

# Identification and culturing of stem cells

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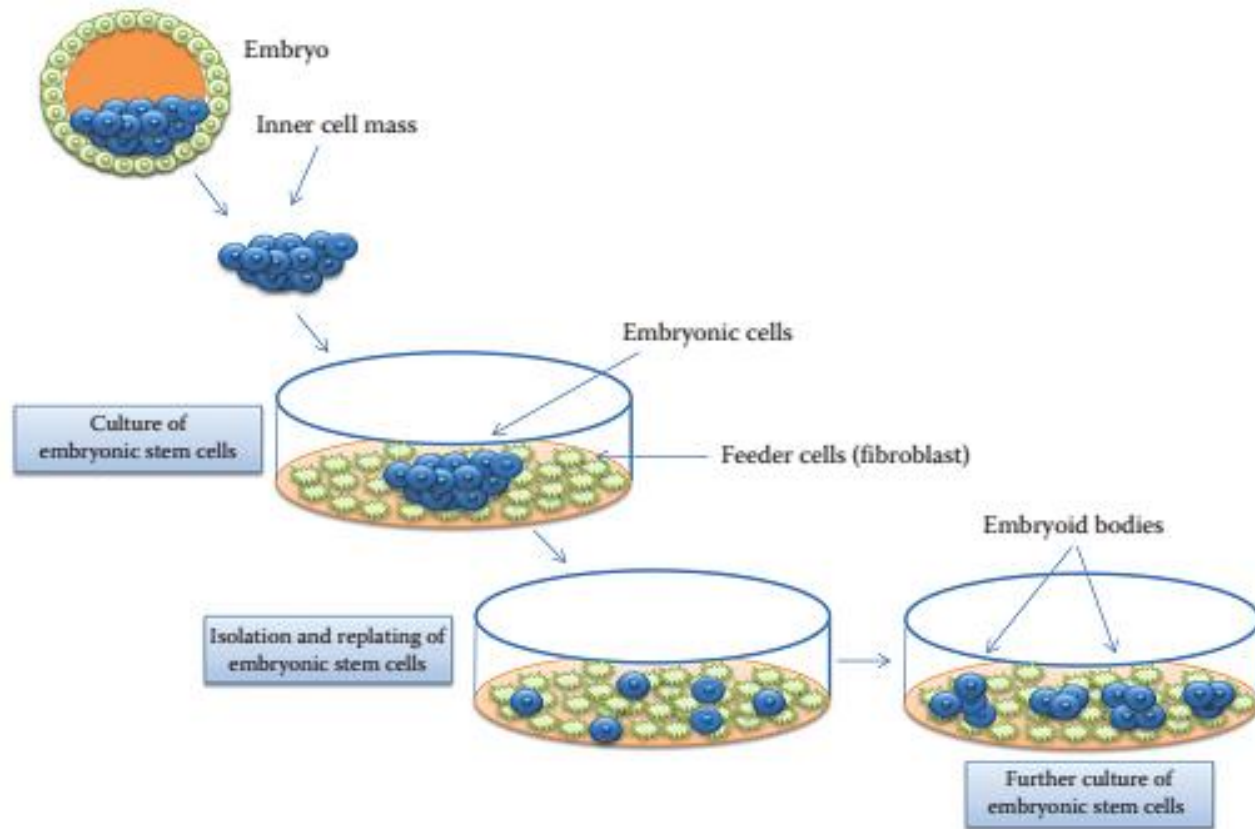
- **Isolation and culture of ESCs**
- **ESCs can be isolated from inner cell mass of early embryo that is donated by mother.**
- **The method of isolating ESCs involves the delay in embryo implantation, which allows the inner cell mass to increase in size.**
- **The isolation process includes removing donor mother ovaries and treating her with hormone progesterone, which cause the embryos to remain free in uterus.**

- **Isolation and culture of ESCs**
- **Furthermore, after 4-6 days of culture, the embryo are harvested and grown in petri plates until the inner cell mass forms an egg or cylinder like structures.**
- **These egg or cylinder shaped inner cell mass is then detached into a single cell population, and plated on fibroblast cells that previously treated with mitomycin-c.**
- **The single cells are then processed for isolating clonal cells. Moreover, clonal cells grown from these in vitro cell cultures can form three germ layers.**
- **After successful isolation and culture of mouse ESCs, efforts have also been made to isolate ESCs form humans.**
- **For that purpose, a method to derive human ESCs and cultured embryonic cells was established.**

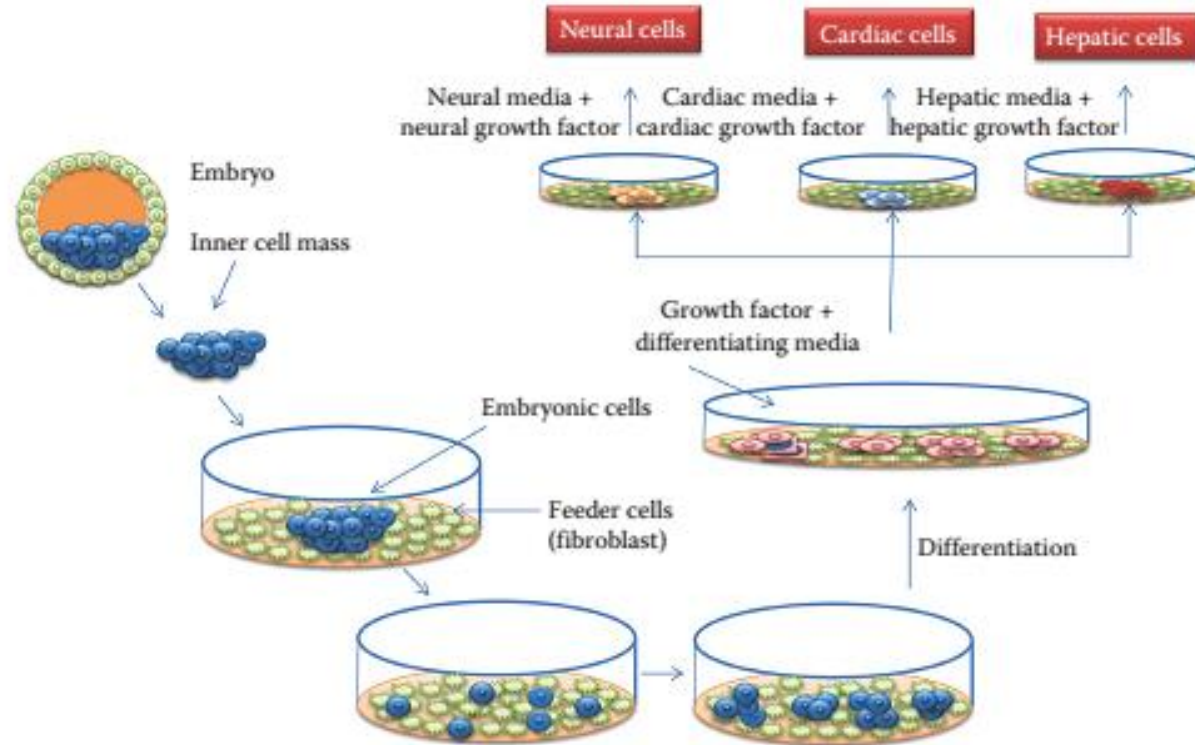
- **Isolation and culture of ESCs**
- **In the first step, embryos are removed from donor mother at approximately 76h after fertilization and cultured overnight in media containing serum and nutrients.**
- **After inner cell mass is removed from late blastocyst using microsurgery, the extracted inner cell masses are cultured on fibroblasts treated with mitomycin-c in media that contains serums.**
- **One week later, colonies of cells grow out and these cells demonstrates pluripotent. The colonies of cells have ability to form three germ layers, differentiate in vitro, and form embryoid bodies.**
- **These embryoid bodies are commonly known as ESCs.**
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- **Isolation and culture of ESCs.**
- **Additionally, to maintain the pluripotency of stem cells, the culture media is delivered with leukemic inhibitory factor and bone morphogenetic proteins that are necessary to prevent ES cells from differentiating.**
- **Moreover, these factors are extremely important for efficiency of deriving ES cells.**

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*Figure 7.7* Isolation and culture of embryonic stem cells.



**Figure 7.8** Differentiation of embryonic stem cells.

- **Identification of ESCs**
- **While isolating and deriving ESCs, it is very important to know the identity of stem cells and their cellular, molecular and functional characteristics.**
- **The various characteristics of ESCs**
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*Table 7.2* Various Characterizations of Stem Cells

Tools	Characterization
Microscopic observation	During culture, the cellular or morphological characteristics of stem cells can be observed through a microscope. This method confirms that the cells are capable of long-term growth and self-renewal.
Cellular markers	Stem cells can be identified by the presence of cell-membrane markers or cell-surface markers by using immunocytochemistry or immunofluorescence techniques.
Molecular markers	Stem cells can be analyzed by PCR techniques to know the molecular identity of cells. The PCR technique allows you to determine the presence of transcription factors (Nanog and Oct4) that are typically produced by undifferentiated cells. It has been reported that these transcription factors turn genes <i>on and off</i> , which is an important process of cell differentiation and embryonic development stages.
Karyotype technique	This method is used to assess whether the number of chromosomes has changed and it does not detect genetic mutations.
Teratoma formation	This method is used to test whether ESCs are pluripotent or not and this can be done by injecting ESCs in the animals; if these stem cells are pluripotent, they will form three germ layers, ectoderm, mesoderm, and endoderm, which are also known as teratoma, and if the stem cells are not pluripotent stem cells, they will not form three germ layers.
Animal studies	To check the functionality of stem cells, stem cells are injected in the animal model of diseases.

## **- Differentiation of ESCs**

- One of the main characteristics of ESCs is to give rise to many cell types, which have been demonstrated in a number of experiments conducted using in vitro cell method**
- Under specific culture conditions and balanced nutrients presence, these ESCs can be differentiated to form various body cell types such as heart, skin, kidney and nerve and many other cell types.**
- Over the last few years, efforts have been made to develop basic methods to differentiate ESCs into some specific cell types.**
- These differentiated cells such as neural cells or cardiomyocytes can be used to treat Parkinson disease and heart infarction.**

# *Types of Stem cells*

## **1. Embryonic Stem cells**

**-- Isolated from the inner cell mass of blastocytes**

## **2. Adult Stem cells**

**They are found in various tissues.**

**-- In adult organisms, stem cells and progenitor cells act as a repair system for body, replenishing adult tissues.**

**-- In a developing embryo, stem cells can differentiate into all the specialized cells- ectoderm, endoderm and mesoderm but also maintain the normal turnover of regenerative organs such as blood, skin or intestinal tissues.**

## ***Sources of autologous adult stem cells in human***

**--bone marrow**

**--adipose tissue**

**-- blood**

**Stem cells can also be taken from umbilical cord blood just after birth.**

**--Adult stem cells are frequently used in various medical therapies as bone marrow transplantation.**

**--Stem cells can now be artificially grown and transformed or differentiated into specialized cell types with characteristics consistent with cells of various tissues such as muscles or nerves.**

**-- Embryonic cells lines and autologous embryonic stem cells generated through somatic cell nuclear transfer or dedifferentiation have also been proposed as promising candidates for future therapies.**

## ***Properties***

### **Self renewal**

**the ability to go through numerous cycles of cell division while maintaining the undifferentiated state.**

### **Potency**

**-- the capacity to differentiate into specialized cell types.**

# ***Self renewal***

## **1. Obligatory asymmetric replication**

**--A stem cell divides into one mother that is identical to original stem cell, and another daughter cell that is differentiated.**

**--when stem cell self renews it divides and does not disrupt the undifferentiated state. This self renewal stem demands control of cell cycle as well as upkeep of multipotency or pluripotency, which all depends on stem cell.**

## **2. Stochastic differentiation**

**-- when one stem develop into two differentiated daughter cells, another stem cell undergoes mitosis and produce two stem cells identical to original.**