

Cloning in farm animals: Concepts and applications

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ABSTRACT

Cloning is a scientific term used to describe the process of genetic duplication. The most commonly applied and recent technique is nuclear transfer from somatic cell, in which the nucleus from a body cell transferred to an egg cell to create an embryo that is virtually genetically identical to the donor nucleus. Cloning has started early in the development of biological science during the Theodor Schwann period of cell theory. The application of animal cloning are rapid multiplication of desired livestock, animal preservation, research models, human cell based therapies, xenotransplantation and gene farming. However, cloning of animals is restricted to highly advanced laboratories in developed countries. The problems associated with farm animal cloning has been reviewed and discussed in this paper which includes incomplete reprogramming, placental abnormalities, parturition difficulties, postnatal death, clone specific phenotype and lung emphysema as well as socio-cultural problems. Most of the problems were molecularly explained as the failure of genetic reprogramming. In Ethiopia this type of science is not yet conceived and concerned body should pay attention to such a valuable aspect of biotechnology.

Keywords: Embryo twinning, dolly, recombinant DNA technology, xenotransplantation.

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INTRODUCTION

Molecular biology has enhanced our knowledge of genetically phenomena in an extra-ordinary manner in a brief period although it is still the basic tools of classical genetic that are utilized to handle problems of heredity. The most spectacular spin-off molecular genetics is the birth of the recombinant DNA technology (gene cloning). So that, genetic engineering is direct manipulation hereditary material based on a set of molecular techniques (Mitra, 1996).

Cloning refers to producing genetically identical individual to donor cells and copying gene, which involves the creation of an animal or individual that derives its genes from a single other individual; it is also referred as "Asexual reproduction" (Baurdon, 1997; Reik and Wolf, 2007).

No reproductive technology has greater potential to change the way we breed animal than cloning. If cloning becomes feasible, the commercial herd and flocks of today could be replaced by clonal lines, population of individual (presumably highly select ones) that are genetic equivalent of identical twins (Baurdon, 1997).

Cloning technology has the potential to stimulate development of the animal biotechnology industry in many countries of the world, as well as provide conservationists with an additional tool to assist with conserving critically endangered wild life species (Vajita et al., 2004).

Cloned offspring in human and farm animals sometimes produced in nature when early embryo splits in to two (or sometime, more) species of just a few days after fertilization, before the cells have become too specialized. However, there are a number of artificial methods to produce genetically identical mammals. Of these, the nuclear cloning technology is considered to have the greatest potential application for animal agriculture and medicine (Wells, 2005).

Scientists have been attempting to clone animals through nuclear transfer of somatic cell (SCNT) for several decades. They cited several potential benefits, for example, an efficient way to create herds of genetically modified cloned animals, preservation of endangered species, production of human therapeutic proteins in

genetically modified clone animals, use of genetically modified cloned animals as a source of organs for human transplantation, gaining a better understanding of cellular differentiation and reprogramming capabilities that could be the basis for human cellular therapies, and better models to study new treatments for human disease. However, SCNT cloning thus far has been very inefficient process and cloned animals have exhibited serious health problems (Wilmut, 2003).

HISTORY OF CLONING

The story really begins in 1839, when Theodor Schwann launched the cell theory, "all cells come from cells." As applied to development, the cell theory requires two identical daughter cells. After the first cleavage division each cell on its own could produce a whole embryo, or would one produce a left and one a right half, or one a front and one a back (Weismann, 1902).

In 1888, Wilhelm Roux attempted to answer this question by damaging one cell of a two-cell frog embryo with a hot needle. The cell stayed in place but did not develop further; its partner developed in to a left or right half-embryo. Sadly, this pioneering effort gave a misleading result. It supports his long-held, erroneous belief that all development and cell differentiation depends on loss of hereditary material (Vajita et al., 2004). Hans Driesch, before the SCNT technique was developed, used other method to obtain genetically identical organism, soon disproved this theory. In late 19th century, Hans Driesch created genetically identical sea urchins by dividing a two-cell stage embryo into two separate cells, which each formed a new embryo that developed in to sea urchins (Gail and Javitt, 2006).

In 1901, Hans Speimann found that if the first two cells of amphibian embryos were separated, two normal tadpoles could develop. It seemed that Roux's result was unfortunate artifact due to the inhibitory effect of the damaged cells (Maclaren, 2000). In 1902, Walter Sutton proves that chromosomes contain genetic information. Hans speimann ran a series of experiments using salamanders. Speimann separated a two-celled embryo of a salamander to create two individual salamanders. Speimann took the process a step further when he was able to produce a salamander from one cell that he had removed from a 16-cell stage embryo. From this experiment, he concluded that an early embryo cell contains all the genetic information necessary to create a new organism (Gail and Javitt, 2006).

In 1928, Hans Speimann was conducted the first nuclear transfer experiment from point view of previous his trial. Experiments along these lines emboldened speimann in 1938 to propose a "tough experiment". Cloning with differentiated cells nuclear transfer first envisioned and then he published his book "Embryonic development and induction," where he wrote up the result

of the nuclear transfer experiment in 1928. He also proposed that experiments on cloning organisms by extracting the nucleus of a differentiated cell and inserting it in to an enucleated fertilized egg. However, Speimann did not know how to do it and technique to carry out such a traumatic series of manipulation. After 58 years, Speimann articulated his fantastic experiments, when Dolly was born (Maclaren, 2000).

In 1952, Robert Briggs and Thomas king electrified the biological world by reporting successful nuclear transfer in the frog *Ran* pipes. The nuclei came initially from undifferentiated blastula cells; they were transferred to unfertilized eggs from which the nuclei had previously been removed. Once the eggs have been stimulated to develop, some produced normal tadpoles. Where nuclei were taken from gastrula cells, the next developmental stage after blastulae, and the position of normal tadpoles was much lower. By 1960, Briggs and king concluded that differentiation was accompanied by progressive restriction of the capacities nuclei to promote all the various types of differentiation on required for normal development (Brigg and King, 1952).

In 1973, Boyer and Cohen created the first recombinant DNA organism using recombinant DNA technology, or gene-splicing, which allow the manipulation of DNA. They remove plasmid from a cell. Using restriction enzymes, they cut the DNA at precise positions and then recombined the DNA strands in their own way using DNA ligase enzyme. They then inserted the altered DNA into *E. coli* bacteria to produce specific protein. This technology was a major break through for genetic engineering (Wells, 2005).

In fact, nuclear transfer was used in 1952, to study early development in frogs (Brigg and King, 1952). In 1990s, the technique was used to clone cattle and sheep, using cell taken from early embryos. In 1995, Ian Wilmut, Keith Campbell, and colleagues at the Roslin institute in Scotland created live lambs-Megan and Morag-from embryo derived cells that had been cultured in the laboratory for several weeks. In 1996, Dolly was born a nucleus extracted from mammillary cell of a six-year old sheep (Gail and Javitt, 2006). Though Dolly was the first cloned mammal, the first vertebrate to be cloned was a tadpole in 1952 (MCFarland and Dougla, 2000). With the advancement of the cloning the world has reaped the benefit in several ways including *in vitro*-fertilization to production of numerous therapeutics.

TECHNIQUES OF CLONING

Artificial embryo splitting (embryo twinning)

Embryo splitting is a method of cloning, where an embryo is splitting in the maturation (i.e. into single cell or blastomere) soon after fertilization of the egg by the sperm before embryo transfer. It is optimally performed at

the 6-to 8-cell stage, where it can be used as an expansion of in vitro fertilization (IVF) to increase the number of available embryos. To implant the embryo in a surrogate mother, allowing it to develop fully off spring produced by embryo splitting will be identical (mono zygote) twins (Morley, 2002).

Embryo splitting changes neither the age nor the (toti-) potency of the cell used. The two embryos from the splitting are in the same stage of development, exactly the same age as the same stage of development, exactly the same age as the undivided embryo would have been and genetically completely identical (TAB, 1998).

Somatic cell nuclear transfer (SCNT)

The transfer of a cell nucleus from a body cell into an egg from which the chromosomes have been removed or inactivated; this method is used for cloning of organism. Once the transferred genome in the egg cell and one cell embryo is created, the process of cloning is completed and further development of the clone can occur (Reik and Wolf, 2007).

Reproductive cloning

SCNT, pass through process, to create embryo that are implanted into surrogate mother and brought to full term. The resulted organism will be clones of the cell donor, e.g. dolly (Morley, 2002). In SCNT the nucleus, which contains the organism's DNA, of somatic cell (body cell other than sperm or egg cell) is removed and at the same time, the nucleus of an egg cell is removed. Then the nucleus of somatic cell is inserted into an unfertilized enucleated egg cell. After being inserted into the egg, the somatic cell nucleus is reprogrammed by host cell. The egg, now containing the nucleus of a somatic cell, is artificially induced (stimulated) with electrical shock and will begin to divide after many mitotic divisions in culture, forms a blastocyst. Thus the embryo is implanted (transferred to a uterus) and brought to term, in an organism that contains the identical nuclear DNA as the donor of the somatic cell, although it may not contain the same mitochondrial DNA as the donor since the presence of mitochondrial DNA in the enucleated egg cell, which will differ from that of the mitochondrial DNA in the somatic cell donor (Gail and Javitt, 2006; Morley, 2002).

Therapeutic (research) cloning

The goal is to harvest embryo stem cell at blastocyst stage that have a genome identical to the donor's cell, which can be used to study human development and potentially to treat disease. While a clonal human,

blastocyst has been created, stem cell lines are yet to be isolated from a cloned source (Morley, 2002; Semb, 2005).

A potential use of genetically customized stem cell should be to create cell lines that have genes linked to the particular disease. For example if a person with Parkinson's disease donated his/her somatic cells, the stem cell resulting from SCNT would have gene that contribute to Parkinson's disease. In this situation, the disease specific stem cell lines would be studied in order to better understand the disease. In another situation, genetically-customized stem cell lines would be generated for cell-based therapies to transplant to the patient. The resulting cells would be genetically identical to the somatic cell donor, thus avoiding any complication from immune system rejection (Semb, 2005).

Use of recombinant DNA technology (transgenic animal)

The most important outcome of the application of transgenic biotechnology has been the production of recombinant proteins from bacteria. The first successes involved the production of recombinant growth hormone and insulin for human replacement therapy (Bacci, 2007).

The ability to produce offspring from cultured cells opens up relatively easy way to make genetically modified or transgenic animals. Such animals are important for research and can produce medically valuable human proteins. By introducing key human genes into mammals, biologists can induce dairy animals to produce therapeutic proteins in the milk (Jacnich, 1998).

The genetic engineer first constructs a transgene containing the gene of interest plus some of extra DNA, which correctly controls the function of the gene in the new animal. This transgene has then to be inserted into the new animal (Gravin et al., 1998).

Several transgenic techniques have been optimized to obtain transgenic animals, with different levels of efficiency in function of the species in which they had been employed. But out of them, two principal techniques are now available in order to modify an organism as a whole and subsequently to obtain a germ line transgenic animal; the delivery of exogenous DNA has to be performed at the one cell stage embryo (Wheeler, 2003).

Techniques used

Male pronuclear microinjection: The microinjection of male pronuclear with naked DNA is the first technique successfully utilized to obtain transgenic animals (Gordon et al., 1980; Krimpenfort et al., 1991).

In this method, eggs (oocytes) are harvested from super ovulated (matured) animal and fertilized *in vitro*. A

microtubule is used to hold the fertilized egg in place and extremely fine needle used to inject a tiny amount of solution, which containing many copies of the foreign DNA (transgene), into the male pronuclear. These eggs are then introduced into the oviducts of surrogate females. This is the main method currently used to produce genetically modified animals and involves the physical injection of 200-300 copies of the foreign gene into surrogate mothers. Genes can only be added (not deleted) by this method (Gravin et al., 1998).

The insertion of the DNA; however, is a random process and there is a high probability that the introduced gene will not insert it self into the desired site on host DNA, that will permit its expression. Fertilized ovum is transferred into the oviduct of recipient female or foster mother that has been induced to act as a recipient by mating with a vasectomized male (Gordon et al., 1980).

Sperm mediated gene transfer (SMGT): This technique relies on the intrinsic ability of sperm cells to bind and internalize exogenous DNA and to transfer it into the oocyte at fertilization. This technique is the first optimizer for application in small laboratory animals, showing high efficiency for mice. Subsequently, SMGT was successfully adapted and optimized for use in large animals. The team produced human decay accelerating factor (hDAF) transgenic pig lines with high efficiency. Those transgenic pigs showed good protection against hyperacute rejection challenge in ex-vivo experiments. The researchers obtained a multigene transgenic pig in a one step protocol, simultaneously introducing three reporter genes. More recently, researchers' study showed that SMGT is suitable for the transformation of the animal genome with a non-viral episomal vector (Bacci, 2007).

SO FAR CLONED ANIMALS

Megan and Morag: The first mammal cloned from differentiated cell

Megan and Morag, two domestic sheep, were the first mammals to have been successfully cloned from differentiated cells. Megan and Morag were cloned at the Rollin institute in Edinburgh, Scotland in 1995 (Wilmut, 1998).

The team chooses to combine two approaches: microinjection and embryonic stem cells. In order to achieve this they decide to try to transfer the nucleus from one cell to another and stimulate this new cell to grow and become an animal, a process known as nuclear transfer. The team of the Roslin institute tried to make immortalized and undifferentiated embryonic stem cell line in sheep, but failed. As the result, they decide to work with cultured blastocyst cells. The nuclear material of these blastocyst cells would be transferred in to unfertilized sheep egg cell, an oocyte where the nucleus

had been removed to optimize the chance of successful nuclear transfer; they put the cultured cells in to a state of quiescence, which was similar state to that of the unfertilized egg cell (Hogland et al., 1997).

Nuclear transfer was done, using electrical stimuli both to fuse the cultured cell with enucleated egg and to kick start embryonic development from 244 nuclear transfers, 34 developed to a stage where they could be placed in the uterus of surrogate mothers. In the summer of 1995, five lambs were born, of which two-Megan and Morag-survived to become healthy fertile adults. These were the first mammals cloned from differentiated cells. They were born with the name 5LL2 and 5LL5 in June 1995 (Hogland et al., 1997).

The production of Megan and Morag demonstrated that nuclear transfer from cells, which have been cultured *in vitro*, could produce viable sheep. They signified the technical break through that made Dolly the sheep possible. The birth of Megan and Morag, a year before Dolly, with their huge beneficial potential, made no headlines. As of 2005, Megan was still alive and was the oldest cloned animal at the time (Gail and Javitt, 2006).

Dolly: The first mammals cloned from another adult animal

Dolly, a Finn Dorset ewe, was the first mammal to have been successfully cloned from an adult cell by using somatic nuclear transfer (Gail and Javitt, 2006; Maclaren, 2000; Wilmut et al., 1997). In 1996, Ian Wilmut and colleagues at the Roslin Institute in Scotland used a nucleus extracted from a mammary cell of a six-year-old sheep to create Dolly, born on July 5, 1996 and she lived until the age of six. She has been called "the world's most famous sheep" (Gail and Javitt, 2006; Lehrman and Sally, 2008).

In somatic nuclear transfer the cell nucleus from adult cell (e.g. Udder cell) is transferred in unfertilized oocyte (developing egg cell) that has had its nucleus removed. The hybrid cell is then stimulated to divide by an electric shock, and when it develops in to a blastocyte implanted in to a surrogate mother (Campbell et al., 1996). The production of Dolly showed that genes in the nucleus of such a mature differentiated somatic cell are still capable of reverting back to an embryonic (toti-) potent state, creating a cell that can then go on to develop in to any part of an animal (Neimann et al., 2008). However, this reprogramming process is not perfect and embryos produced by nuclear transfer often show abnormal development and because of these difficulties, cloning mammals by nuclear transfer is still highly inefficient, with Dolly as the only lamb that survived to adulthood from 277 attempts (278th attempt). However, her birth is still recognized as one of the major stepping-stones in the development of modern biology (Wilmut et al., 1997; Jaenich et al., 2005). Since the birth of Dolly, reproductive cloning using SCNT has been used to

produce a variety of mammals (Gail and Javitt, 2006).

Polly and Molly: The first genetically engineered (genetic modification carried out) cloned sheep, which were born in 1997

By 1997, Polly and Molly, two ewes born, have been successfully cloned from an adult somatic cell (a fetal fibroblast) in to which had been inserted agene (transgene) for valuable pharmaceutical protein, the human blood-clotting factor IX (Semb, 2005; Graven et al., 1998).

The two ewes were the first mammals to have been cloned from an adult somatic cell and to be transgenic animal at the same times, which building the experiments of somatic nuclear transfer that had led to the cloning of Dolly. Creating Polly and Molly injected in their DNA a new gene which gene chosen of a therapeutic value to human to demonstrate the potential of such recombinant DNA technology combined with animal cloning to produce pharmacological and therapeutic proteins to treat human diseases (Semb, 2005).

The sheep Dolly, Megan and Morag have been cultured *in vitro*. In case of Polly and Molly, somatic cells were cultured *in vitro*, but additionally in this case they were transferred with foreign DNA, and transfected cells which stably integrated this new piece of genetic information were selected. The nuclei of these somatic cells were then transferred into an empty oocyte, as in the procedure of nuclear transfer and this was used to produce several transgenic animals. Hence, Transgene that was inserted in the donor somatic cell was designed to express human clotting factor IX protein in the milk of sheep (Schnieke, 1997).

Futhi (replicate in Zulu): African's first cloned (nuclear transferred) healthy calf, produced with handmade cloning (HMC)

The first used of this method was establishment of donor cell lines from ear skin notches from 9-year old Holstein cow, a former South Africa milk production record holder. Then for nuclear transfer, actively growing fibroblast cultures were made available for the nuclear transfer process. Bovine oocyte from slaughter house (abattoir) derived ovaries were collected and matured for 21 h. In modified TCM-199 medium supplemented with 15% of cattle serum, 10IU MI-I ECG and 15I Uml-1hCG nuclear transfer was performed using the HMC technique (Vajita et al., 2003). At 21 h after the start of maturation, cumulus cells and zona pellucidiae were removed and oocytes were randomly bisected by hand. Then reconstructed embryos were activated with calcium ionosphere and dimethylaminopurine. Finally for embryo transfer, in two consecutive experiments, six blastocysts

were produced from 52 reconstructed embryos. On 7th day, five blastocysts were selected and transferred into three previously synchronized recipients. All the three recipients become pregnant, but two of the recipients aborted at six and seven months, respectively. The third pregnancy went to term, and a healthy calf, weighing 27 kg, was delivered by caesarean section (Figure 1). The three-month-old calf was raised by a surrogate Jersey cow under standard dairy conditions. Microsatellite DNA analysis of hair sample showed that the offspring matched 100% with that of the somatic cell donor cow (Vajita et al., 2004; Farin and Zuelke, 2004).

Cloning extinct and endangered species

In 2001, Asian Noah guar the first endangered species cloned, which nucleus cells was taken from a cow named, Bessie, gave birth to a cloned Asian guar, but the calf died after two days (Pence and Gregory, 2005). In 2002, geneticists at the Australian museum announced that they had replicated DNA of the Thylacine (Tasmanian tiger), extinct for about 65 years previous, using polymerase chain reaction (BBC News, 2003; Holloway and Grant, 2002).

In 2003, abanteng was successfully cloned, followed by three African wild cats from a thawed frozen embryo (Pence and Gregory, 2005). In 2009, the Pyrenean ibex, a form of wild mountain goat, which was officially declared extinct in 2000, was cloned. Using DNA from skin samples kept in liquid nitrogen the scientists manage to clone the ibex from domestic goat egg cells. The newborn ibex died shortly after birth due to physical defects in its lungs." The Frozen Zoo" at the San Diego Zoo has stores frozen tissue from the world's rarest and most endangered species (Pence and Gregory, 2005).

APPLICATION OF CLONING TECHNIQUE

Rapid multiplication of desired livestock

Cloning could enable the rapid dissemination of superior genotypes from nucleus breeding flock and herds, directly to commercial farmers. Genotype could be provided that are ideally suited for specific product characteristics, disease resistance or environmental conditions. Cloning could be extremely useful in multiplying outstanding F1 cross, breed animals or composite breeds, to maximize the benefit of both hetrosis and potential uniformity with in the clonal family. These genetic gains could be achieved through the controlled release of selected lines of elite live animals or cloned embryo (Wells, 2005).

Disease resistance could be an early target, but the most immediate impacts of nuclear transfer cloning that they are likely to see will be animal producing valuable



Figure 1. Futhi ('replica' in Zulu), African's first cloned animal 6 months after the birth. Source: (Gravin et al., 1998).

pharmaceutical products in their milk or even urine ("pharming"), or producing milk lacking proteins to which babies allergic, or milk or meat with enhance nutritional value or with high quality (Maclaren, 2000).

Animal preservation

Cloning can be used along with other forms of assisted reproduction to help preserve indigenous breed of livestock, which have production trial and adaptability to local environments that should not be lost from the global gene pool (Wells et al., 1998).

By using cloning, it may be possible to recover species that became extinct, provided some of their cells are preserved by freezing. This possibility provides a strong incentive to maintain tissue banks for endangered species of animals (Pence and Gregory, 2005).

Cloning becomes a viable tool for preserving endangered species. E.g., in January 2009, scientists from the Centre of Food Technology and Research of Aragon, in Zaragoza announced the cloning of the Pyrenean ibex (a form of wild mountain goat), which was officially declared extinct in 2000. Using DNA from skin samples kept in liquid nitrogen (frozen) the scientists managed to clone the ibex from domestic egg cells newborn ibex died shortly after birth due to physical

defect in its lungs. However, it is the first time an extinct animal has been cloned, and may open doors for saving endangered species and newly extinct species by resurrecting them from frozen tissue (Pence and Gregory, 2005; Trounson, 2006).

Research models

Set of cloned livestock animals could be effectively used to reduce genetic variability and reduce the numbers of animals needed for some experimental studies. This could be conducted on a larger scale than is currently possible with naturally occurring genetically identical twins (Mackle et al., 1999). Either e.g., lamb cloned from sheep selected for resistance or susceptibility to nematode worms will be useful in studies aimed at discovering, novel genes and regulatory pathway in immunology (Hein and Griebel, 2003).

Human cell based therapies

This is the direct application of NT technology in human medicine; principally therapeutic cloning (Colman, 1999). Patient with particular disease or disorder in tissues that neither repair nor replace themselves effectively (e.g.

insulin dependent diabetes, muscularly dystrophy, spinal cord injury, certain cancers and various neurological disorders, including Parkinson's disease) could potentially generate their own immunologically compatible cell for transplantation, which would offer lifelong treatment with out tissue rejection. Initially, this approach could employ human NT and embryonic stem cells, the use of which is controversial (Collas and Hakelien, 2003).

Xenotransplantation

Xenotransplantation is the transplantation/implantation of cells, tissues or organs from one species to another such as from pigs to human. Such cells, tissue or organs are called xenografts/xenotransplant (Sachs, 1994). Transplantation makes it possible to alleviating the shortage of human heart and kidney, which are scarce in supply (Hogland et al., 1997).

For example, the most abundant non-human primate, the baboon, the hope was, that the baboon bone marrow which is resistance to HIV and a source of immune cells, could provide a replacement for the patients damaged immune system (White and Largford, 1998).

For long-term use, pigs may be a better choice of organ donor. Because pig organs are more similar to that of human in anatomical structure, physiological and biochemical characteristics, they are generally healthier than most primates and extremely easily to breed, producing a whole litter of piglets at a time and moral objection to kill pigs are fewer since they are slaughtered for food. In addition to the selection of pig as preferred donor based on the above consideration, an additionally significant and, perhaps most important advantage, is the ability to genetically modify the pig. This allows focusing on immunological point of view not only on the recipient of the graft but also on donor (Sachs, 1994).

Gene farming (pharmaceutical protein production)

Pharming is a production of human pharmaceuticals in farm animal. It has been undergoing application among biotechnologists since the development of the first mice in 1982 to produce a human TPA (tissue plaminogic activator) to treat blood clot (David, 1992). Animals as drug producers, involve the use of transgenic animals to manufacture (human) proteins with therapeutic use, in their milk (TAB, 1998). Transgenic sheep, goat and cattle are being used as a bioreactor to produce important human protein in milk. It use a method of transferring bacteria by inserting the gene coding, for proteins needed for the treatment of various hereditary diseases, such as special form of diabetes (Gravin et al., 1998).

Pharmaceutical products from transgenic animals, e.g. transgenic sheep, are α -antitrypsin or α PI, which its inherited deficiency leads to emphysema disease. It was

derived from blood plasma of human before the production in transgenic animals (Gravin et al., 1998).

DIFFICULTIES OF CLONING

In so far cloned animals, the success rate of cloning was found to be very rare. Most of the cloned animals have died shortly after delivery or in short age. There has been different reasons incriminated as the factor of inefficiency of cloning. One of the most explained factors is incomplete reprogramming. Furthermore, there are technical problems and socio-cultural difficulties. Here under is explanation of some of these problems.

Incomplete reprogramming (epigenetic reprogramming)

Epigenetic is the changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the under lying DNA sequence. These changes may remain through cell divisions for the remainder of then cells life and may last for multiple generations. However, there is no change in the underlying DNA sequence of the organism (Bird, 2007); instead, non-genetic factors cause the organism" genes to behave or express themselves differently (Hunter, 2008). It is done by inactivating some genes while inhibiting others (Reik and Wolf, 2007). Epigenetic reprogramming with clones shows inappropriate pattern of DNA methylation (David, 1992; Slotkin and Martiniessen, 2007), Chromatic modification (Santos et al., 2003), X-chromosomes in activation (Xue et al., 2002), expression of imprinting and non-imprinting genes (Humphrey et al., 2002).

The pattern of mortality and clone phenotypes observed presumably reflect the inappropriate expression of various genes, whose harmful effects are exerted at various different stage of development. Aberration that occurred early in embryonic or fetal development may impair health in adult hood (Wells, 2005; Slotkin and Martiniessen, 2007). There is a wide spectrum of phenotypic outcomes which appear natural and do not compromise the health and welfare of the animals, but epigenetic variations reduce the informality of in the clonal family, which may undesirable for some application. The common consequence of incomplete reprogramming following somatic cell NT are: placental abnormalities, high rate of pregnancy, difficulty parturition, high rate of post natal mortality, and some specific clone-associated phenotype adult hood (Wells, 2005).

Placental abnormalities

A failure of the placenta to develop and function correctly

is a common feature amongst clones. The majority of early pregnancy failures, before placentome formation, are attributed to an inadequate transition from yolk sack to allantoises derived nutrition, with poor allantoises vasculature in sheep. Furthermore, there is report indicated that evidence of immunological rejection contributing to early embryonic loss. Typically in cattle, 50 to 70% of pregnancies at day 50 are lost throughout the remainder of gestation and up to term. More commonly, cloned placenta only have half the normal numbers of placentomes, display compensatory over growth and are becomes edematous (Lee et al., 2004).

Of particular concern are the losses in the second half of gestation; especially the occurrence of Hydroallantoises. It does occur with other forms of assisted sexual reproduction in cattle, ranging incidence between 0.07 and 5% for artificial insemination (AI) and *in vitro* production (IVP) respectively. Comparatively with clones, however, typically 25% of those pregnant cows at day 120 of gestation develop clinical hydroallantosis (Wells, 2005).

Parturition difficulties (Dystocia)

Gestation period (length) in NT pregnancies is typically prolonged and birth weight of cloned calves may be 25% heavier than the normal. New born cloned calve display functional adrenal glands. Therefore, this extended gestation may be due to failure of the placenta to respond to fetal cortisol near term or lack of adrenocorticotrophic hormone release from fetus (Charatte-Palmer et al., 2002). The studies indicated that oversized cloned offspring add to the birth complication. They are comparatively larger than IVP, AI and natural mating (Rhind et al., 2004). Furthermore, the somatic cloned calve somatic cloned calve are heavier than embryonic clones (Wells, 2005).

Postnatal death

The viability of cloned offspring at deliver and up to weaning is reduced compared to normal, and this is despite greater than usual veterinary care. Data shows that around 80% of cloned calves delivered at term are alive after 24 h (Wells *et al.*, 2004). Two-third of mortality within this period is due to a spinal cord fracture syndrome through the cranial epiphyseal plate of the first lumbar vertebrae or to deaths that occurred either in utero or from dystocia due to placental abnormalities, new born cloned have altered neonatal metabolism and physiology (Charatte-Palmer et al., 2002).

Agricultural research shows, typically on additional 15% of calves initially born alive die before weaning (Wells et al., 2004). The most common mortality factors during this period are gastroenteritis and umbilical infections. Other

abnormalities like cardiovascular, musculoskeletal and neurological system, as well as lung infection and digestion disorder. Hydronephrosis is particularly common in sheep, with correspondingly elevated serum urea level in some surviving clones (Wells, 2005; Wells, 2003).

Clone specific phenotype

Although whilst studies indicated that clones can be physiologically normal and apparently healthy (Wells et al., 2004; Lanza et al., 2001). E.g. Dolly was euthanized at young age due to developed arthritis and lung tumour, which resulted largely from her indoor housing and handling rather than the fact she was a clone other observations and reports in literature of abnormal clone associated phenol types that become apparent during the juvenile and adult phase of life. The incidence of these anomalies may vary according to the particular species, genotype or cell type, or according to specific aspect of the NT and Culture protocol used (Shiels et al., 1999).

The cloned offspring syndrome is continuum, in that lethality or abnormal phenotype may occur at any phase of development, depending up on the degree of deregulation of key genes, presumably due to fundamental errors in epigenetic reprogramming (Humphrey et al., 2002).

Wells (2005) indicated that females mice cloned from cumulus cells develop an increased body weight phenotype, commencing 8 to 10 weeks after birth, which was directly attributable to increased adipose tissue. Evidence of compromised immuno-system is a clone phenotype noted in some species. Thymic aplasia has been documented in cloned cattle and low level of antibody production in cloned mice and of cytokines in cloned pig are direct indicators of a reduced immune response. This may increase their susceptibility to infection and disease.

Lung emphysema

Emphysema is a lung disease, which commonly occurs in transgenic cloned sheep. The lung tissue is damaged and cause breathing difficulties lung contains large numbers of neutrophil (WBC) that secrete enzymes which broken down foreign proteins. One of the important enzymes that the neutrophil secrete is elastase, which digests any foreign proteins or irritants, which enter the lung via air stream. The more irritants are in the air, the more elastase is produced are broken down the wall of the alveoli, which maintain the elasticity of the lungs due to imbalance of blood serum enzyme or deficiency of α 1-proteinase inhibitor which can bind to elastase, thus inactivating it and protecting the lung tissue from damage. Since large space, appear in the lungs, gas

exchange through surface area reduced, the lung become inefficient and susceptible to damage by pollutants (Gravin *et al.*, 1998).

Ethical and socio-cultural problems

Creating animals with genetic defects rises challenging ethical questions (White and Largford, 1998). The recent technological advancements that have allowed for cloning of animals and human have been highly controversial. Many religious organizations oppose all form of cloning. It is regarded as playing with God and to act against the will of God in all religions. On the grounds that life begins at conception. Most scientific, governmental and religious organizations oppose reproductive cloning since serious ethical concerns have been raised by the future possibility of harvesting organs from clones (Pence and Gregory, 2005).

The legal ethical considerations are associated with ethical considerations of fellow-creature status and an ethical judgment on animals cloning is an interference with creation which humans have no right to undertake. Anyone who feels that animal have intrinsic value or creature dignity will generally regard cloning as morally dubious, at least. The different social positions represent an effort to attract support for a specific moral or ethical ideal and also influence the political climate as well (TAB, 1998).

Currently, cloning is a matter of public discussion. It is rare that a field of science causes debate and challenge not only among scientists, but also among religious scholars, ethicist, governments, and politicians. One important concern is religious argument, various religions have different attitude towards the morality of these subjects, and even with in a particular religious tradition there is a diversity of opinions. The moral status of human embryo, the most sensitive and disputed point in the debate, is also discussed according to the Holy Qur'an teaching. The majority of Muslim jurists distinguish between reproductive and therapeutic cloning. Since cloning is an unnatural born of an individual, no one has the right to undertake it except God (Larijani and Zehedi, 2009).

CLONING IN ETHIOPIAN SITUATION

In Ethiopia no reports can be found about cloning. It is so difficult to perform or apply the technology not only because of technological insufficiency and financial limitation but also due to lack of adequate skill and knowledge. If possible, to apply the technology, it will be advantageous to recover species that have been extinct, provided some of their cells were preserved by freezing and it can be used to improve livestock production and in gene therapy.

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