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Agricultural and Biomedical Application of Animal Cloning: Review

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Abstract
Cloning is a creation of an organism which is called clone that that is a genetic copy of another organism or the donor by using different technique such as somatic cell nuclear transfer and embryo splitting. The advanced applications of cloning are biomedical applications which comprehend research model, bioreactors, and xenotransplantation. Rapid multiplication of desirable livestock and conservation of gene pools are enclosed under agricultural application. Particularly Cloning has the potential to improve the efficiency of trans-genesis in these applications, as well as a role for the multiplication of animals of proven production. In developing country science is not yet conceived and the concerned body should pay attention to such valuable aspects of biotechnological advancement.
Keywords: Bioreactors, Cloning, Embryo Splitting, Gene Pools

1. Introduction

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 (Reik, 2007). 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 (Wells, 2005).

Cloning farm animals has been technically feasible for several years. Several techniques are used, embryo splitting which has successfully been established for several livestock species. In sheep, 36% of embryos split as 2- and 4-cell embryos developed to term following transfer to recipient females. In cattle, embryos split into blastomeres at the 4-cell stage could further develop to term giving rise to multiple monozygotic healthy calves (Wells, 2005), but the most well-known being somatic cell nuclear transfer. This technology was used to create the sheep 'Dolly' in 1996. Since then this technology has been successfully applied to several other species (EGE, 2008).

Cloning holds the promise of by passing conventional breeding procedures to allow creation of thousands of precise duplicates of genetically engineered animals. In remote areas, where sampling and storage of adequate samples of semen and embryos is not practical, one could use clone samples from diverse animals for conservation of the available genetic diversity. The local breeds may contain valuable genes that confer adaptation, especially to heat tolerance or disease resistance, and there is an urgent need to prevent their extinction which can achieve by cloning techniques (Duszewska and Reklewski, 2007).

Most recently, there is growing scientific and public interest in using nuclear transfer techniques to facilitate production improvement and the rescue of endangered species, or even to restore them after the extinction of intact organisms (Rudenko *et al*., 2004). In view of this, the current review was undertaken with the following main objectives:

- ✓ To highlight agricultural and biomedical application of farm animal cloning and
- ✓ To provide a comprehensive and well-illustrated techniques of producing cloned animal

2. Techniques of Farm Animal Cloning

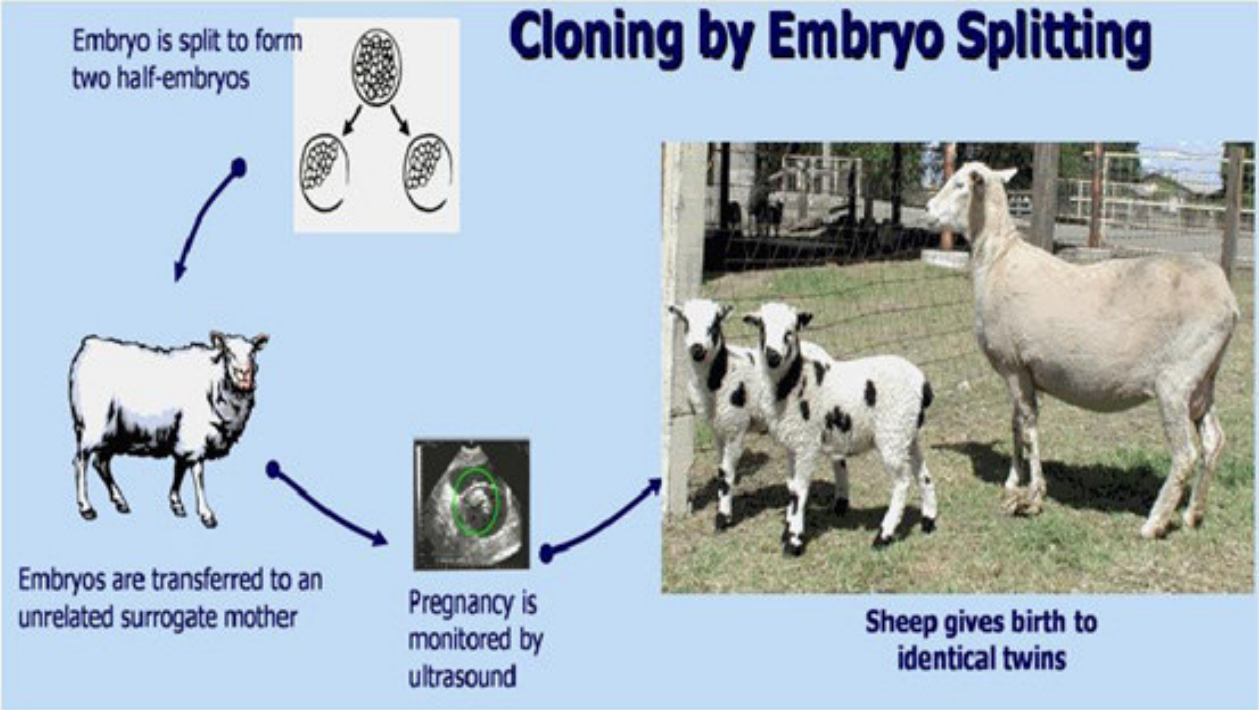
Domestic animals can be cloned using techniques such as embryo splitting and nuclear transfer to produce genetically identical individuals. Although embryo splitting is limited to the production of only a few identical individuals, nuclear transfer of donor nuclei into recipient oocytes, whose own nuclear DNA has been removed, can result in large numbers of identical individuals (Keefer, 2015).

2.1. Embryo Splitting (Blastomer Separation)

Embryo splitting may be considered the first true cloning procedure involving human intervention, and was first described by Willesden and Polge in 1981, when monozygotic twin calves were produced. Embryo splitting or the mechanical separation of cells can be used in very early embryos. Two-cell embryos derived from either in vitro fertilization or embryo rescue following in vivo fertilization are held in place with micro pipettes under a microscope. The zonapellucida (the clear layer of protein surrounding the oocyte and fertilized ovum) of these embryos is opened, and the two-celled embryo is then split into individual cells with a finely drawn needle or pipette. One of the cells is left in the original zonapellucida and the other is either placed into an empty zonapellucida or allowed to develop without a zonapellucida. These so-called demi-embryos can be cultured in

vitro for a few days, inspected for appropriate growth and then transferred directly to synchronized recipient dams or frozen for future use (Eyachew et al., 2017).

Figure 1.Technique of cloning via Embryo splitting

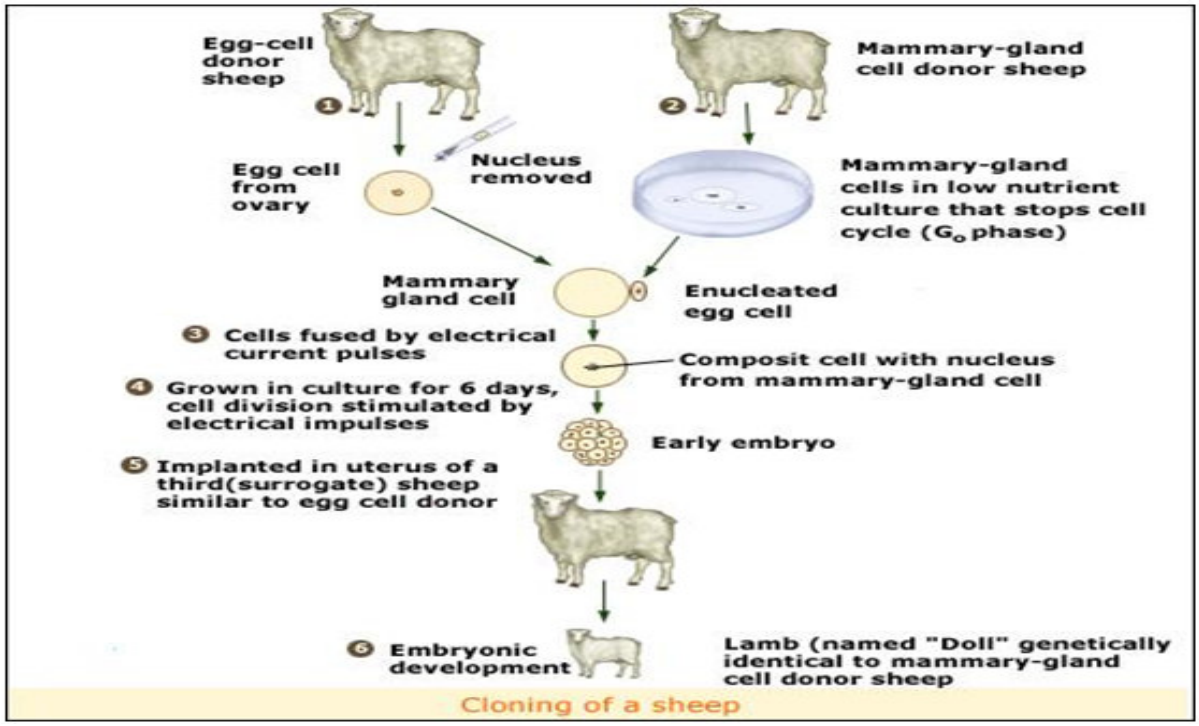


2.2. Somatic Cell Nuclear Transfer

Somatic cell cloning (cloning or nuclear transfer) is a technique in which the nucleus (DNA) of a somatic cell is transferred into an enucleated metaphase-II oocyte for the generation of a new individual, genetically identical to the somatic cell donor. The transfer of a cell nucleus from a body cell into an egg from which the chromosomes have been removed or inactivated; is method used for cloning of organisms. Once the genome transferred with the egg cell then one cell embryo is created and the process of cloning is completed and further development of the clone can occur (Tamashiro *et al*., 2002).

Technical Procedures of Somatic Cell Nuclear Transfer; There are three basic procedures in somatic cell nuclear transfer. Inoculation is the first procedure which is performed by mechanical fixation of oocyte in appropriate position with polished end of holding pipette slight vacuum. Aspiration of chromatic containing part of the oocyte into sharp inoculation pipette is other option to enoculat somatic cell. After enoculation the insertion will take place. Common way of inserting somatic cell by injecting them under zona pellucide and then using an electric impulse to induce the membrane fusion between inoculated oocyte and somatic cell. Activation step is the third step use to embryo activation during reconstruction. Increase cytoplasmic calcium level may use for activation but the usual trigger of activation are electric impulse or short exposure a chemical agent followed by extended block on protein synthesis. At the end of activation the embryos start to cleave (Vajta and Gjerris, 2005).

Figure 2. Technique of cloning via somatic cell nuclear transfer



3. Application of Farm Animal Cloning

Application possibilities of cloning in research, industry and agriculture are theoretically almost limitless. However, low level of efficiency is hampering most applications from a technical point of view (Vajta and Gjerris, 2005). Recently the current as well as the potential applications of farm animal cloning have been reviewed (Lewis *et al*., 2004), and it has been suggested that these applications can be divided into two general areas: biomedical and agricultural.

3.1. Biomedical Application

The greatest potential of farm animal cloning seems to be the biomedical application. Since the 1980s it has been possible to genetically modify mammals by injecting copies of desired genes into one of the two pronuclei in the zygote. This method is, however, very inefficient. Most of the injected embryos do not develop, and less than 1% of the individuals born have the desired genetic change. Furthermore, the method can only introduce new genes into the genome and these often cause problems, because the integration site is random. In spite of the various alternative attempts, SCNT is at present the most efficient way to produce genetically modified farm animals. By introducing the genetic changes into cells, and then choosing the ones with the desired changes for the cloning procedure, other and more precise genetic modifications can be introduced into the genomes of farm animal species. Additionally, SCNT can also be used for rapid multiplication of individual transgenic animals that are known to carry desired changes (Paterson *et al*., 2003).

The two applications that seem the most realistic within the next 3–5 years are creation of disease models and bioreactors. A third application, xenotransplantation will also be discussed here, not because it is a realistic possibility within the next few years, but because it is often mentioned when the perspectives of farm animal cloning are listed, and because it is one of the most controversial applications from a societal and ethical point view (Vajta and Gjerris, 2005).

3.1.1. Disease (Research model)

Disease models are animals designed to express, either at the genotypic or phenotypic level, a certain human disease. They can be used both to further understanding of the disease and to do initial tests on possible treatments. Sheep and especially pig are ideal animals because of the similarities in physiology and size, of their organs to those of humans (Paterson *et al*., 2003). Sets of cloned 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 (Rudenko *et al*., 2004).

3.1.2. Bioreactor

Gene ‘pharming’: production of recombinant human proteins in the mammary gland of transgenic animals. The

conventional production of rare human therapeutic proteins from blood or tissue extracts (Kues and Niemann, 2004). Bioreactors are transgenic animals that have had genes that produce human proteins insert into their genome. These proteins can subsequently be harvested from the animal and used within the biomedical sector as medicine (Vajta and Gjerris, 2005).

The most promising site for production of recombinant proteins is the mammary gland, but other body fluids including blood, urine and seminal fluid have also been explored (Dyck et al, 2003). Specifically, the ability to express transgenes in milk-producing animals has resulted in the creation of "bioreactors," or animals that produce large amounts of a given recombinant protein in their milk, in fully biologically active form through proper posttranslational modification (PTM), for purification and therapeutic use (Ko *et al*., 2000).

Most of the research in this field has been designed to get the transgenic animals to express the desired proteins in their milk. Goats, cows, sheep and pigs have been genetically modified and cloned in attempts to create such proteins. Potentially, proteins for the treatment of a range of human diseases can be produced this way in the future. Protein such as human coagulation factor IX, Human anti-thrombin and Alfa-1-antitrypsin have all been harvested in an experimental setting from transgenic and cloned animals today (vajta and Gjerris, 2005).

3.1.3. Xenotransplantation

Xenotransplantation is the transplantation of organs or cells from one species to another. Human to human transplantation are sometimes difficult due to scarcity of donor organ (Tseng *et al*., 2004). For reasons similar to those listed at disease models, pigs appears to be the most suitable donor animal: it has organs of a similar size to those found in humans; porcine anatomy and physiology are not too different from their human counterparts; pigs have short reproductive cycles and large litters; pigs have rapid growth; practical maintenance is relatively cheap; and pigs are a domesticated species (Pinkert, 1994). However, one of the problems associated with using pig organs for xenotransplantation is that the immune system of the human recipient attacks the transplanted organ, causing transplant rejection (Lai *at al*., 2002).

The process of generating and evaluating transgenic pigs as potential donors for xenotransplants involves a variety of complex steps and is time, labor and resource intensive. Essential prerequisites for successful xenotransplantation are: (i) Overcoming the immunological hurdles, (ii) Prevention of transmission of pathogens from the donor animal to the human recipient,(iii) Compatibility of the donor organs with the human organ in terms of anatomy and physiology (Kues and Niemnn , 2004).

3.2. Agricultural Application

According to Vajta and Gjerris in 2005, the application of SCNT technology in agriculture seems to be a possibility that lies further in the future than biomedical uses. Although the technical and scientific problems are similar, agricultural applications obviously have to be highly productive to become eventually cost-efficient and viable. This requirement restricts seriously the potential areas to some special fields. Cloning can be used to create copies of animals with highly valued traits, such as a dairy cow with high milk production or bulls with especially good meat (Paterson *et al*., 2003).

The potential to clone adult animals creates entirely new dimension for animal agriculture. A desirable and unique specimen can be precisely reproduced, capturing traits that are difficult to develop through traditional breeding practices. For example, a dairy cow that produces milk with unusually high milk protein content (which is important for making cheese), or with an unusually low percentage of saturated fat (which has human health benefits), could be cloned (Murray and Anderson, 2000). Cloning can be used to create copies of animals with highly valued traits, such as a dairy cow with high milk production or bulls with especially good meat. Alternatively, it can also be used to create copies of animals whose traits are especially in demand in breeding programs, thereby bypassing the need for repeated breeding cycles (Paterson *et al*., 2003).

Cloning could enable the rapid dissemination of superior genotypes from nucleus breeding flocks and herds, directly to commercial farmers. Genotypes 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 crossbred animals, or composite breeds, to maximize the benefits of both heterosis and potential uniformity with in the colonal family (Wells., 2005). The ability to clone genetically elite females, while possibly increasing the level of inbreeding, also increases the intensity of genetic selection (Murray and Anderson, 2000).

Several studies regarding the biological and biochemical properties of products from cloned animals in relation to risks to human health have already been published. In 2004, Takahashi and Ito have examined the differences in meat samples taken from embryonic cloned, somatic cloned and non-cloned cattle and find no significant biological differences. Examinations of the nutritional value of milk and meat products derived from cloned cattle and find no significant differences in products from cloned and non-cloned animals (Tome *et al*., 2004). Use of cloning in animal genetic improvement for milk production may increase the rates of selection progress in certain cases, particularly in situations where artificial insemination is not possible, like in pastoral

systems with ruminants (Montaldo, 2006).

Cloning can be used along with other forms of assisted reproduction to help preserve indigenous breeds of livestock, which have production traits and adaptability to local environments that should not be lost from the global gene pool (Rudenko, 2004). Another possibility is to produce animals that can reduce negative agricultural effects on the environment. The most known of these today is the EnviropigTM: a pig that has the capability to digest plant phytate, leading to less phosphate in the manure from the animal and thus less environmental pollution. This animal is currently under development in Canada, and is often mentioned in the literature as an example of an environmentally friendly use of biotechnology being close to the market (Kues and Niemann, 2004).

Conclusion and Recommendation

Cloning of farm animals can be considered as suitable way of reproducing animals with superior genetic makeup by serving as another form of assisted reproduction. Splitting of embryo (blastomer separation) to produce identical clones by dividing fertilized embryo in to two or three cells which grow individually, and somatic cell nuclear transfer which results clones through transferring DNA containing nucleus in to inoculated oocyte to generate new individuals are the techniques used to clone farm animals. Applications of cloning were numerous in farm animals but biomedical and agricultural applications are the most significant. Genetically engineered and cloned animals are conducted to produce therapeutic drugs for the treatment of disease, production of organ for human transplantation, and to study the effects of that individual gene on body function. In livestock production, clones can be used as elite breeding animals and also able to produce copies of highest yielding and animals with desirable traits.

- ✓ Based on the above review, further investigations are needed to be conducted to improve the drawbacks of the techniques and increase the success rate of farm animal cloning which are imperative to realize the ultimate benefits of somatic cell nuclear transfer of animal in agriculture and biomedical application.

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