FACULTY OF ENGINEERING & TECHNOLOGY

LT.14 Organ culture, Totipotency, Nuclear Transfer

Content outline

1. Organ culture
2. Methods of organ culture
3. Totipotency
4. Somatic cell nuclear transfer

LT.14 Organ culture, Totipotency, Nuclear Transfer

Organ Culture

Organ culture is a development from tissue culture method

• The organ culture is able to accurately model functions of an organ in various states and conditions by the use of the actual *in vitro* organ itself.

• Not whole but pieces of organs can be cultured on artificial medium.

The culture media on which organ is cultured are the same as described for cell and tissue culture.

• Culture of whole or part of animal organ is difficult because these require high amount of O2(about 95%).

• Special serum-free media *(e.g.* T8) and special apparatus (Towell's Type II culture chamber) are used for adult culture.

Organ culture on plasma clot

A plasma clot is prepared by mixing five drops of embryo extract with 15 drops of plasma in a watch glass placed on a cotton wool pad.

• The cotton wool pad is put in a Petri dish. Time to time cotton is moistened so that excessive evaporation should not occur.

• Thereafter, a small piece of organ tissue is placed on the top of plasma clot present in the watch glass.

• In the modified technique the organ tissue is placed into raft of lens paper or ryon. The raft makes easy to transfer the tissue, excess fluid can also be removed.

Organ culture on agar

Solidified culture medium with agar is also used for organ culture.

• The nutrient agar media may or may not contain serum.

• When agar is used in medium, no extra mechanical support is required.

Agar does not allow to liquefy the support.

• The tumors obtained from adults fail to survive on agar media, whereas embryonic organs grow well.

• The media consist of ingredients: agar (1% in basal salt solution), chick embryo extracts and horse serum in the ratio of 7:3:3.

Raft methods

In this approach the explant is placed onto a raft of lens paper or rayon acetate, which is floated on serum in a watch glass.

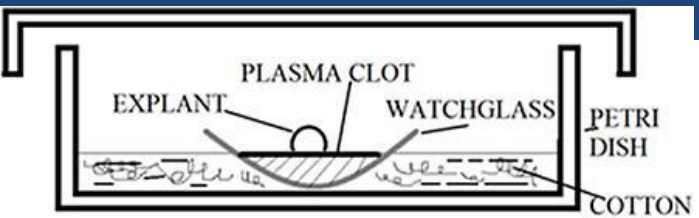
• Rayon acetate rafts are made to float on the serum by treating their 4 corners with silicone.

• Similarly, floatability of lens paper is enhanced by treating it with silicone.

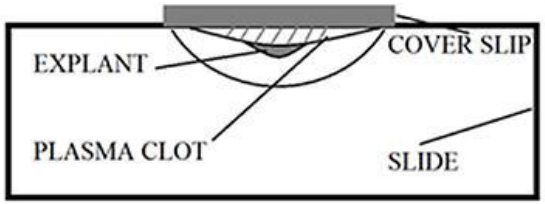
On each raft, 4 or more explants are usually placed.

• In a combination of raft and clot techniques, the explants are first placed on a suitable raft, which is then kept on a plasma clot.

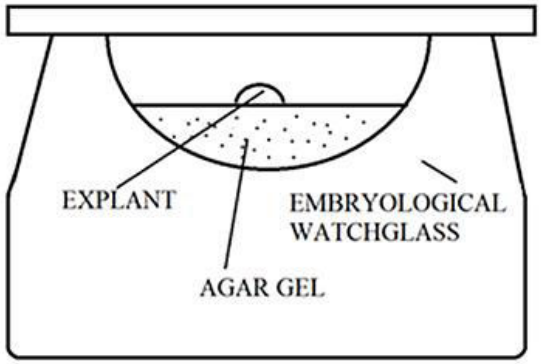
• This modification makes media changes easy, and prevents the sinking of explants into liquefied plasma.



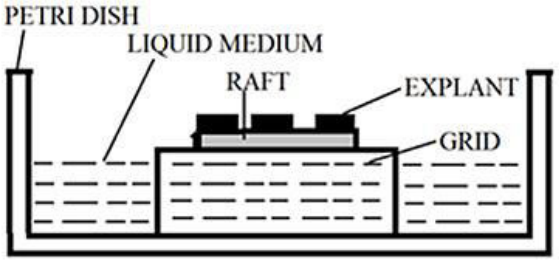
A. WATCHGLASS METHOD



B. SINGLE SLIDE TECHNIQUE

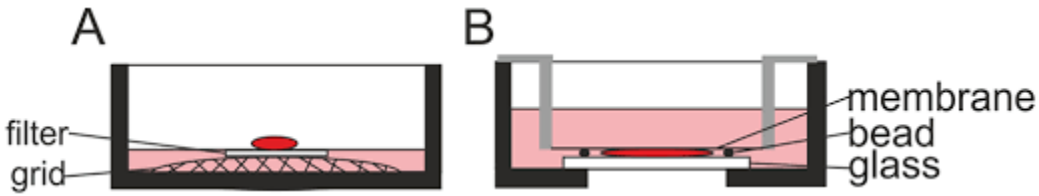


C. AGAR GEL IN EMBRYOLOGICAL WATCHGLASS



D. GRID METHOD

Raft culture



Grid method

Grid methods

Initially devised by Trowell in 1954, the grid method utilizes 25 mm x 25 mm pieces of a suitable wire mesh or perforated stainless steel sheet whose edges are bent to form 4 legs of about 4 mm height.

• Skeletal tissues are generally placed directly on the grid but softer tissues like glands or skin are first placed on rafts, which are then kept on the grids.

The grids themselves are placed in a culture chamber filled with fluid medium up to the grid; the chamber is supplied with a mixture of O2 and CO2 to meet the high O2 requirements of adult mammalian organs.

• A modification of the original grid method is widely used to study the growth and differentiation of adult and embryonic tissues.

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Organ culture in liquid media

The liquid media consist of all the ingredients except agar.

• When liquid media are used for organ culture, generally perforated metal gauze or cellulose acetate or a raft of lens paper is used.

• These possibility provides support

Totipotency & Somatic cell nuclear transfer

Totipotency of animal cells

Totipotency means cells should have required genetic material in the nucleus to achieve full range of developmental capability. *Totipotency*, measured by the ability of a single isolated *cell* to give rise to live. The cells of human embryo is said to be totipotent because it can give rise to any cell type.

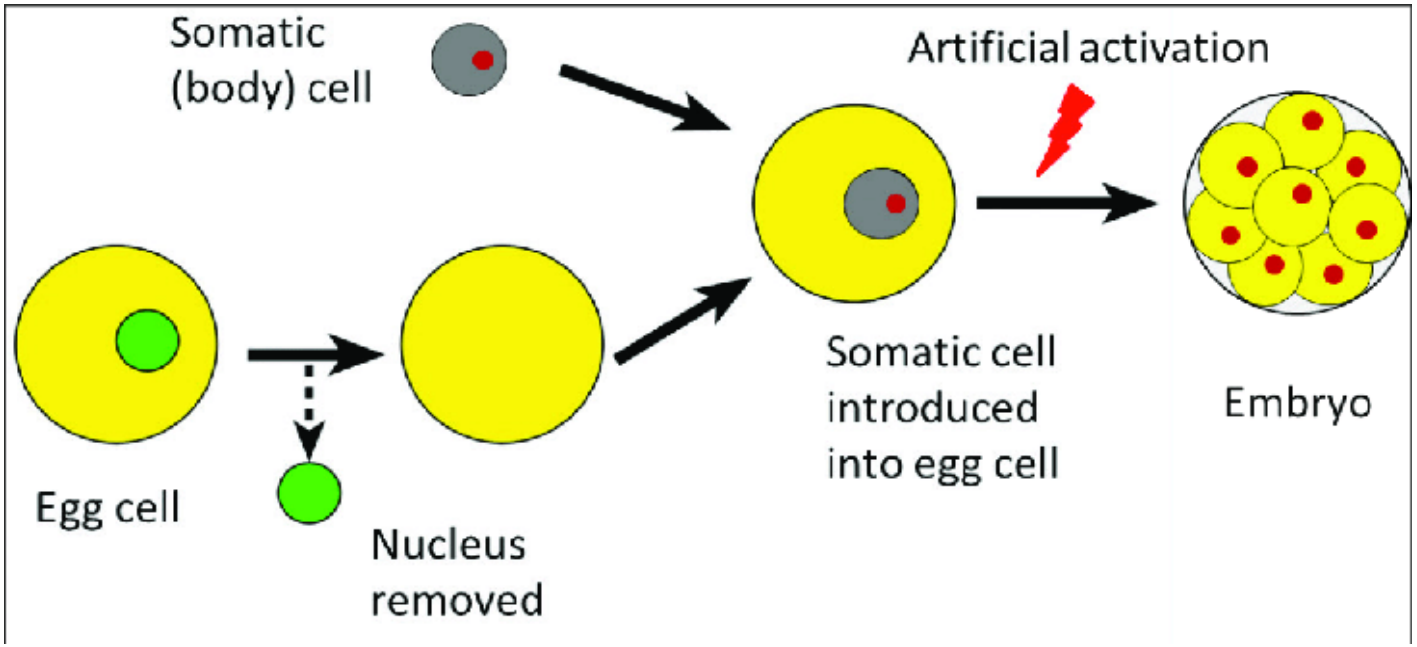
Somatic cell nuclear transfer

Somatic : Any diploid cells in the body

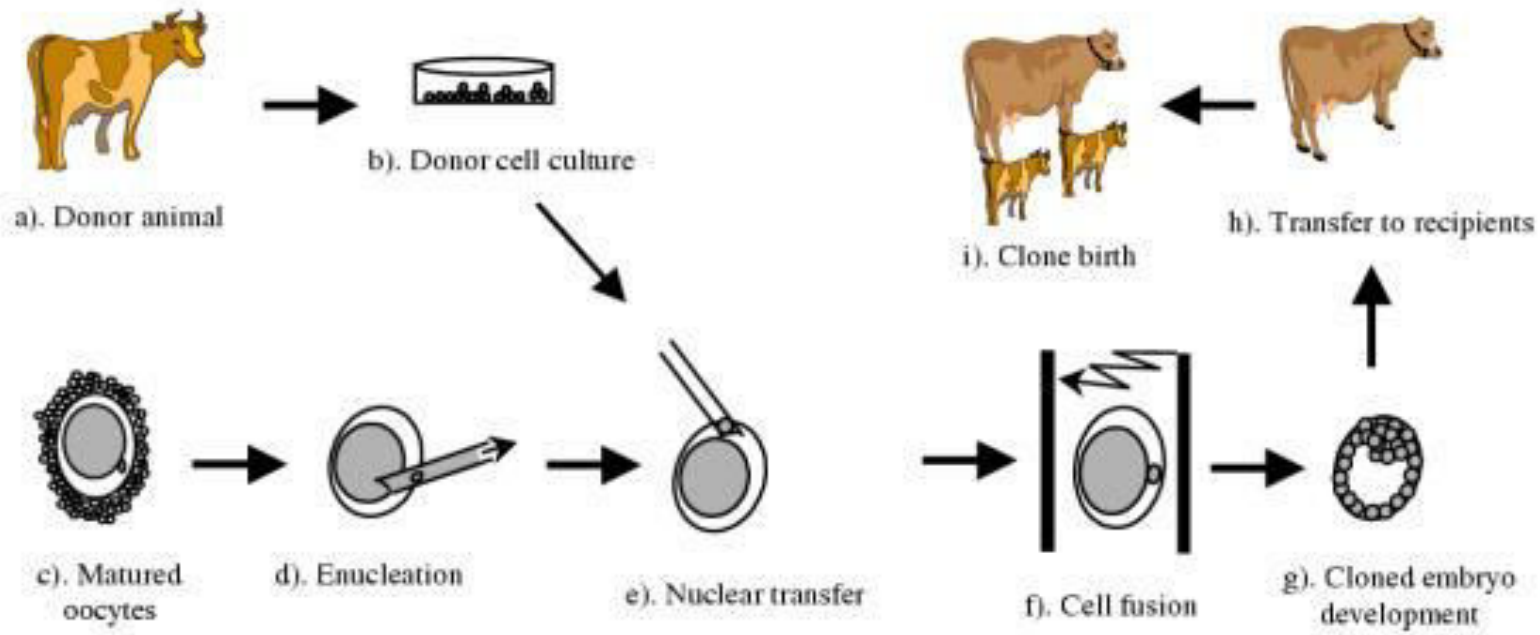
Nuclear: Relating to the nucleus of a cell

•Nuclear transfer is a technique used to create a genetically identical copy – a clone – of a given animal, that implicate the transfer of a donor nucleus into an enucleated MII oocyte (metaphase stage II of meiosis). In this technique a nucleus of a somatic cell is transferred into an oocyte or egg, whose nucleus is removed (enucleated oocyte) or inactivated before implantation of the somatic nucleus.

•SCNT is responsible for the cloning of the first large animals first from a nucleus of the blastomere, and eventually from a fully differentiated nucleus, giving birth to the famous sheep, Dolly. Many different mammals have been cloned by SCNT such as mice, cow, dog, and pigs. The latest cloned species by SCNT is the macaque monkey in 2018.

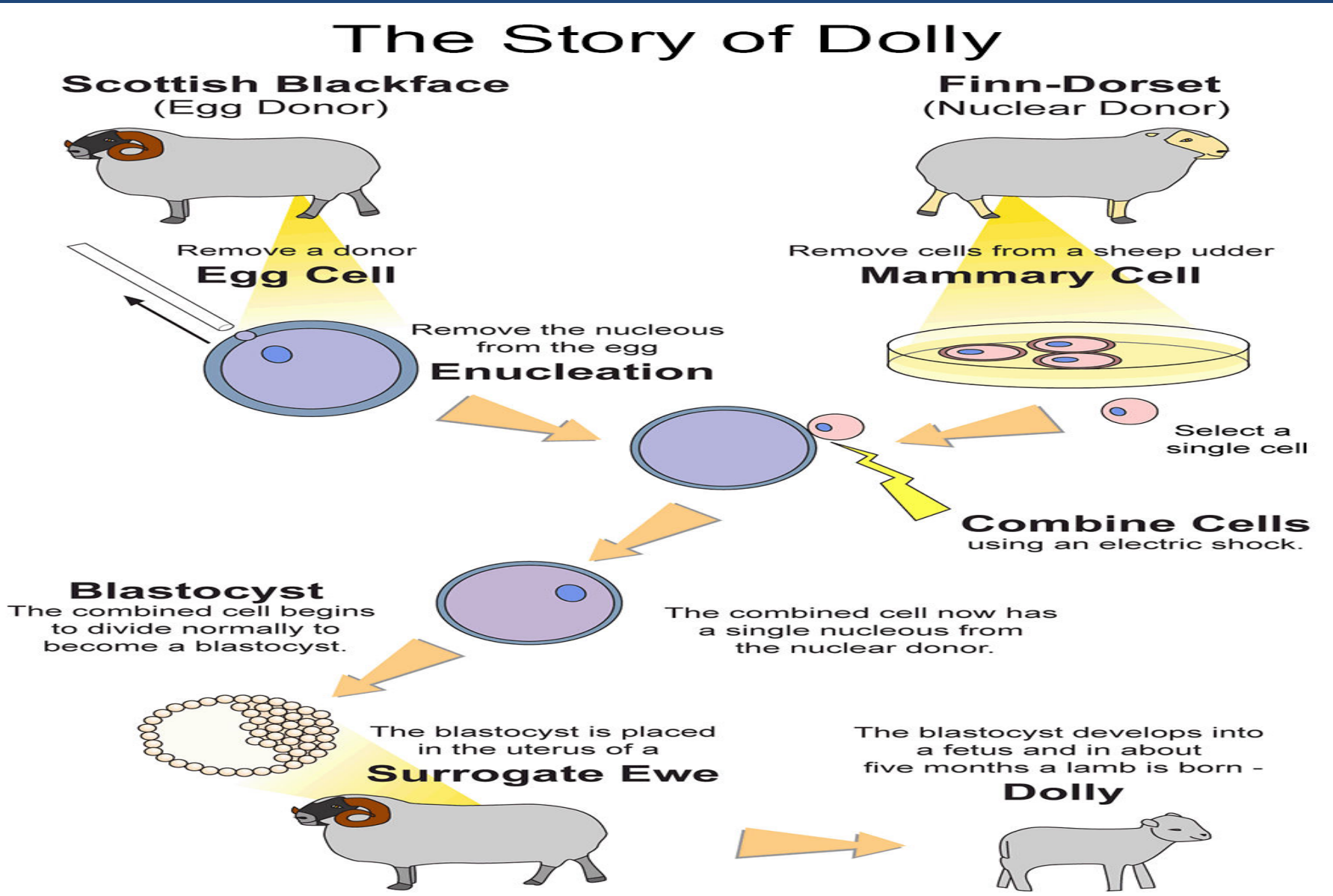


Cloning animals by somatic cell nuclear transfer



Schematic diagram of the somatic cloning process. Cells are collected from donor (a) and cultured in vitro (b). A matured oocyte (c) is then enucleated (d) and a donor cell is transferred into the enucleated oocyte (e). The somatic cell and the oocyte is then fused (f) and the embryos is allowed to develop to a blastocyst in vitro (g). The blastocyst can then be transferred to a recipient (h) and cloned animals are born after completion of gestation (i)

The story of Dolly



References & Further reading

1. https://www.slideshare.net/neeru02/organ-culture-technique-in-synthetic-media-animal-tissue-culture?qid=01c316dd-025f-4d6b-9035-0a0060da772f&v=&b=&from_search=6
2. Organ culture. Saurabh Bhatia, Tanveer Naved and Satish Sardana . 2019 IOP science
3. https://askabiologist.asu.edu/content/story-dolly
4. Image source : Google