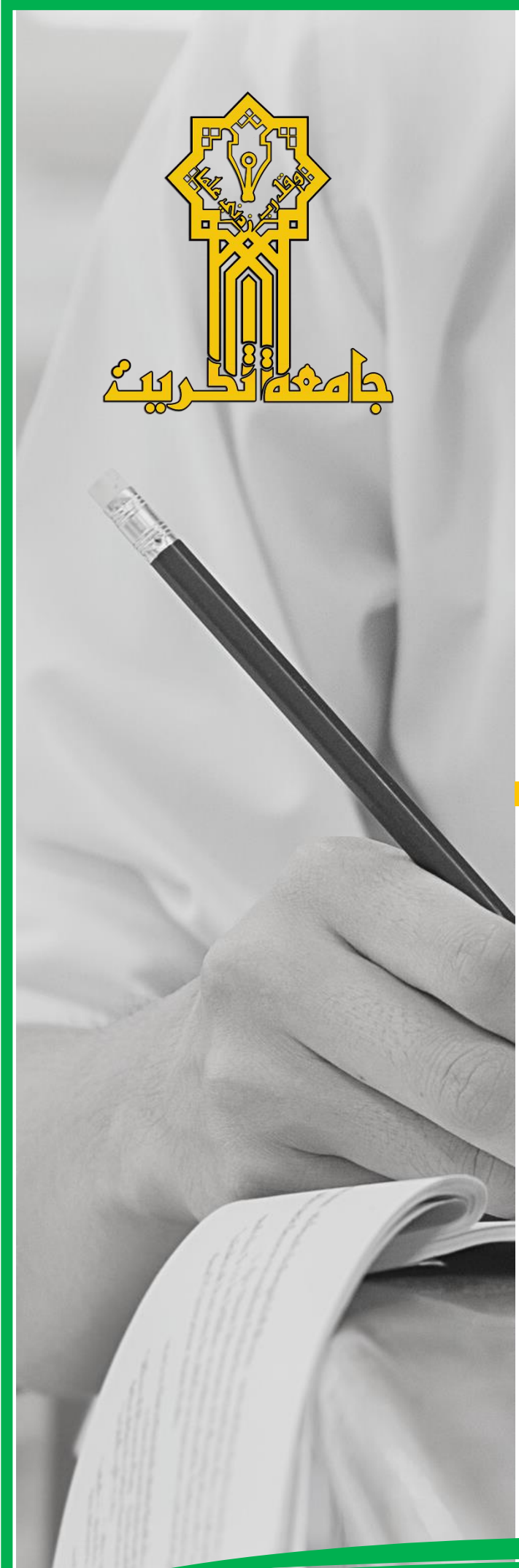

Tikrit University
College of Veterinary Medicine

Cloning

Subject name: Reproductive techniques

Subject year:5

Lecturer name: Dr.Ali Aziz Abd

Academic Email:

aliaziz2235@tu.edu.iq

SCAN ME
Lecturers link

Cloning: it’s a technique which permit to produce an organism a sexually from a clone cell.

Somatic cell nuclear transfer (SCNT): refers to the production of genetic copies of individual animals by using nuclear transfer (NT). More specifically; a nucleus from a somatic cell of the donor individual is inserted into an oocyte whose own nuclear DNA has been removed (enucleation). In nuclear transplantation cloning there is a single genetic "parent," unlike sexual reproduction where a new organism is formed when the genetic material of the egg and sperm fuse. Dolly ewes is produced from mammary gland cell.

Advanced reproductive methods involving microsurgery, embryo culture, and transfer into recipients (surrogate mothers) are required to produce animal clones.

The implications and potential benefits of cloning farm animals

1. Contribution to increasing the rate of genetic gain in selection programs and livestock production (genetic and economic gains).

2. Possibility of producing multiple copies of embryo.

3. To introduce genetic change into nucleus herds or for increasing the rate of genetic progress in the general population.

4. In (2001) cloned a 21-year old Brahman steer from skin cells to produce a bull calf.

The Animal nuclear transplantation cloning steps:

1. Cell preparation and culture

A primary cell line is isolated from a mature animal. Dermal skin fibroblasts have been commonly used. Dermal fibroblasts are the cells that

make collagen and the extracellular matrix that provide the structural framework around epithelial tissue. Bovine skin fibroblasts have maintained a stable karyotype in long-term culture and have been successfully used for nuclear transfer. After isolated, cells are washed in Dulbecco minimal essential medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 1% (v/v) penicillin/streptomycin, the cells are seeded into six-well culture plates following typical cell culture techniques.

2. Oocyte preparation, maturation and enucleation

Recipient oocytes are washed and selected after they are removed from animal. Only oocytes that have a homogenous cytoplasm and at least three layers of cumulus cells are selected for in vitro maturation. In vitromaturation medium consisted of tissue culture medium (TCM 199)supplemented with 10% FCS, luteinizing hormone (LH), and folliclestimulating hormone (FSH). After maturation, oocytes are vortexed to remove expanded cumulus cells and stained with Hoechst for the observation of DNA (chromatin). To make sure the enucleation, ultraviolet light is used to check DNA located in the polar body.

3. Nuclear transfer unit fusion and activation

A. The donor cell and recipient cytoplasm of the nuclear transfer couplets are fused approximately 22– 24 hours post maturation by a single direct electrical pulse (40 V) delivered through needle-type electrodes. B. Fusion took place in Zimmermann cell fusion medium byplacing an electrode on each side of the nuclear transfer couplet. A sample of couplets is examined 1 hour after the pulse to determine fusion efficiency. C. This reconstructed oocyte is activated to continue embryonic development, activation of the couplets is performed beginning 2 hours after fusion by

using TCM 199 plus FCS D. Following activation, reconstructed embryos are cultured in BARC medium under low oxygen (5.0%) for 7 or 8 days.

4. Embryo transfer

Embryos that reaches the blastocyst stage are transferred into recipient animals approximately 7 days after synchronized estrus. One or two embryos per recipient are nonsurgically introduced into the uterine horn ipsilateral to the ovary containing a palpable corpus luteum. Pregnancy evaluation is performed using transrectal ultrasound approximately 21 days following embryo transfer (Day 28 of gestation).

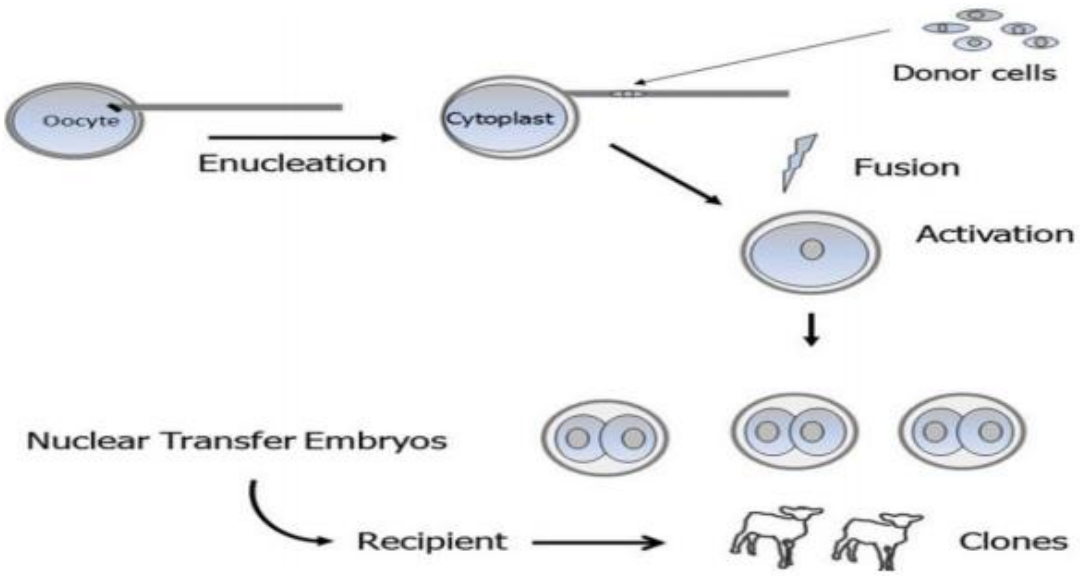

Fig. 1: Somatic cell nuclear transfer process

Limitation of Cloning (Somatic cell nuclear transfer)

- Decrease Genetic Diversity

The mixing of parental genetics resulting from conventional reproduction increases genetic variability, providing diversity in phenotypic traits, but cloning decrease the genetic diversity.

- Efficiency and Cost Effectiveness

Embryonic and fetal death is a major reason for the low success rates and high costs of somatic cell nuclear transfer. Currently, only around 17% of cloned embryos transferred into surrogate mothers result in viable calves at weaning. Peri-natal and post-natal mortality rates with cloned offspring are greater than normally expected.

- Abnormal Development

Data show that 14% of cloned calves die within the first 2 hours of delivery. Failure of the placenta to develop and function correctly is a common feature among clones. Most calves die during this period because of respiratory distress. Clones have altered neonatal metabolism and physiology possibly due to placental abnormalities. Additional losses throughout the post-natal period are mostly due to abnormalities of the cardiovascular, skeletal and central nervous systems, umbilical and lung infections, along with digestive and kidney disorders.

- Premature Aging

One important question is whether cellular aging will affect the ability of somatic cell nuclei to program normal development. As somatic cells divide they progressively age and there is normally a limit number of cell divisions that they can undergo before senescence. Part of this aging process involves the progressive shortening of the ends of the chromosomes called telomeres. Germ cells (eggs and sperm) evade telomere shortening by expressing an enzyme called telomerase that can keep telomeres full length. It seems likely that returning an adult mammalian nucleus to the egg environment will expose it to sufficient telomerase activity to reset telomere length, since oocytes have been found to be potent sources of telomerase activity. However, there has been much discussion about Dolly's

premature arthritis and consideration that this could be due to premature cellular aging due to shortened telomeres.

Embryo splitting or embryo twinning:

General aspects:

- Prior to the blastocyst stage, cells in the early embryo (2-4 cells), called blastomeres.

- Cells divide without increasing in mass between each division: thus the term cleavage divisions—each cell cleaves in half.

- Embryogenesis: is the process by which the embryo forms and develops. A fertilized egg is called a zygote. The zygote is a diploid single cell which divides and grows. One cell divides to become two, which divide to become 4 and so on until the cell mass is called a blastocyst.

- A blastocyst is made of an inner cell mass which subsequently forms the fetus and an outer layer of cells (trophectoderm) which forms the placenta.

Embryo splitting or embryo twinning: The simplest form of artificial cloning, its mean separating the blastomeres of an early embryo and forming two or more smaller embryos. Possibility of obtaining artificially produced monozygotic sheep twins developed from microsurgically separated blastomeres from two-cell embryos. They induced a specific hatching sequence by zona drilling that led to the formation of two incomplete demi embryos; these were finally separated with a glass needle. Then, the embryos will culture and transferred to recipient.

Early studies in amphibians, rabbits and mice suggested that the very early cleavage stages very soon after fertilization (two-cell to four-cell);were flexible and that each blastomere could yield a viable blastocyst, this

called “embryo twinning”. At later stages; the blastomeres could no longer independently form a viable blastocyst due to the loss of mass as each blastomere underwent cleavage division. These blastomeres (2 cells) are considered to be totipotent (completely growth), that is they possess the total potential to make an entire new organism. This totipotency allows scientists to split animal embryos into several cells to produce multiple organisms that are genetically identical to one another.

Steen Willadsen demonstrated that twins could be produced in sheep and cattle after splitting of cleavage-staged embryos, culturing and transfers it into recipients.

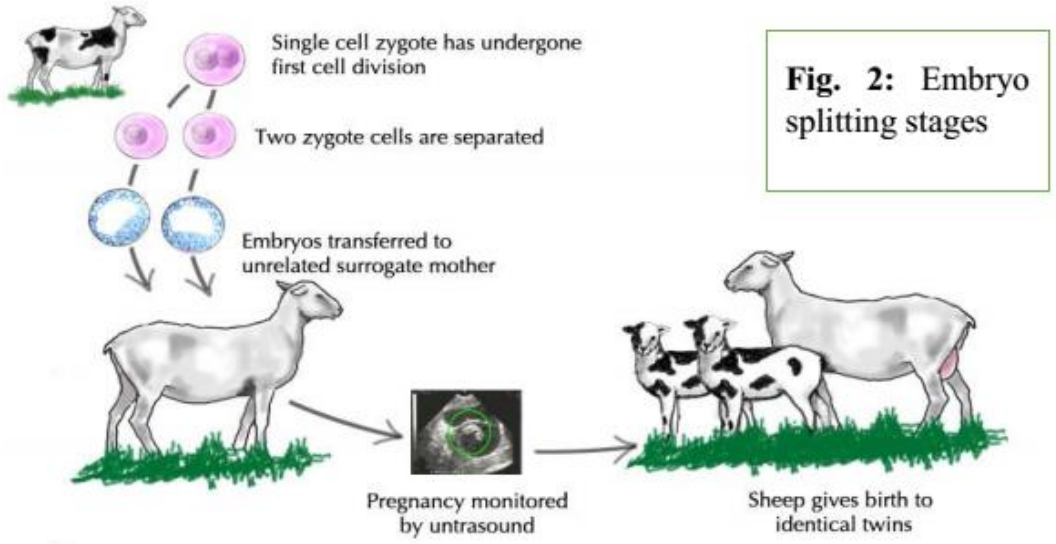

Fig. 2: Embryo splitting stages