MICE CLONING : ITS APPLICATION AND ETHICAL ISSUES

SUBJECT : ZOOA

SEMESTER : 6TH

PAPER : DSE(A)-6-2-P

UNIVERSITY REGISTRATION NUMBER : 115-1211-0713-18

UNIVERSITY ROLL NUMBER : 183115-11-0164

INDEX

INTRODUCTION

What is Cloning- **Cloning** is the process of producing individuals with identical or virtually identical DNA, either naturally or artificially. In nature, many organisms produce clones through asexual reproduction. Cloning in biotechnology refers to the process of creating clones of organisms or copies of cells or DNA fragments (**molecular cloning**).

There are three different types of cloning:

1. **Gene cloning**, which creates copies of genes or segments of DNA.
2. **Reproductive cloning**, which creates copies of whole animals.
3. **Therapeutic cloning**, which creates embryonic stem cells.

Advantages Of Cloning:

1. Ensure the continuity of hereditary traits from the parent to the clones.
2. Increase the rate of production and the quality of the products.
3. Good qualities of the plants and animals can be selected and maintained in the clones.
4. Many clones are produced in a short time.
5. Can be carried out any time of the year.

Disadvantages Of Cloning:

1. Raise ethical issue on human. The resistance of the clones towards diseases and pests is the same. If a clone is infected with a disease or attacked and die.
2. Raise ethical issues on human cloning.

History – The first study of cloning took place in 1885, when German scientist Hans Adolf Eduard Driesch began researching reproduction. In 1902, he was able to create a set of twin salamanders by dividing an embryo

into two separate, viable embryos, according to the Genetic Science Learning Center. Since then, there have been many breakthroughs in cloning.

In 1958, British biologist John Gurdon cloned frogs from the skin cells of adult frogs. On July 5, 1996, a female sheep gave birth to the now-famous Dolly, a Finn Dorset lamb — the first mammal to be cloned from the cells of an adult animal — at the Roslin Institute in Scotland.

Since Dolly, many more animal clones have been born, and the process is becoming more mainstream. Research has also been conducted on human-cell cloning. In 2013, scientists at Oregon Health and Science University took donor DNA from an 8-month-old with a rare genetic disease and successfully cloned human embryonic stem cells for the first time. Unfortunately, the researchers didn't remove the cells to save the child. The project was to prove that mature donor cells could be used to produce new ones. This research has evolved into using stem cells for many different applications, including hair regrowth, treatments for burns and more.

CLONING PROCEDURE

Gene cloning is the process in which a gene of interest is located and copied (cloned) out of all the DNA extracted from an organism.

The basic steps in gene cloning are:

1. DNA extracted from an organism known to have the gene of interest is cut into gene-size pieces with restriction enzymes.
2. Bacterial plasmids are cut with the same restriction enzyme.
3. The gene-sized DNA and cut plasmids are combined into one test tube. Often, a plasmid and gene-size piece of DNA will anneal together forming a recombinant plasmid (recombinant DNA).
4. The recombinant plasmids are transferred into bacteria using electroporation or heat shock.
5. The bacteria is plated out and allowed to grow into colonies. All the colonies on all the plates are called a gene library.
6. The gene library is screened to identify the colony containing the gene of interest by looking for one of three things:
    - The DNA sequence of the gene of interest or a very similar gene
    - The protein encoded by the gene of interest
    - A DNA marker whose location has been mapped close to the gene of interest.

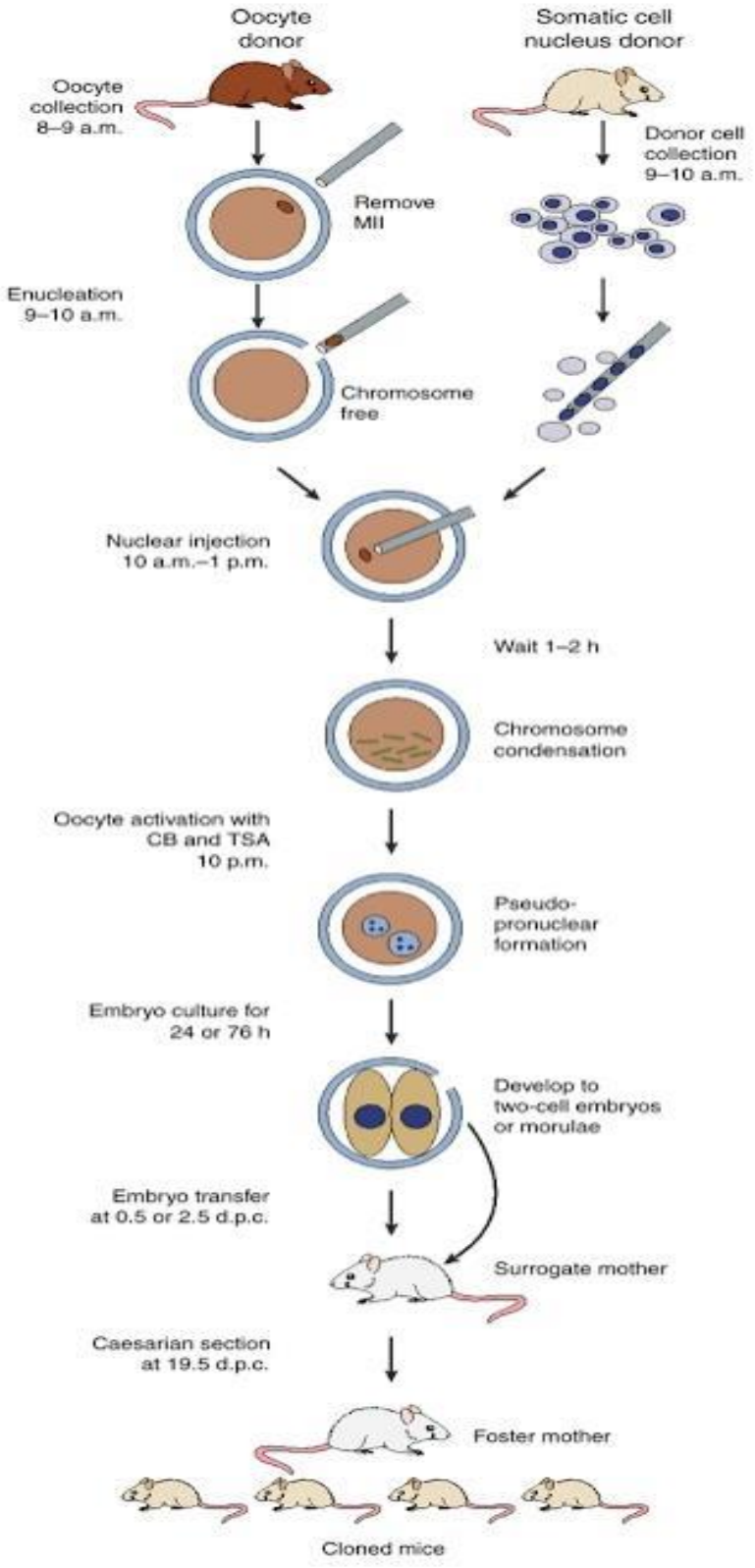

Figure : Mice cloning

According to **Mizutani et al. in 2016** a **Cloned Mice** generation method which has published on **Nature Scientific Report** is given below.

Methods

Animals. Urine-derived cells were collected from the following mice strains: B6D2F1 (C57BL/6× DBA/2) Male and female mice aged 8–10weeks, female mice aged about 1 year, male and female mice aged 8–10weeks, and pCX-eGFP 129/Sv Tg male and female mice aged 8–10weeks. Oocytes were collected from B6D2F1 female mice aged 8–10weeks. The surrogate pseudopregnant females used as embryo transfer recipients (see below) were ICR strain mice mated with vasectomized males of the same strain. B6D2F1, 129/Sv and ICR mice were purchased from **Shizuoka Laboratory Animal Center** (Hamamatsu, Japan). The 129B6F1 strain and Tg mice were bred in our mouse facility. All animal experiments Conformed to the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Committee of Laboratory Animal Experimentation of the University of Yamanashi.

Collection of oocytes. Mature oocytes were collected from the oviducts of 8–10-week-old female mice that had been induced to superovulate with 5 IU pregnant mare serum gonadotropin followed by 5 IU human chorionic gonadotropin (hCG,) 48 h later. Cumulus–oocyte complexes (COCs) were collected from the oviducts about 16h after hCG injection. After collection, COCs were placed in **HEPES-buffered CZB medium (H-CZB)29** and treated with 0.1% bovine testicular hyaluronidase (Sigma-Aldrich, St Louis, MO, USA). After several minutes, the cumulus-free oocytes were washed twice and then moved to a droplet of CZB medium for culture.

Collection of urine-derived cells. Randomly selected mice were caught by hand. Most of the mice dis-charged their urine immediately after being caught, and the urine was collected in 10cm dishes. The volume of urine was measured with a pipette. The urine was placed in a dish covered with mineral oil, and the number of cells was counted for each mouse. In some cases, the urine-derived cells were collected by pipette (inner diameter: 10–15μm) using a micromanipulator and double stained with PI and Hoechst stain to measure the cell Survival rate . For NT, urine-derived cells were collected using a micromanipulator, placed individually and carefully into **Polyvinylpyrrolidone** (PVP) medium, and kept until use.

Nuclear transfer. The NT procedure was performed as described14,30. Groups of oocytes were transferred into a droplet of H-CZB containing 5mg/ml cytochalasin B (CB) on the microscope stage for enucleation of the **Metaphase II (MII)** spindle. Oocytes undergoing microsurgery were held with a holding pipette, and a hole was made in the **zona pellucida** using an enucleation pipette following the application of several piezo pulses

(PrimeTech, Ibaraki, Japan). The **MII chromosome**–spindle complex was aspirated into the pipette with a minimum volume of ooplasm. After enucleation of all oocytes in one group, they were transferred into CZB. For nuclear Injection, urine-derived cells and control cumulus cells were gently aspirated into and out of the injection pipette until their nuclei were largely devoid of visible cytoplasmic membrane. Each nucleus was injected immediately into an enucleated oocyte. The reconstructed oocytes were kept in an incubator until activation.

Activation, culture and embryo transfer. The reconstructed oocytes were activated in 10mM SrCl2 in Ca2+-free CZB medium in the presence of 50 nM trichostatin A (TSA) supplemented with 5 µM latrunculin A (Lat A) for 9h17,18,31. Our original method for oocyte activation was 6h with CB and TSA, but an additional 3h Culture was required without CB after washing of embryos. However, when Lat A was used instead of CB, oocytes could be cultured up to 9 h continuously. Formation on pseudopronuclei was examined, and the oocytes were cultured in **CZB** until embryo transfer at the two-cell stage. In additional experiments, some cloned embryos were cultured for 96h to examine their potential for development to blastocysts or for 72h to establish the ntES cell lines.

After the cloned embryos had developed to the two-cell stage the next day, they were transferred into the oviducts of pseudopregnant ICR strain female mice at 0.5 days post coitum (dpc) that had been mated with a vasectomized male the night before transfer. At 19.5dpc, the offspring were delivered by caesarean section. After growing to adulthood, these mice were mated with other cloned mice as a measure of their fertility.

Establishment of ntES cell lines. Cloned embryos were produced as described above and, when they reached the **morula/blastocyst** stage, were used to establish ntES cell lines as described 20–23. The ICM was allowed to grow normally and, at passage 5–6, they formed as ES cell-like colonies. At this time, we considered these cells to be established and then randomly selected six cell lines for examination with AP staining and immunostaining to determine their pluripotency.

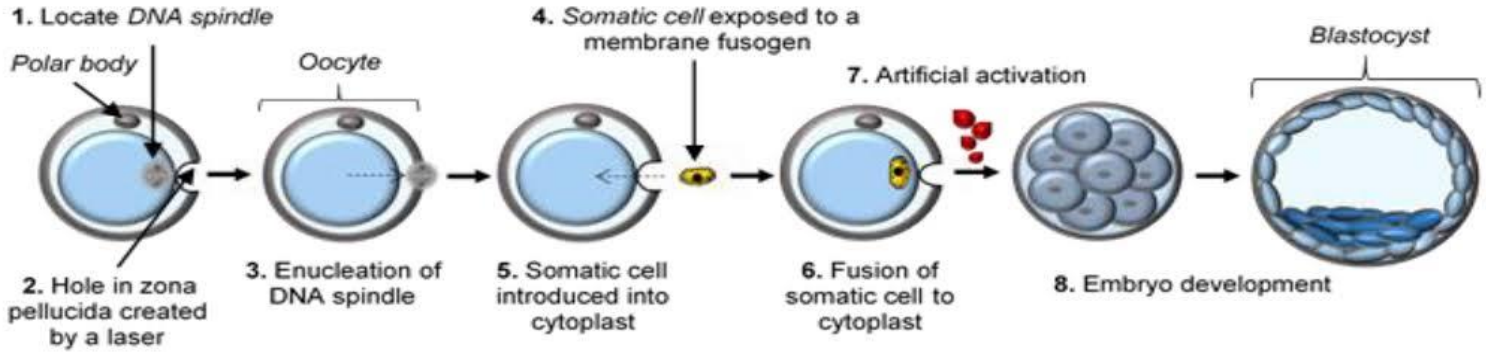

FIGURE : SNCT PROCESS OF MICE CLONING

Why Scientists Choose Mice for Animal Cloning:-

The Mouse is the only mammal that provides such a rich resource of genetic diversity coupled with the potential for extensive genome manipulation, and is therefore a powerful application for modelling human disease."—Justice et al. (2011).The physiology and size of mice — they're small enough to handle and house easily — are the main reasons for their popularity in the lab. Physiologically, mice are very like humans, albeit around 3,000 times smaller (Partridge, 2013) but with similar basic body functions such as blood cell production (haematopoiesis), digestion, respiration and the cardiovascular system. Although differences do exist, mice respond similarly to humans when they are sick or undergo treatment. Mice also breed easily, with short pregnancies and large litter sizes that are important in helping researchers create their own modified mice. However, most laboratories in Canada source non-specialized mice from commercial breeders, receiving purpose-bred animals with a full breeding history. For researchers, this is very important: Working with animals that show very little difference among individuals increases the value of experimental results, since all the animals respond the same. For even more consistency, we've also been able to clone mice since 1997.

APPLICATION

Gene cloning has made a phenomenal impact on the speed of biological research and it is increasing its presence in several areas of everyday life. One of the reasons why biotechnology has received so much attention during the last decade is because of gene cloning.

Proteins that are normally produced in very small amounts include growth hormone, insulin in diabetes interferon in some immune disorders and blood clotting factor VIII in haemophilia are known to be missing or defective in various disorders. Prior to the advent of gene cloning and protein production via recombinant DNA techniques this molecules are purified from animal tissues or donated human blood. But both sources have drawbacks , including slide functional differences in the non-human proteins and possible viral contamination (eg – HIV,CJD) . Production of protein gene from a cloned gene in a defined non pathogenic organism would circumvent these problems. A gene for an important animal or plant protein can be taken from its normal host inserted into a cloning vector and introduced into a bacterium. If she manipulation are performed correctly then the gene will be expressed and the protein is synthesized by the bacterial cell then it is possible to obtain large amounts of the protein. But in practice obtain recombinant protein is not as easy as theoretically it sounds. For this special types of cloning vectors are needed.

ETHICAL ISSUES

In bioethics, the ethics of cloning refers to a variety of ethical positions regarding the practice and possibilities of cloning, especially human cloning. While many of these views are religious in origin, some of the questions raised by cloning are faced by secular perspectives as well. Perspectives on human cloning are theoretical, as human therapeutic and reproductive cloning are not commercially used; animals are currently cloned in laboratories and in livestock production.

Advocates support the development of therapeutic cloning in order to generate tissues and whole organs to treat patients who otherwise cannot obtain transplants, to avoid the need for immunosuppressive drugs, and to stave off the effects of aging. Advocates for reproductive cloning believe that parents who cannot otherwise procreate should have access to technology.

What are the risks of cloning ?

1. Expensive and highly inefficient
2. more than 90% of cloning attempts fail to produce a viable offspring.
3. In addition to low success rates cloned animal tend to have more compromised immune function and higher rates of infection tumor growth and other disorders.
4. Many cloned animals have not lived long enough to generate good data about clones age.
5. Appearing healthy at a young age unfortunately is not a good indicator of long-term survival.
6. Clones have been known to die mysteriously.

CONCLUSION

The term “Clone” has many meanings but in its simplest and most scientific sense it means the making of identical copies of molecules , cells , tissues and even entire animals. In this process the nucleus from an adult somatic cell is transplanted into an enucleated ovum to produce a developing animal that is a “ delayed” genetic twin of the adult.

There are many applications that nuclear transfer cloning might have for biotechnology, live stock production and new medical approaches. Work with embryonic stem cells and genetic manipulation bor early embryos in animal species(including nuclear transfer) is already providing unparalleled insights into fundamental biological processes and promises to provide great practical benefit in terms of improved livestock, improved means of producing pharmaceutical protein and prospects for regeneration and repair of human tissues.

However, the possibility of using human cloning for the purposes of creating a new individual entails significant scientific uncertainty and medical risk at this time. Potential risks include those known to be associated with the manipulation of nuclei and egg and those yet unknown, such as the efforts of aging, somatic mutation , and improper imprinting. These effects could result in high rates of failed attempts at pregnancy as well as the increased likelihood of developmentally and genetically abnormal embryos.

ACKNOWLEDGMENT

I would like to express my special thanks of gratitude to all my respected teachers of **Zoology Department** of Zoology especially **DR. Subhadipa Majumder** and **DR. Subhadra Roy** to give me the golden opportunity to do this wonderful project on cow cloning; its application and ethical issues.

I would also like to extend my thank of gratitude to the principal sir **DR. Indranil Kar** and our Head of the Department **Professor Suman Tamang** sir and all my respected teachers for providing me with all the facilities that was required.

I would also like to thank my parents and my friends who had helped me with their valuable suggestions and guidance has been helpful in various phases of the completion of the project.

Date - 05/08/21

Anuska Mukherjee

(6^{th} semester)

REFERENCE

- Alan O Trounson. Methods Mol Biol. 2006: Future and applications of cloning.
- Armin Schumacher, Cynthia Faust, Terry Magnuson ; Positional cloning of a global regulator of anterior–posterior patterning in mice .
- Atsuo Ogura, Cold Spring, Harb Protoc. Cloning Mice, 2017.
- Giovanna Sebastiani, Gary Leveque, Line Larivière, Line Laroche, Emil Skamene, Philippe Gros, Danielle Malo ; Cloning and characterization of the murine toll-like receptor 5 (Tlr5) gene: sequence and mRNA expression studies in Salmonella-susceptible MOLF/Ei mice
- Jean Chen Shih ; Cloning, after cloning, knock-out mice, and physiological functions of MAO A and B
- Kazuhiro Furukawa, Hidehito Inagaki, Yasuo Hotta ; Identification and cloning of an mRNA coding for a germ cell-specific A-type lamin in mice.
- Kevin Eggan, Hidenori Akutsu, Janet Loring, Laurie Jackson-Grusby, Martina Klemm, William M Rideout, Ryuzo Yanagimachi, Rudolf Jaenisch ; Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation .
- Matti Häyry. Br Med Bull. 2018 ; Ethics and cloning .
- Rika Azuma, Kei Miyamoto, Mami Oikawa, Masayasu Yamada, Masayuki Anzai; Combinational treatment of trichostatin A and vitamin C improves the efficiency of cloning mice by somatic cell nuclear transfer.
- Teruhiko Wakayama, Ivan Rodriguez, Anthony CF Perry, Ryuzo Yanagimachi, Peter Mombaerts; Mice cloned from embryonic stem cells.
- Teruhiko Wakayama, Ryuzo Yanagimachi, Cloning of male mice from adult tail-tip cells, 1999
- Teruhiko Wakayama, Yoichi Shinkai, Kellie LK Tamashiro, Hiroyuki Niida, D Caroline Blanchard, Robert J Blanchard, Atsuo Ogura, Kentaro Tanemura, Makoto Tachibana, Anthony CF Perry, Diana F Colgan, Peter Mombaerts, Ryuzo Yanagimachi; Cloning of mice to six generations.
- Tetsuo Ono, Chong Li, Eiji Mizutani, Yukari Terashita, Kazuo Yamagata, Teruhiko Wakayama ; Inhibition of class IIb histone deacetylase significantly improves cloning efficiency in mice.
- Tomomi Aida, Keiho Chiyo, Takako Usami, Harumi Ishikubo, Risa Imahashi, Yusaku Wada, Kenji F Tanaka, Tetsushi Sakuma, Takashi Yamamoto, Kohichi Tanaka ; Cloning-free CRISPR/Cas system facilitates functional cassette knock-in in mice.