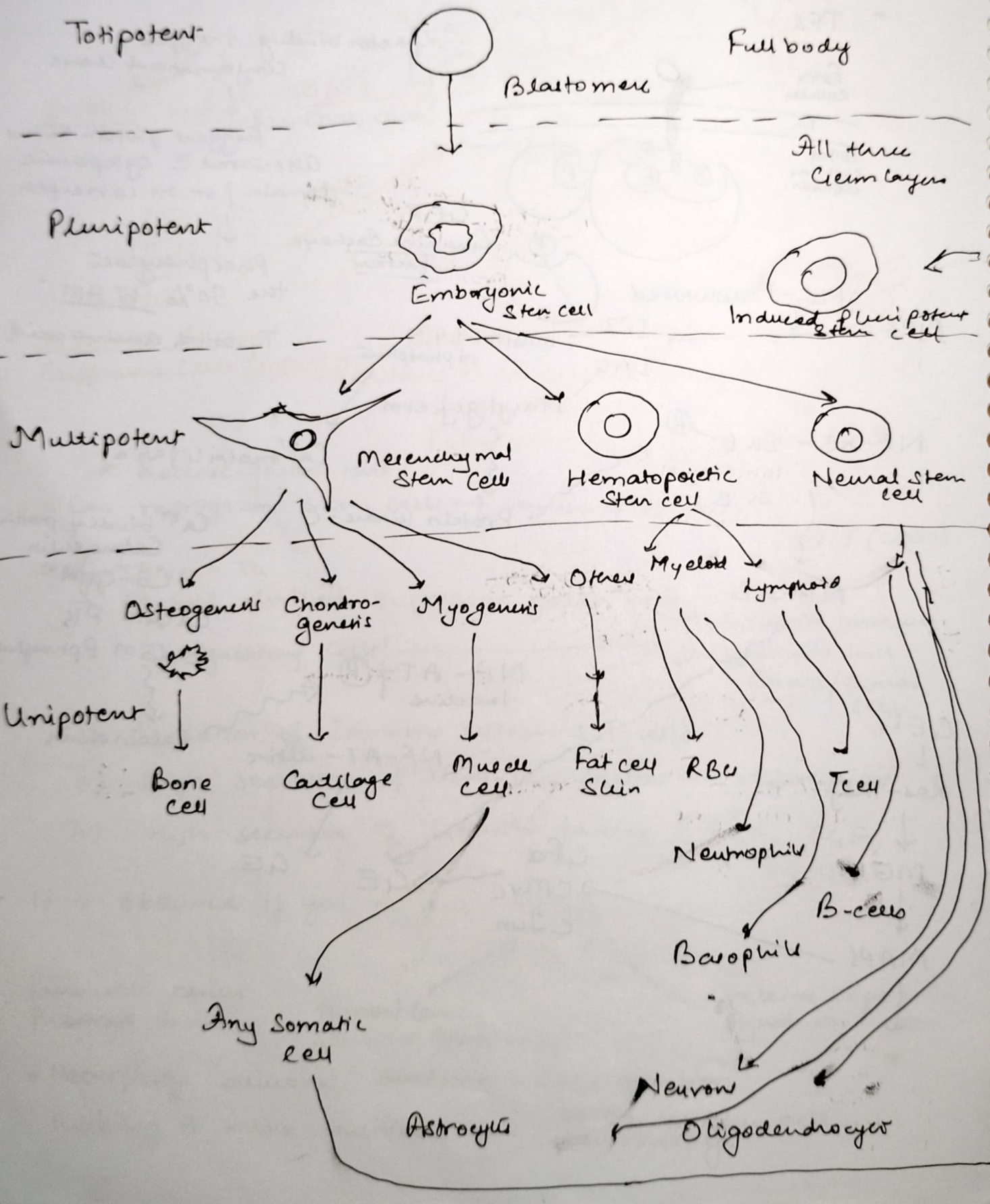
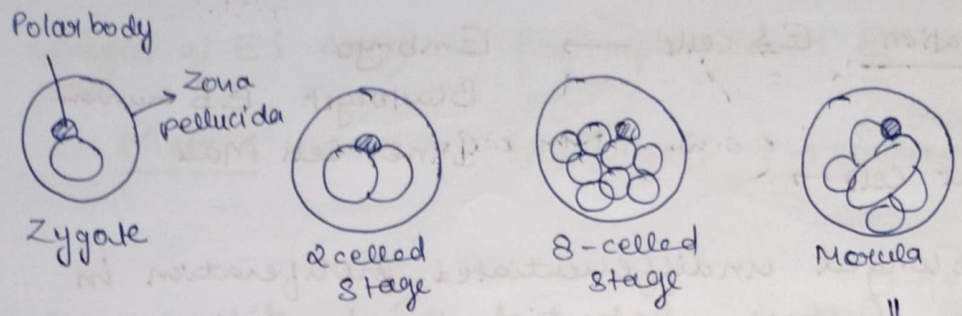
13/09/22

Stem Cell Engineering

Embryonic Stem cells





ESC derivation

Compaction & Cavitation

ICM

Trophoectoderm

Cavity

(Extra embryonic tissue – placenta umbilical cord)

TE

Trophoectoderm

Blastocyst

Epiblast

Primitive endoderm

Blastocyst Inner Cell mass

Late blastocyst

E3.5 mouse / E5 human

Trophoblast

Visceral endoderm

Epiblast

Parietal endoderm

Early Egg cylinder

Induced Pluripotency

Pluripotent Cell

Give rise to all cells of the body

Embryonic Stem cell

Reprogramming factors

Sox 2
Oct 4
Klf-4
Nanog
c-myc

Derivation ES cells → Embryo
Blastocyst ES human
Inner cell mass

Pluripotent cells →

i) Prolonged undifferentiated proliferation in culture, potential ~~to~~ into differentiation to create ~~to~~ derivatives of all 3 germ layer.

ii) ES → Specialized somatic cells
↓
Cardiomyocytes

How to identify that you are using ES cells

3 pluripotent cell types

i) Human embryonic carcinoma (EC) cells
ii) Human EG cells
iii) Human ES cells.

Undifferentiated cells → Culture condition → differentiate → 3 germ layer.

EC cells → Self renewal
differentiate
↓
3 germ cells
Potency ↓
Aneuploidy ↑

3 germ layer.
Potency ↓
Aneuploidy ↑

- Culture cond.
- 3 germ layers.
- Potency → Capability of differentiation ↑
- Euploidy ↑
- Invitro condition → teratocarcinoma formation
↓
3 Germ layers
↓

Origin of ES cells → Pre implantation Embryo

Method → TE (blastocyst) → selectively removed → Specific antibodies (Immunosurgery)

ICM cells are isolated

Placed on mitotically inactivated mouse embryonic fibroblast layer (MEF) (Feeder layer)

Till homogenous colonies appear

Selected Passaged Expanded for ES Cell lines.

- Undifferentiated proliferative Potential (Self renewal)
- Potency
- 3 germ layers.
- Stable Karyotype.
- High level of telomerase

How can we establish pluripotency of ES cells.

i) Mouse ES cells can be retransferred into early mouse embryo → give rise to all 3 germ layers. When they develop all somatic cells → Chimeric embryo
Chimera

ii) 3 germ layers → formation

iii) hES cells → Immunodeficient mice → teratomas (differentiated tissue representing 3 germ layers)

iv) In vitro differentiation

Mouse ES cells / Human ES cells → Feeder layer removed → Embryoid bodies (having 3 germ layers)

- Ectoderm
- Mesoderm
- Endoderm