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Role of Reproductive Cloning in livestock and their Applications: A review

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Abstract

Cloning is the process of creating genetically identical copies of biological matter include genes, cells, tissues or entire organisms. In molecular biology, cloning is the process of producing similar populations of genetically identical individuals that occur in nature when organisms like bacteria, insects or plants reproduce asexually. The first major breakthrough has been the pioneering work of Wilmut, Campbell and their colleagues in 1996 through Somatic Cell Nuclear Transfer (SCNT) technology, followed by the birth of Dolly the sheep. This was the first reported mammalian clone from a fully differentiated adult cell. The birth of Dolly, the prospects of cloning technology have extended to ethically hazier areas of mammalian cloning and embryonic stem cell research. This review hopes to bring the reader closer to the science and the ethics of reproductive technology, and what the implications are for the medical practitioner.

Key words: Cloning; DNA; Somatic Cell Nuclear Transfer (SCNT); Dolly

Introduction

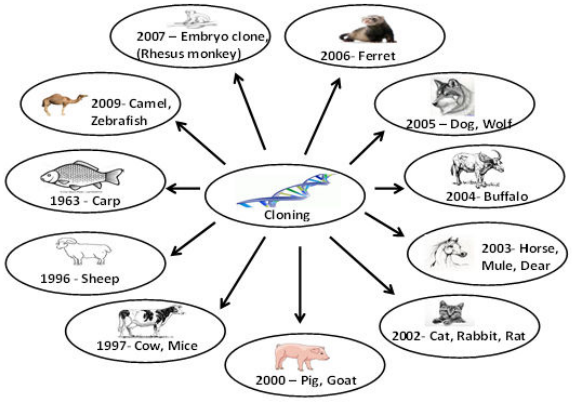
The clone word derived from Ancient Greek word that's mean "twig". Some organisms generate clones naturally through asexual reproduction. Plants, algae, fungi, and protozoa produce spores that develop into new individuals that are genetically identical to the parent organism. Prokaryotes are capable of producing to clones through binary fission, where the bacterial DNA is replicated and the original cell is divided into two identical cells. In humans and other mammals, the formation of identical twins is also a type of natural cloning. In this case, two individuals develop from fertilized embryo [1].

In animals there are various in-vitro techniques for improvement of the farm animals such as artificial insemination (AI), natural breeding through good quality of animals or by cloning [2]. Cloning is one of the novel in-vitro techniques to produce superior quality of livestock farm animals [3, 4]. Clones may be produced by naturally like identical twins or they can produce in-vitro techniques. The best thing of cloning is that it does not manipulate or change the animal's genetic material as DNA [5]. It is simply another form of reproduction under in-vitro and aseptic conditions. Clones are superior breeding, used to produce healthy and disease-free offspring [6].

Dolly (the sheep) was cloned by Ian Wilmut, Keith Campbell and colleagues at the Roslin Institute, Edinburgh, be successfully cloned from an adult cell (udder cell) by using Somatic Cell Nuclear Transfer Technology (SCNT). Doll was born on 5 July 1996 and announced officially on 22 February 1997. Dolly's embryo was produced by mammary somatic cell and placed into a sheep ovum [7]. The in-vitro produced healthy embryos were then placed inside the sheep ovum and that went through a natural pregnancy. Dolly sheep was died in 14 February 2003 when she was six years old due to some respiratory problems.

In 1963, the Chinese embryologist Tong Dizhou cloned the world first fish to insert the male DNA into an egg from a female carp [8]. After that subsequently, in 1997 the first cloned Calf was born [9], five Scottish Piglets (Jose, Josue, Juan, Amber and Jose) were cloned in 2000 [10], first cloned Cat was produced by David in 2002 [11], Shaoni (2003) was cloned world's first Horse in 2004 [12], world's first Water Buffalo was cloned by Sinha in the year of 2009 and in 2009 [13]. In India, Samrupa the world's first cloned Water Buffalo Calf at NDRI, Karnal in 2004 [14]. In 2012, Blurtit were produced the clones of Ferret via nuclear transfer by cell fusion [15], Rhesus monkey cloned embryos were produced by transfer of DNA from adult cells on [16], Wani and coworkers (2009) were cloned Camel and Zebra fish were [17] (Figure 1). The scientists were still working to improve and produce other important livestock farm and wild animals.

Figure 1: Important milestones in cloning history



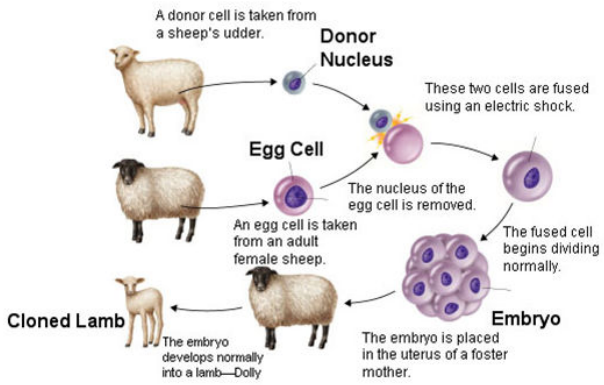
Reproductive Cloning

Reproductive cloning is used to generate an animal with the same nuclear DNA as another existing animal. The Dolly was cloned using reproductive cloning. Through Somatic Cell Nuclear Transfer (SCNT), a donor's nucleus genetic material is transferred to an egg whose nucleus has been removed [18]. The cloned egg is then treated with growth factor chemicals to initiate cell division. When the embryo reaches a specific stage like morula or blastocyst, it is then transferred to a surrogate mother for further embryonic development until birth.

The Roslin Technique/ Somatic Cell Nuclear Transfer Technique

The Roslin Institute is an animal sciences research institute at Easter Bush, Midlothian, Scotland University of Edinburgh, sponsored by the Biotechnology and Biological Sciences Research Council (BBSRC) (The Roslin Institute-Home page, 2011) [19]. The Roslin Technique is also known as the Somatic cell Nuclear Transfer Technique (SCNT), which is used to create one of the world's most famous cloned Sheep, Dolly (Ian Wilmut, Keith Campbell and their colleagues) [4, 19]. Dolly was born on 5 July 1996 and died on 14 February 2003. Dolly was the first mammal which was produced by SCNT (Figure 3).

Figure 2: Cloning procedure of Dolly, the first mammal clone



Mechanism

Generally, there are three types of SCNT techniques were used to create cloned offspring. Most widely used technique is classical cloning, second is the employing micromanipulators [20] and third is handmade cloning [21].

In handmade cloning all steps are performed manually without using the micromanipulators. Removal of the zona pellucida from the embryos is creating the main difference in handmade cloning technique and other technique.

Zona pellucida free reconstructed embryos are to be cultured under aseptic and sterile in vitro conditions up to the blastocyst stage prior to transfer into recipients.

In addition, Piezo-driven injection of the donor nucleus has been successfully accomplished in oocytes retrieved from horse, cattle, swine, mice and rabbit [22, 23, 24].

Classical cloning method involves following steps

Enucleation retrieved oocytes from recipient - Oocytes at metaphase II stage (MII) of meiosis is the most appropriate stage for the creation of in-vitro fruitful cloned mammalian embryos. Oocytes were retrieved from slaughterhouse derived ovaries in in-vitro aseptic and sterile conditions.

Retrieved oocytes were in-vitro matured in tissue culture media containing tissue culture media, follicle stimulating hormone, luteinizing hormone, estradiol, gentamicin, and sodium private.

Stage of in-vitro maturation (MII stage) of oocytes varies species to species as in case of bovine, cattle and porcine this will 24 hours, respectively. Now in-vitro matured oocytes are enucleated by sucking of out small portions of the oocyte ooplasm/cytoplasm, closely attached to the primary polar body, where the MII chromosome is situated [24, 25].

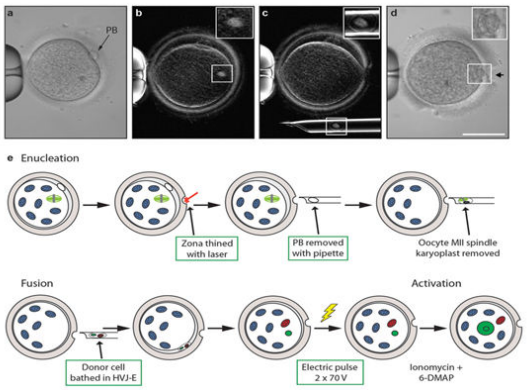
Transfer of donor nucleus into enucleated recipient cell - Nucleus of donor cell was transfer to penetrate the zona pellucida which is in close contact to the cytoplasmic membrane of the oocyte using suitable fine bore micropipette.

Now a day, a large variety of somatic cells has been successfully used for cloning of livestock animals.

Many literatures say that theoretically it is possible that somatic cell can be successfully reprogrammed. But, in-vitro, regarding cloning efficiency between the different types of somatic cells it is not possible yet.

Fetal cells, like fetal fibroblasts are commonly used for somatic cloning as they have less genetic errors and a higher proliferative capacity than adult somatic cells [26], figure 4.

Figure3: Enucleation of mammalian oocytes.



(a) Brightfield image (100X, Arrow indicates the first polar body) of an oocyte arrested at MII. (b) The MII spindle of oocyte (100X). (c) Oocyte enucleated by aspirating the MII spindle into a fine glass pipette using polarized light microscope (100X). (d) Brightfield image of an enucleated oocyte showing the donor fibroblast bathed in HVJ-E solution positioned under the zona pellucida. Inset shows enlarged image of a fibroblast positioned for fusion. (e) Schematic drawing of the NT procedure [27].

Fusion of two cells - Enucleated oocyte and the donor cell are fused by short high voltage pulses (few seconds) through the point of contact between the two cells within in suitable and aseptic environment, figure 4 [13].

Activation of cells - Activation of cell after fusion can be achieved by short electrical pulses (2×70V) or by brief exposure to chemical substances like dimethylaminopurin or ionomycin, as they can regulate the calcium inflow into the complexes or the cell cycle, figure 4.

In-vitro culture cell – In-vitro culture of reconstructed cell is cultured up to the blastocyst stage to achieve the initial developmental competence prior to transfer into a recipient. The success rates of the in-vitro culture are variable in species to species. Culture systems for bovine embryos are allowed to routine production of 30-40% blastocysts from in-vitro fertilized bovine oocytes retrieved from slaughterhouse ovaries [28, 29].

Transfers of an embryo to recipient - Bovine embryos are transferred at the morula to blastocyst stage by using nonsurgical techniques. In the small ruminants, embryos are transferred by endoscopic or surgical into the uterus of recipient [8, 30, 31].

Applications of Somatic Cell Nuclear Transfer (SCNT)

In Stem cell research: Somatic cell nuclear transplantation technology plays an important role of study in stem cell research to diagnose various types of treatment and diseases. The main aim of stem cell research is to obtain pluripotent stem cells from a cloned embryo because these cells are genetically matched to the donor organism from which they are obtained. The resion behind this that, they give them the ability to develop a patient specific pluripotent cells, which could then be used in therapies or disease diagnosis [14, 32].

Agricultural Benefits: In livestock farm animals as cow, buffalo, goat, sheep having specific livestock beneficial characteristics. These beneficial characteristics will increase the health of livestock as well as consumer population. Through SCNT technology we can improve these characters in their offspring's. SCNT also plays an important role to preserve such beneficial characteristics in livestock animals [33, 34, 35].

Life-Saving Technology: Now a days this is possible through tissue engineering technology we can cloned valuable organs and tissues like hearts and liver. Animal cloning also beneficial for the production of drug by gene farming [4, 36].

SCNT is very useful in reproductive biology to produce new offspring with healthy charterstics, therapeutic cloning, and basic research. There are some conflicts between scientist, some believe that cloning will never work for humans while others believe that human cloning is easier to do that animal cloning [14, 32, 35].

Human cloning

On 2 February 2004, as per report of American Cable News Network (CNN) South Korean scientists reported that they have created human embryos by cloning and isolate embryonic stem cells, scientist expected that the universal cell will help to improve to diagnose may disease and helpful to understand money pathways of gene regulations. But it was banned by United States (US) to clone human cells and the offense is punished in Michigan of USA with $1 million (10 lakh in Indian rupee) fine and 10 years jail. However, it is not banned in animal to the purpose of study and research as well as for the livestock productions [37].

Is that cloned animals are safe to eat

On 15 January 2008, U.S. Food and Drug Administration (FDA), endorse the safety of meat and milk products from cloned animal and their next generation for human ingesting. The FDA Risk determination is the most widespread science-based evaluation of livestock cloning conducted in the world to date [38, 39, 40].

Conclusions

The potential applications of biotechnology in livestock production are endless and need more research in various streams. The utility of biotechnology in livestock production is limited and it has to be come. We can improve the livestock production by using molecular biology with gene modification and cloning. For that we have to need advance biotechnology tools which will improve the livestock offspring. Procedures and policies for evaluation of the risk, food safety, efficacy, and consumer benefit of products produced by these technologies need to be developed, discussed, and implemented. There is need to use potential of biotechnology for the improvement of livestock. Researcher need to be update with advance technology and concept behind the technology. Cloning is a very powerful tool of biotechnology for the Scientist/researchers to improve the new generation of livestock. Now, there is a need to be implying a right guidance, strategies and regulations for the livestock animals.

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