production

Transgenic animals in biomedical research can aid in the production and subsequent collection of insulin, growth hormone, blood anticlotting factors, and other biological products in the milk of cows, sheep, and goats. Dairy cows, for example, have a yearly milk output of approximately 10 000 l, making it possible for a single-lactating cow to produce tens of kilograms of therapeutic proteins. Relatively small herds of a few hundred lactating transgenic cows or goats can produce several hundred kilograms of purified protein per year. In fact, it has been estimated that only 60 transgenic pigs would be needed to supply the entire factor IX protein required in the US. This is referred to as biopharming, and is gaining momentum as a potential route for the production of products for medical use.

The first therapeutic protein produced in the milk of transgenic animals to be approved for human use was antithrombin, an anticoagulant protein that can treat patients with a congenital deficiency. GTC Biotherapeutics (Framingham, MA) markets recombinant antithrombin purified from the milk of transgenic goats. In 2006, the European Medicines Agency approved the drug and then in 2009, the US FDA also gave approval. In addition, the production of transgenic pigs whose milk contains human factor VIII and IX, hemoglobin, human protein C, human erythropoietin, human granulocyte-macrophage colony stimulating factor, and Von Willebrand factor are being researched.

In 1997, the first transgenic cow was produced whose milk was enriched with the human protein α-lactalbumin. The transgenic milk, being more similar to human breast milk, is more nutritionally balanced than natural bovine milk and could be given to babies or the elderly with special nutritional or digestive needs. In addition, cows have been produced that secrete human lactoferrin, a glycoprotein involved in innate host defense, in their milk (Van Berkel *et al.*, 2002). Because of lactoferrin's antibacterial, antifungal, antiendotoxin, and antiviral activities, a number of medical uses for this glycoprotein have been considered, such as the treatment of infectious or inflammatory diseases. The ability of these researchers to produce and purify human lactoferrin from the milk of these animals shows the potential of transgenic animals for large-scale production of biopharmaceutical products.

Human Disease Models

An area of biomedical research that has huge potential for transgenic animals is their use as human disease models. Although mice have traditionally been used as the go-to animal model for human diseases, many of the breakthroughs in mice have not translated to humans. Because of their similar size and physiology, there has been increasing interest in using pigs as human disease models. Conventional pigs are already used to study cardiovascular disease, atherosclerosis, cutaneous pharmacology, wound repair, cancer, diabetes, and ophthalmology. Using transgenic technology, pig models are currently being produced for such diseases as Alzheimer's disease, cystic fibrosis, retinitis pigmentosa, spinal muscular atrophy, diabetes, and organ failure (Aigner *et al.*, 2010). Once these animal models have been characterized, new drugs and therapies can be tested before clinical trials.

Xenotransplantation

An estimated 45 000 Americans under age 65 could benefit from a heart transplant each year, but only approximately 2000 human hearts are available. To close this gap, researchers have begun to study xenografts, the transplanting of organs and tissues from animals into humans. Although nonhuman primates such as chimpanzees are genetically closest to humans, reducing the chances of graft rejection, primates are endangered in the wild and their use as a source of replacement organs raises ethical concerns because of their high level of intelligence and the increased risk of disease transmission between such closely related species. As an alternative, some have proposed using pigs as a source of organs because they have large litters, a short gestation time, are anatomically and physiologically similar to humans, are already produced in high volume as a food source, and are currently used to provide some replacement tissues such as heart valves.

Xenotransplantation would have to overcome many technical and ethical obstacles before it can become a reality. One of the first technical issues researchers have focused on are the antigens on the surface of pig cells. These surface antigens are similar to the ABO blood group antigens that trigger severe immune responses called hyperacute rejection. To address this, scientists have inserted human genes into single-cell pig embryos in an attempt to make their cell-surface proteins more similar to human ones so the tissues are no longer antigenic. However, even if this procedure reduces the risk of hyperacute rejection, other immunological barriers to xenotransplantation, such as acute humoral xenograft rejection, thrombotic microangiopathy, and coagulation dysregulation still exist.

In addition, there are concerns of cross-species infections caused by exogenous viruses, such as porcine cytomegalovirus, present in the xenotransplanted organs (Fishman and Patience, 2004). In 1997, Robin A. Weiss, a virologist at University College London, discovered a new class of pig viruses called porcine endogenous retroviruses (PERVs) and determined that they have the ability to infect cultured human cells. The transplantation of a pig organ into a human host would therefore create the opportunity for the transmission of PERVs, potentially enabling such viruses to evolve into human pathogens. Retrospective studies of patients who received heart valves from pigs identified the DNA of PERVs in some recipients. Therefore there is real concern that xenografts from pigs could provide a path for the transmission of novel viruses from animals to humans. Until this issue is resolved definitively, clinical trials of xenotransplantation are unlikely to move forward.

Transgenic Animals with Increased Disease Resistance

The ability to enhance disease resistance in animals holds enormous potential for the continuing field of animal biotechnology. Currently, numerous studies are being performed to induce disease resistance in a variety of animals. Some of the diseases being studied include mad cow disease, foot and mouth disease (FMD), porcine reproductive and respiratory syndrome (PRRS), and avian influenza viruses (AIVs).

Mad Cow Disease

Mad Cow Disease, or more formally bovine spongiform encephalopathy (BSE), is a specific type of a transmissable spongiform encephalopathy (TSE). TSEs are progressive, degenerative diseases of the brain, spinal cord, and central nervous system. They are also characterized by a long period of time between infection and detectable symptoms. The abnormal folding of the prion protein (PrP) is thought to cause TSEs. Abnormally folded PrPs can be transmitted and cause host PrPs to adopt abnormal configurations. Although the first TSE identified in cattle was in 1986 (BSE), TSEs have been documented in a variety of species (Wells *et al.*, 1987). The

human form of the disease is called Creutzfeldt–Jakob Disease (CJD), which was first characterized in 1920. However, scientists linked a new variant of CJD to BSE in 1996. Owing to the seriousness of the disease and the public health concerns, many studies are underway to induce resistance to these prion diseases. The primary method for inducing resistance is to silence the PRNP gene, which encodes for the normal PrP. These knock out studies, performed in cattle and mice, have shown that animals without the PrP are unable to produce and transmit the infectious form of the protein (Büeler *et al.*, 1993; Hirata *et al.*, 2004; Richt *et al.*, 2007).

Foot and Mouth Disease

FMD is a highly contagious disease that infects cloven-hoofed animals (those with divided hoofs). The pathogen responsible for FMD, foot and mouth disease virus (FMDV), is easily transmitted through direct contact, aerosols (air-borne), and ingestion. The virus also replicates rapidly once inside the host, and symptoms typically appear with 2–3 days. There are a variety of symptoms, but lesions on the tongue and feet characterize the virus. The virus is typically only lethal for younger animals. FMD poses enormous economic losses on the global livestock and trade industries. Not only does FMD wipe out herds of animals, many countries refuse to trade livestock with countries that experience FMD epidemics. Currently, vaccines provide the primary method to induce resistance to FMD. Researchers have recently created entirely synthetic vaccines to protect against FMD. However, vaccines remain problematic for eradicating FMD because of the fact there are more than 7 serotypes and more than 60 strains of the virus. This has sparked many studies that explore producing transgenic livestock that are resistant to FMD. Multiple studies have shown that RNAi is a viable antiviral strategy *in vitro* and *in vivo*, either through the use of small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) (Haasnoot *et al.*, 2003; Grubman, 2005). Currently, no siRNA or shRNA transgenic livestock have been produced that are resistant to FMD, although many studies are being performed.

Porcine Reproductive and Respiratory Syndrome

PRRS is a viral disease that affects swine. PRRS is the largest economic hurdle the US swine industry faces, as the virus costs the industry approximately US$600 million each year. The two primary symptoms of PRRS are reproductive failure and respiratory complications for younger animals. The porcine reproductive and respiratory syndrome virus (PRRSV) is most often transmitted through direct contact, often appearing in high concentrations in semen, urine, feces, mammary secretions, and nasal secretions. Vaccines have been developed to help control the spread of PRRS, but the efficacy of the vaccines vary. The reason many vaccines are not effective is the virus's ability to generate a high degree of genetic diversity and its remarkable ability to evade host defenses. Owing to the unreliability of vaccines, other methods are being studied to control the disease. Some studies are focused on creating breeding programs that only breed swine with a high resistance to PRRS. Other studies are focused on creating transgenic pigs that are resistant through RNAi.

Avian Influenza Viruses

AIVs are a very diverse group of viruses that infect a wide variety of birds. However, because of their high rate of mutation, AIVs can also infect other species, such as humans. One example is the AIV strand H5N1. This is a fatal strand that can infect humans and many other species. These human health risks have sparked research into creating disease resistant fowls. Transgenic studies are at the forefront of this field. Transgenic chickens that are unable to transmit AIVs to other birds have recently been produced. This is a monumental achievement for genome editing and disease resistance.

Antimicrobials

The immune system of newborn piglets is immature, and thus they are susceptible to many bacterial infections, some of which cause diarrhea. These infections can also significantly reduce newborn survival rate. Although it is common to use antibiotic feed additives for newborns, this has led to a drastic increase in the number of antibiotic-resistant bacterial strains. This has required alternative approaches to prevent bacterial infections in piglets. Transgenic approaches offer great promise. Transgenic goats have been produced that make milk with the same concentration of lysozyme, the natural antimicrobial agent, as human breast milk (Brundige *et al.*, 2008). This milk was fed to piglets and it helped protect against *Escherichia coli* and improved gastrointestinal health. Transgenic cattle have also been produced that make human lysozyme and milk in their milk so that it is nutritionally similar to human breast milk.

Antimicrobial peptides (AMPs) exhibit another crossroad of transgenics and disease resistance. Cecropin B, the AMP that originates from the giant silk moth, has many antimicrobial effects. Many of these antimicrobial effects are against gram-negative bacteria. The gene encoding for cecropin B was transfected into catfish and the Asian medaka fish. Both transgenic fish breeds showed increased bacterial resistance to numerous pathogens.

Biotechnology Enhances and Advances Selective Breeding

Genetic Screening of Breeding Stock

Traditional animal breeding (TAB) exploited variations that existed within breeds and animal populations to bring about genetic improvement in traits of economic importance such as milk yield, growth traits, and egg numbers. TAB has been very successful over the years by utilizing records of the phenotype of an animal and a number of its relatives to estimate the likelihood that an animal will pass on its good traits to its offspring. An obvious example of the success of TAB is the doubling of dairy cow milk yields over the past 40 years (Oltenacu and Broom, 2010). However, for traits that are difficult to measure such as disease resistance, fertility, and feed efficiency, these traditional breeding methods have not been successful.

Genomics on the Dairy Farm: More Accuracy and Less Expense

Older pedigree tests used by the US Department of Agriculture (USDA) Animal Improvement Programs Laboratory were based on rating the genetic fitness of dairy cows by analyzing milk production records and assessing the quantity of certain cells in the cow's udder. Over the past 30 years, farmers and companies have selected for the best dairy cows using these tests. These tests can predict the genetic merit of a cow with 30% accuracy at birth. Breeders used the milk production records of a bull's relatives (mother, aunts, sisters, and daughters) to select good bulls to mate with their best dairy cows. Farmers with good bulls had to pay $50 000 for their bulls to be evaluated by breeding companies, and the breeding companies would create a detailed pedigree showing the bull's ancestry. However, in November 2006 the development of a new test based on genetic markers started. Led by Van Tassel, the USDA has determined approximately 38 000 genetic markers that aid in identifying cows that have the best genes for milk production and pass these genes to their offspring. A small single nucleotide polymorphism (SNP) chip can be used to track these 38 000 genetic markers in cows. This new genetic test can predict the genetic merit of a cow with 70% accuracy, compared to only 30% accuracy using traditional pedigree tests. Furthermore, instead of farmers paying US$50 000 for their bulls to be evaluated by breeding companies, it now costs only US$250.00 to genotype the bulls using this new genetic test.

The idea of using marker-assisted selection (MAS) to overcome the shortfalls of TAB has been around since 1923. MAS is the selection of traits of interest indirectly by selecting genetic markers associated with desired qualities, as opposed to traditional methods of finding desired qualities by observing phenotypic traits. Sax (1923) observed an association between seed color and seed weight and concluded that the gene controlling seed color must be linked to genes that control seed size. Thoday and Boam (1961) attempted to map and characterize polygenes affecting sternopleural chaeta number in *Drosophila melanogaster*. By estimating breeding values based on marker, pedigree and phenotypic information, MAS can bring genetic improvement in traits of animals where TAB alone has failed. In a French MAS program in dairy cattle, estimated breeding values (EBVs) using MAS for all traits considered were more reliable than EBVs estimated from classical selection methods (Guillaume *et al.*, 2008), demonstrating that MAS may lead to increases in genetic improvement as compared with traditional breeding methods.

Beginning in the late 1970's many molecular genetic markers were discovered and developed, including allozymes, restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), microsatellite DNA and SNPs. The ability to analyze these markers was developed over several decades and has made the mapping of quantitative trait loci (QTL) feasible on a large scale (Brumlop and Finckh, 2010). Out of these genetic markers, SNPs are currently the marker of choice because of their large numbers spaced across the genome. The decreasing cost and rapid progress in next-generation sequencing methods that employ massively parallel approaches in sequencing several hundred thousands to millions of reads simultaneously have led to the identification of many SNPs in livestock species. SNP arrays containing tens of thousands of SNPs distributed throughout the genome are now available for several livestock species (Table 2) and support the interrogation of hundreds of loci at a low cost.

Table 2 Available single nucleotide polymorphism (SNP) chips developed for some animal species

Species	*Identification*	*Provider*	*Number of SNPs*
Cat	Feline	Illumina	62 897
Horse	Equine	Illumina	65 157
Sheep	Ovine	Illumina	5 409
Cattle	BovineHD	Illumina	777 962
Cattle	BovineSNP50v2	Illumina	54 609
Cattle	BOS 1	Affymetrix	648 000
Sheep	OvineSNP50	Illumina	52 241
Cattle	BovineLD	Illumina	6 909
Pig	PorcineSNP60	Illumina	62 163
Dog	CanineHD	Illumina	173 662

Abbreviations: HD, high density; LD, low density.
Source: Reproduced from Eggen, A., 2012. The development and application of genomic selection as a new breeding paradigm. Animal Frontiers 2 (1), 10–15.

A disadvantage in the implementation of livestock MAS is that population-based, genome-wide association studies are unable to detect SNPs associated with a trait if the desired allele has a frequency below 5% or 1% (Brookfield, 2010). Additionally, MAS requires prior knowledge of markers that are associated with traits. Many markers are now known across the genomes of many livestock species, including cows, sheep, and pigs. Genomic selection, as initially proposed by Meuwissen *et al.* (2001), can use all these markers simultaneously to predict the genomic estimated breeding value (GEBV) for traits of animals without needing to know the location of genes in the genome.

Risks and Regulations

As with any foray into a new area of technology, there are several concerns about the use of animal biotechnology in agriculture and biomedical research. There is concern that food products derived from transgenic or cloned animals may pose risks to human health. There are also concerns about potential impacts of animal biotechnology on the environment and on animal welfare, as well as questions about whether the current regulatory structure is adequate to evaluate and control the risks associated with these technologies. Animals pose unique challenges compared to plants, as there is greater concern for the welfare of animals. Animal biotechnology, as well as its regulatory system, has been subjected to increasing attention and discussions among research scientists and the public. (About Bioscience; Animal Biotechnology: Science-Based Concerns, 2002; Cowan, 2010).

Human Health Concerns

Cloned and GE animals can be used as a source of tissues and organs for xenotransplantation and for production of biopharmaceuticals (Animal Biotechnology: Science-Based Concerns, 2002). Although xenotransplantation offers many

benefits, there are some risks including infection and rejection. Recipients of xenotransplantation risk direct exposure to recognized and unrecognized infectious agents such as prions, virus, or bacteria. Additionally, there is potential for future generations to become infected through vertical transmission (transmission of an agent from an individual to its offspring). Secondly, immunologic barriers associated with the use of xenografts are a concern, as in all allograft procedures. Although immune suppression therapies exist, hyperacute rejection is not always blocked (About Bioscience; Animal Biotechnology: Science-Based Concerns, 2002; Medscape).

The use of transgenic animals for the production of biopharmaceuticals for human health purposes has also raised several concerns. First, there is the potential risk of the generation of pathogenic viruses by recombination of vector sequences and related nonpathogenic viruses present in the same animal. The second concern is the possibility that surplus animals or their offspring inadvertently entering the food chain (Animal Biotechnology: Science-Based Concerns, 2002).

Food Safety Concerns

Concerns around all food and food products are based on the concept that they should be free of chemical or biological agents that can affect the safety of the food for the human or animal consumer (Animal Biotechnology: Science-Based Concerns, 2002; Kochhar and Evans, 2007). In 2001, the US producers agreed to keep food products from cloned animals or their offspring out of the food chain until the FDA could evaluate the risks. In 2008, the FDA released their report, which stated that meat and milk from cloned cattle, swine or goats or their offspring are as safe to eat as conventionally bred animals (Cowan, 2010, 2011; Bazer *et al.*, 2010).

Potential food safety concerns about products derived from GE animals, are mainly related to transgene expression. These transgenes could cause proteins to be present in food that could be allergenic, toxic, or have other antinutritional or/and other physiological effects. These concerns are considered a moderate-level concern of food safety and vary according to the gene product, the food product, and the consumer (Animal Biotechnology: Science-Based Concerns, 2002; Kochhar and Evans, 2007).

Animal Health and Welfare Concerns

The impact of genetic manipulation on animal health and welfare are of significant public interest. Ethical discussions are asking if these genetic manipulations can cause unnecessary stress in the animals (Animal Biotechnology: Science-Based Concerns, 2002; Cowan, 2010). For example, biomedical-use animals, specifically for those housed in sterile and isolated environments necessary for production of xenotransplantation tissues may experience stress and develop behavioral abnormalities. Rules are in place to try to alleviate any problems that might be caused by the pigs' environment (Animal Biotechnology: Science-Based Concerns, 2002). Continued evaluation of food safety, environmental safety, and animal welfare issues associated with animal biotechnology will be required as the field evolves.

Environmental Concerns

Several environmental concerns about GE animals are considered of high importance because both early identification and finding solutions to any problem are so difficult. The main concern is the possibility of GE animals entering natural environments (through release or escape) and disrupting ecosystems (Animal Biotechnology: Science-Based Concerns, 2002; Cowan, 2011).

For example, animals with high mobility and that have historic records of causing community damages, such as insects, shellfish, fish, and mice and rats, which can become feral easily and cause a high level of environmental concern. GloFish, a fluorescent red zebrafish, was the first transgenic animal commercially available in the US and a really popular aquarium item. Because this zebrafish is from southern Asia and cannot survive long in the cold US waters, it is believed that these GE zebrafish pose no risk to the environment. However, the AquAdvantage® Salmon (currently being evaluated) grow much faster than any wild salmon and, if released into the wild, could pose significant ecologic and genetic risks to native salmon stocks (Animal Biotechnology: Science-Based Concerns, 2002). This is why the company has proposed to sell only infertile female salmon eggs and which must be grown in inland tanks to reduce any risk of release into the wild. Furthermore, the cloning of extinct species, such as the woolly mammoth, is another focus of recent environmental concerns.

Regulation of Animal Biotechnology

United States

In the US most regulations are generally applied only to the products of biotechnology, not to the processes. It is focused on whether the products are safe for use, or 'generally recognized as safe' (GRAS) (Cowan, 2010, 2011; Bazer *et al.*, 2010). The US FDA and the USDA are primarily responsible for the regulation of animal biotechnology in the US. The FDA is responsible for the regulation of food safety issues for food animals produced by biotechnology, any environmental issues caused by these animals, and the regulation of drug safety. Concurrently, the USDA, with the animal and plant health inspection service (APHIS) and the food safety inspection service, regulates food products produced by animal biotechnology (Cowan, 2010, 2011; Cowan, 2010; Bazer *et al.*, 2010).

In 2009, the FDA released its final guidance statement regarding genetically engineered animals and products regulation. They defined GE animals as "those animals modified by recombinant DNA techniques, including the entire lineage of animals that contain the modification." These modifications are made with the purpose to 'alter the structure or function' of the animals involved. The process of registration and approval for use of new GE animals must follow the FDA's 'new animal drug' procedures. Currently, the FDA is working on the approval of the first GE animal to be used for human consumption. Although AquAdvantage® salmon were declared to pose no risk for human consumers in August 2010 (Cowan, 2010), the process began more than 10 years ago. Final approval will not be given for the salmon until studies of environmental issues are completed, possibly in 2013. However,

the FDA approved the first biopharmaceutical product produced by a transgenic animal (goats) in 2009. This was ATryn®, antithrombin III manufactured by GCC-Biotherapeutics.

When a product from animal biotechnology comes to the market, it is subject to FDA and APHIS labeling requirements. It is considered illegal to introduce food from a GE animal into the food supply without previous approval by FDA. For example, in food biotechnology, those products considered GRAS that are equivalent to food products that are already on the market, such as milk from cows receiving BST do not need to be labeled. However, those foods derived from GE animals that have altered genomes are not considered substantially equivalent and are required to be labeled. Attempts to create state laws for labeling of food produced by biotechnology have failed. Ethical questions regarding such labeling concern the right of the consumers to know the process by which food is produced. FDA is not allowed to consider those ethical questions. Its authority just covers safety issues (Cowan, 2011; Bazer *et al.*, 2010).

European Union and China

Outside the US, the regulation of animal biotechnology can differ a little. The European regulators, in contrast to the American, consider the biotechnology itself as a novel process that requires regulation (Bazer *et al.*, 2010). In the European Union, the European Medicines Agency regulates the approval of pharmaceuticals, whereas the European Food Safety Authority was set up in 2002 to be responsible for scientific risk assessment of food biotechnology. In addition, the European parliament and member states handle the risk management policy. The distribution of genetically modified organisms (GMOs) and GMOs used in food products are regulated by the Directive 2001/18/EC. This Directive requires notification before a product derived from genetic engineering comes to market. Furthermore, it is required that each product containing GMOs be labeled with the sentence: "This product contains genetically modified organisms" (Bazer *et al.*, 2010).

Animal biotechnology in China is regulated by three main agencies: the Ministry of Health, the Ministry of Science and Technology, and the Ministry of Agriculture. Although there is little formal legislation, there have been several statements concerning GMOs, whereas those statements formally apply to animals. Almost all enforcement involves the importation and production of plants instead of animals (CAST, 2010). In China, in contrast to Europe and US, there are no regulations related specifically to animal cloning. Instead, they have regulations regarding human cloning, leaving open all other research. The research in China is more lightly regulated and controlled than clinical and commercial applications. In 2004, an attempt to regulate animal welfare failed. Recently, new attempts have begun, but no regulations have been proposed (Bazer *et al.*, 2010).

Multimedia Annexes

PDF on Artificial Insemination

This PDF gives a basic overview of artificial insemination, beginning with semen collection and ending with the insemination procedure. It includes details such as the optimal thawing temperature for sperm and the accuracy of sperm-sorting procedures. It also details the advantages of disadvantages conferred by artificial insemination.

PDF on *In Vitro* Fertilization

This PDF gives a general overview of the IVF procedure, including the beginning steps of oocyte collection and ending with embryo culture. The PDF was designed for a laboratory course, therefore it includes exact reagents and volumes for the IVF procedure of cattle. It also includes information on embryo culture of other species. ICSI is also discussed.

PDF of the Food and Drug Authorities Final Guidelines on Genetically Engineered Animals

This is the document the FDA released in 2009 (and revised in 2011) that details their current policies on GE animals. These policies include information on introducing transgenic animals into the food supply, environmental concerns, record keeping, and many other topics. The guidelines are very thorough and detailed.

PDF on Molecular Markers

This file has a description of classical technologies involved in the development of molecular markers. Molecular markers discussed include RFLP markers, randomly-RAPD markers, amplified fragment length polymorphism (AFLP®) markers, microsatellite markers, SNP markers, sequence characterized amplified region (SCAR) markers, cleaved amplified polymorphic sequences, intersimple sequence repeat markers, and polymerase chain reaction-based sequence tagged site markers.

See also: Biotechnology: Pharming. Biotechnology: Regulatory Issues. Cloning Animals by Somatic Cell Nuclear Transplantation. Genomics of Food Animals. Stem Cells. Transgenic Methodologies – Plants

References

Aigner, B., Renner, S., Kessler, B., *et al.*, 2010. Transgenic pigs as models for translational biomedical research. Journal of Molecular Medicine (Berlin) 88 (7), 653–664.

Ajmone-Marsan, P., Garcia, J.F., Lenstra, J.A., 2010. On the origin of cattle: How aurochs became cattle and colonized the world. Evolutionary Anthropology: Issues, News, and Reviews 19 (4), 148–157.

Allan, M.F., Smith, T.P.L., 2008. Present and future applications of DNA technologies to improve beef production. Meat Science 80 (1), 79–85.

Andersson, L., 2011. How selective sweeps in domestic animals provide new insight into biological mechanisms. Journal of International Medicine 271, 1–14.

Animal Biotechnology: Science-Based Concerns, 2002. National Research Council of the National Academies. Washington, DC: The National Academy Press.

Bavister, B.D., 2002. Early history of *in vitro* fertilization. Reproduction 124 (2), 181–196.

Bazer, F., Einsiedel, E., Gaskell, G. Murray, J., Riley, M., 2010. Ethical implications of animal biotechnology: Considerations for animal welfare decision making.

Animal Agriculture's Future through Biotechnology, Part 9, Number 46. USA: Council for Agricultural Science and Technology.
Beja-Pereira, A., Caramelli, D., Lalueza-Fox, C., *et al.*, 2006. The origin of European cattle: Evidence from modern and ancient DNA. Proceedings of the National Academy of Sciences of the USA 103 (21), 8113–8118.
Brackett, B.G., 2001. Advances in animal *in vitro* fertilization. In: Wolf, D.P., Zelinski-Wooten, M. (Eds.), Contemporary Endocrinology: Assisted Fertilization and Nuclear Transfer in Mammals. Totowa, NJ: Humana Press Inc., pp. 21–51.
Brookfield, F.Y., 2010. Q&A. Promise and pitfalls of genome-wide association studies. BMC Biology 8, 41.
Brophy, B., Smolenski, G., Wheeler, T., *et al.*, 2003. Cloned transgenic cattle produce milk with higher levels of beta-casein and kappa-casein. Nature Biotechnology 21 (2), 157–162.
Brumlop, S., Finckh, M.R., 2010. Applications and Potentials of Marker Assisted Selection (MAS) in Plant Breeding. Bonn, Germany: Bundesamt für Naturschutz (BfN), Federal Agency for Nature Conservation.
Brundige, D.R., Maga, E.A., Klasing, K.C., Murray, J.D., 2008. Lysozyme transgenic goats' milk influences gastrointestinal morphology in young pigs. Journal of Nutrition 138 (5), 921–926.
Büeler, H., Aguzzi, A., Sailer, A., *et al.*, 1993. Mice devoid of PrP are resistant to scrapie. Cell 73 (7), 1339–1347.
Chen, K., Baxter, T., Muir, W.M., Groenen, M.A., Schook, L.B., 2007. Genetic resources, genome mapping and evolutionary genomics of the pig (*Sus scrofa*). International Journal of Biological Science 3 (3), 153–165.
Chen, S.-Y., Duan, Z.-Y., Sha, T., *et al.*, 2006. Origin, genetic diversity, and population structure of Chinese domestic sheep. Gene 376 (2), 216–223.
Chessa, B., Pereira, F., Arnaud, F., *et al.*, 2009. Revealing the history of sheep domestication using retrovirus integrations. Science 324 (5926), 532–536.
Council for Agricultural Science and Technology (CAST), 2010. Ethical Implications of Animal Biotechnology: Considerations for Animal Welfare Decision Making. Issue Paper No. 46. Ames, Iowa: CAST.
Cowan, T., 2010. Biotechnology in Animal Agriculture: Status and Current Issues. Washington, DC: Congressional Research Service.
Cowan, T., 2011. Agricultural Biotechnology: Background and Recent Issues. Washington, DC: Congressional Research Service.
Dalton, J.C., 1999. Factors important to the efficiency of artificial insemination in single-ovulating and superovulated cattle. PhD Dissertation, Virginia Polytechnic Institute and State University.
Damak, S., Su, H., Jay, N.P., Bullock, D.W., 1996. Improved wool production in transgenic sheep expressing insulin-like growth factor 1. Biotechnology 14 (2), 185–188.
Diamond, J., 1997. Guns, Germs, and Steel: The Fates of Human Societies. Norton, NY: WW Norton & Company.
Fernández, H., Hughes, S., Vigne, J., *et al.*, 2006. Divergent mtDNA lineages of goats in an early neolithic site, far from the initial domestication areas. Proceedings of the National Academy of Sciences of the USA 103 (42), 15375–15379.
Fiester, A., 2005. Ethical issues in animal cloning. Perspectives in Biology and Medicine 48 (2), 328–343.
Fishman, J.A., Patience, C., 2004. Xenotransplantation: Infectious risk revisited. American Journal of Transplantation 4 (9), 1383–1390.
Floros, J.D., Newsome, R., Fisher, W., *et al.*, 2010. Feeding the world today and tomorrow: The importance of food science and technology – an IFT scientific review. Comprehensive Reviews in Food Science and Food Safety 9 (5), 572–599.
Gray, M.M., Granka, J., Bustamante, C., *et al.*, 2009. Linkage disequilibrium and demographic history of wild and domestic canids. Genetics 181 (4), 1493–1505.
Grubman, M.J., 2005. Development of novel strategies to control foot-and-mouth disease: Marker vaccines and antivirals. Biologicals 33 (4), 227–234.
Guillaume, F., Fritz, S., Boichard, D., Druet, T., 2008. Estimation by simulation of the efficiency of French marker-assisted selection program in dairy cattle. Genetics, Selection, Evolution 40 (1), 91–102.
Haasnoot, P.C., Cupac, D., Berkhout, B., 2003. Inhibition of virus replication by RNA interference. Journal of Biomedical Science 10 (6), 607–616.
Hale, E.B., 1969. Domestication and the evolution of behavior. In: Hafez, E.S.E. (Ed.), The Behaviour of Domestic Animals. London: Baillière, Tindall & Cassell, pp. 22–42.
Havlicek, V., Lopatarova, M., Cech, S., *et al.*, 2005. *In vivo* culture of bovine embryos and quality assessment of *in vivo* vs. *in vitro* produced embryos. Veterinární Medicína 50 (4), 149–157.
Hirata, M., 2004. Forage crop production. Encyclopedia of Life Support Systems (EOLSS). Oxford, UK: Eolss Publishers.
Hirata, R.K., Xu, C., Dong, R., *et al.*, 2004. Efficient PRNP gene targeting in bovine fibroblasts by adeno-associated virus vectors. Cloning Stem Cells 6 (1), 31–36.
Horiuchi, T., Numabe, T., 1999. Intracytoplasmic sperm injection (ICSI) in cattle and other domestic animals: Problems and improvements in practical use. Journal of Mammalian Ova Research 16 (1), 1–9.
Jabed, A., Wagner, S., McCracken, J., Wells, D.N., Laible, G., 2012. Targeted microRNA expression in dairy cattle directs production of β-lactoglobulin-free, high-casein milk. Proceedings of National Academy of Science of the USA 109 (42), 16811–16816.
Jensen, P., 2006. Domestication – From behaviour to genes and back again. Applied Animal Behaviour Science 97 (1), 3–15.
Joung, J.K., Sander, J.D., 2013. TALENS: A widely applicable technology for targeted genome editing. Nature Reviews Molecular Cell Biology 14 (1), 49–55.
Kochhar, H.P.S., Evans, B.R., 2007. Current status of regulating biotechnology-derived animals in Canada – Animal health and food safety considerations. Theriogenology 67 (1), 188–197.
Lai, L., Kang, J.X., Li, R., Wang, J., *et al.*, 2006. Generation of cloned transgenic pigs rich in omega-3 fatty acids. Nature Biotechnology 24 (4), 435–436.
Larson, G., Albarella, U., Dobney, K., *et al.*, 2007. Ancient DNA, pig domestication, and the spread of the Neolithic into Europe. Proceedings of the National Academy of Sciences of the USA 104 (39), 15276–15281.
Long, D.B., Zhang, K.Y., Chen, D.W., Ding, X.M., Yu, B., 2009. Effects of active immunization against myostatin on carcass quality and expression of the myostatin gene in pigs. Animal Science Journal 80 (5), 585–590.
Luikart, G., Gielly, L., Excoffier, L., *et al.*, 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. Proceedings of the National Academy of Sciences of the USA 98 (10), 5927–5932.
Meuwissen, T.H.E., Hayes, B.J., Goddard, M.E., 2001. Prediction of total genetic values using genome-wide dense marker maps. Genetics 157 (4), 1819–1829.
Oltenacu, P.A., Broom, D.M., 2010. The impact of genetic selection for increased milk yield on the welfare of dairy cows. Animal Welfare 19 (1), 39–49.
Pedrosa, S., Uzun, M., Arranz, J.-J., *et al.*, 2005. Evidence of three maternal lineages in near eastern sheep supporting multiple domestication events. Proceedings of the Royal Society B: Biological Sciences 272 (1577), 2211–2217.
Prather, R.S., Sutovsky, P., Green, J.A., 2004. Nuclear remodeling and reprogramming in transgenic pig production. Experimental Biology and Medicine 229 (11), 1120–1126.
Reh, W., Maga, E.A., Collette, N.M., *et al.*, 2004. Hot topic: Using a stearoyl-CoA desaturase transgene to alter milk fatty acid composition. Journal of Dairy Science 87 (10), 3510–3514.
Richt, J.A., Kasinathan, P., Hamir, A.N., *et al.*, 2007. Production of cattle lacking prion protein. Nature Biotechnology 25 (1), 132–138.
Sax, K., 1923. The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8 (6), 552–560.
Senger, P.L., 2003. Pathways to Pregnancy and Parturition, second ed. Pullman, WA: Current Conceptions, Inc.
Skoglund, P., Götherström, A., Jakobsson, M., 2011. Estimation of population divergence times from non-overlapping genomic sequences: Examples from dogs and wolves. Molecular Biology and Evolution 28 (4), 1505–1517.
Tessanne, K., Golding, M.C., Long, C.R., *et al.*, 2012. Production of transgenic calves expressing an shRNA targeting myostatin. Molecular Reproductive and Development 79 (3), 176–185.
Thoday, J.M., Boam, T.B., 1961. Regular responses to selection. I. Description of responses. Genetics Research 2, 161–176.
Urnov, F.D., Rebar, E.J., Holmes, M.C., Zhang, H.S., Gregory, P.D., 2010. Genome editing with engineered zinc finger nucleases. Nature Reviews Genetics 11 (9), 636–646.
Van Berkel, P.H., Welling, M.M., Geerts, M., *et al.*, 2002. Large scale production of recombinant human lactoferrin in the milk of transgenic cows. Nature Biotechnology 20 (5), 484–487.
Vigne, J.D., Zazzo, A., Saliège, J.F., *et al.*, 2009. Pre-neolithic wild boar management and introduction to Cyprus more than 11,400 years ago. Proceedings of the National Academy of Sciences of the USA 106 (38), 16135–16138.
Von Baeyer, E., 2010. World Environmental History. The Development and History of Horticulture. Encyclopedia of Life Support Systems (EOLSS). Oxford, UK: Eolss Publishers.
Wells, G.A., Scott, A.C., Johnson, C.T., *et al.*, 1987. A novel progressive spongiform encephalopathy in cattle. Veterinary Record 121 (18), 419–420.
Wheeler, M.B., Bleck, G.T., Donovan, S.M., 2001. Transgenic alteration of sow milk to improve piglet growth and health. Reproduction Supplement 58, 313–324.
Wu, X., Ouyang, H., Duan, B., *et al.*, 2012. Production of cloned transgenic cow expressing omega-3 fatty acids. Trasgenic Research 21 (3), 537–543.

Zeder, M.A., Emshwiller, E., Smith, B.D., Bradley, D.G., 2006. Documenting domestication: The intersection of genetics and archaeology. Trends in Genetics 22 (3), 133–159.

Relevant Websites

http://www.nature.com/news/animals-engineered-with-pinpoint-accuracy-1.11506?WT.ec_id=NEWS-20121002
Animals engineered with pinpoint accuracy.

http://www.nifa.usda.gov/nea/animals/in_focus/reproduction_if_assisted.html
Assisted reproductive technologies.

http://emedicine.medscape.com/article/432418-overview#a1
Biologic barriers to xenotransplantation.

http://europa.eu/legislation_summaries/agriculture/food/l28130_en.htm
Deliberate release of genetically modified organisms.

http://www.roslin.ed.ac.uk/public-interest/dolly-the-sheep/
Dolly the sheep.

http://www.fao.org/docrep/004/T0117E/T0117E02.htm#ch2.6
Embryo transfer.

http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/PoliciesProceduresManual/default.htm
FDA's animal policies and procedures manual.

http://cmr.asm.org/content/17/2/465.long
Foot and mouth disease review.

http://www.clonesafety.org/cloning/facts/process/
Process of cloning.

http://www.prrs.org/default.aspx#.UVx-doXHVG5
PRRS eradication project.

http://mmbr.asm.org/content/67/4/657.full
RNA interference.

http://www.fda.gov/animalveterinary/safetyhealth/animalcloning/ucm124765.htm
Somatic cell nuclear transfer.

http://www.nature.com/news/synthetic-vaccine-could-prevent-future-outbreaks-of-foot-and-mouth-disease-1.12700
Synthetic vaccine for FMD.