Lecture 15

BT 632

Stem Cells, Cancer and Therapy

(3-0-0-6)

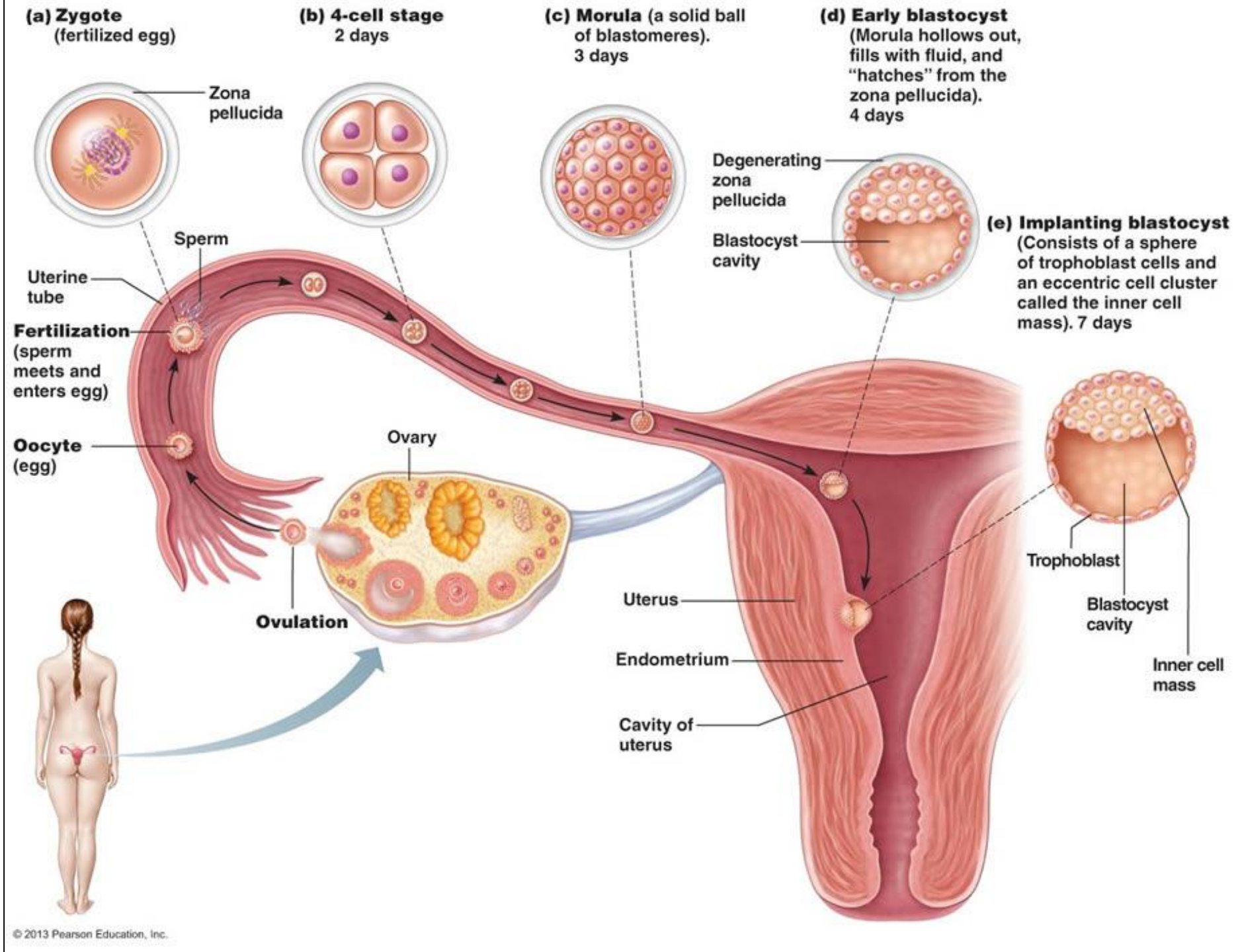
Rajkumar P. Thummer
"O" Block – Room 006; BSBE
Phone: 3208;
Email: rthu@iitg.ac.in

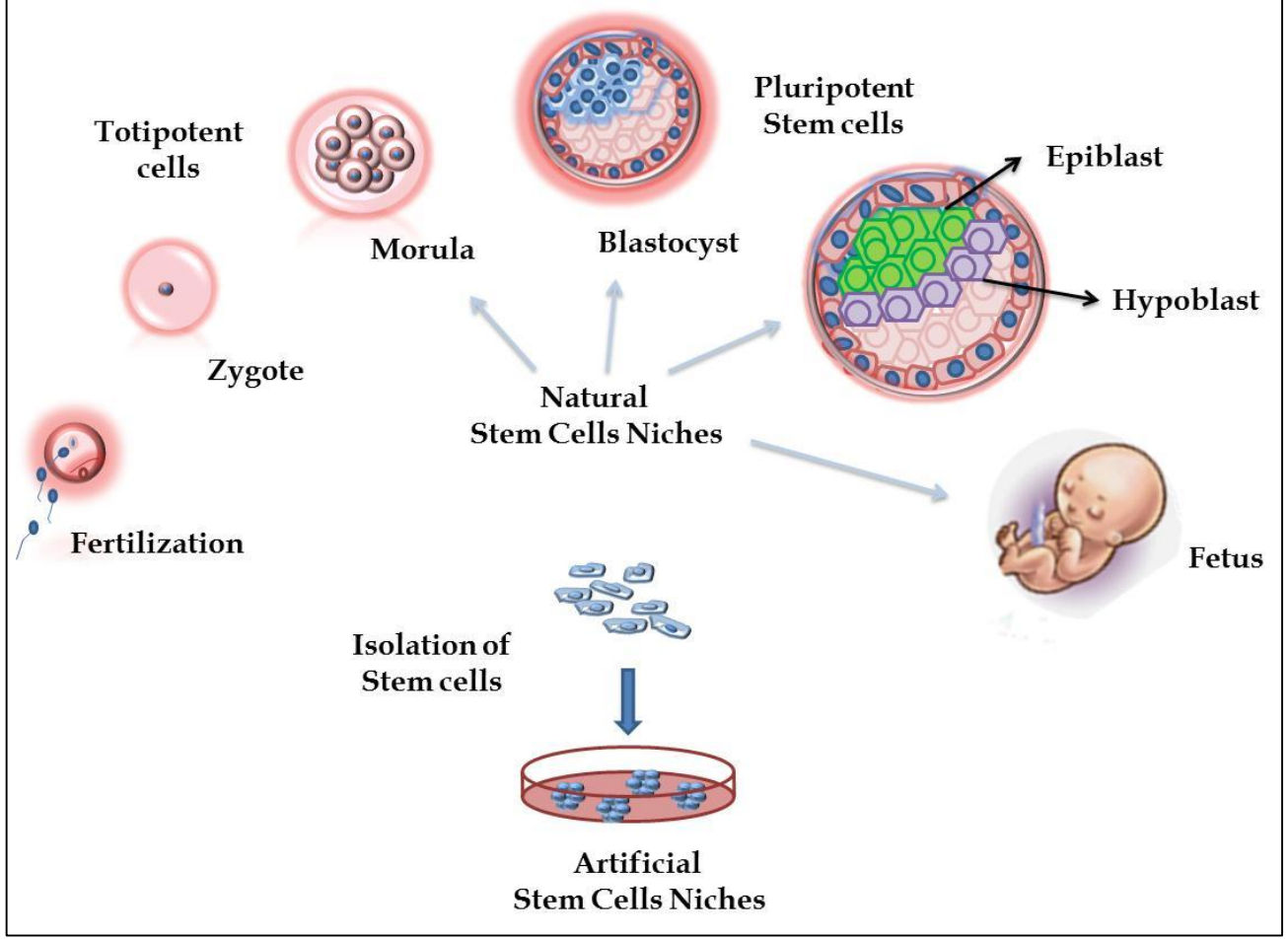
Dr. Rajkumar P Thummer
Assistant Professor
Department of Biosciences and Bioengineering
IIT Guwahati
Guwahati

Epiblast Stem Cells (EpiSCs)

Zona pellucida
(also egg coat or pellucid zone)

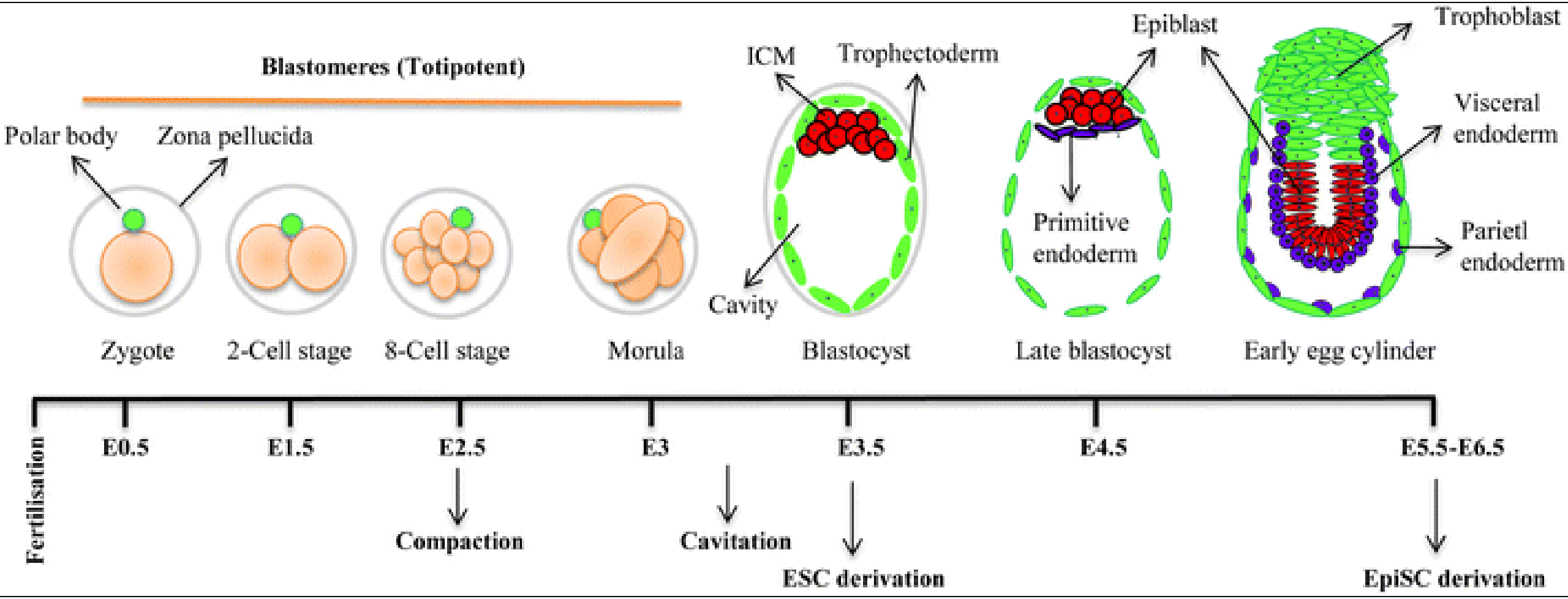
- is a **glycoprotein layer** surrounding the plasma membrane of mammalian oocytes.
- This structure **binds spermatozoa.**
- In humans, **five days** after the fertilization, the blastocyst performs zona hatching; the zona pellucida degenerates and decomposes, to be replaced by the underlying layer of trophoblastic cells.
- The zona pellucida is essential for oocyte death and fertilization.





- After the blastocyst stage, once an embryo implanted in endometrium (in case of rodent), the inner cell mass (ICM) of a fertilized embryo segregates into two layers: **hypoblast and epiblast**.
- The epiblast cells are the functional progenitors of somatic and germ cells. These cells later differentiate into 3 layers, definitive endoderm, mesoderm and ectoderm.
- Stem cells derived from epiblast are pluripotent. These cells are called epiblast-derived stem cells (EpiSC) and have several different cellular and molecular characteristics with Embryonic Stem Cells (ESC) (De-Miguel et al., 2009).

Epiblast Stem Cells (EpiSCs)

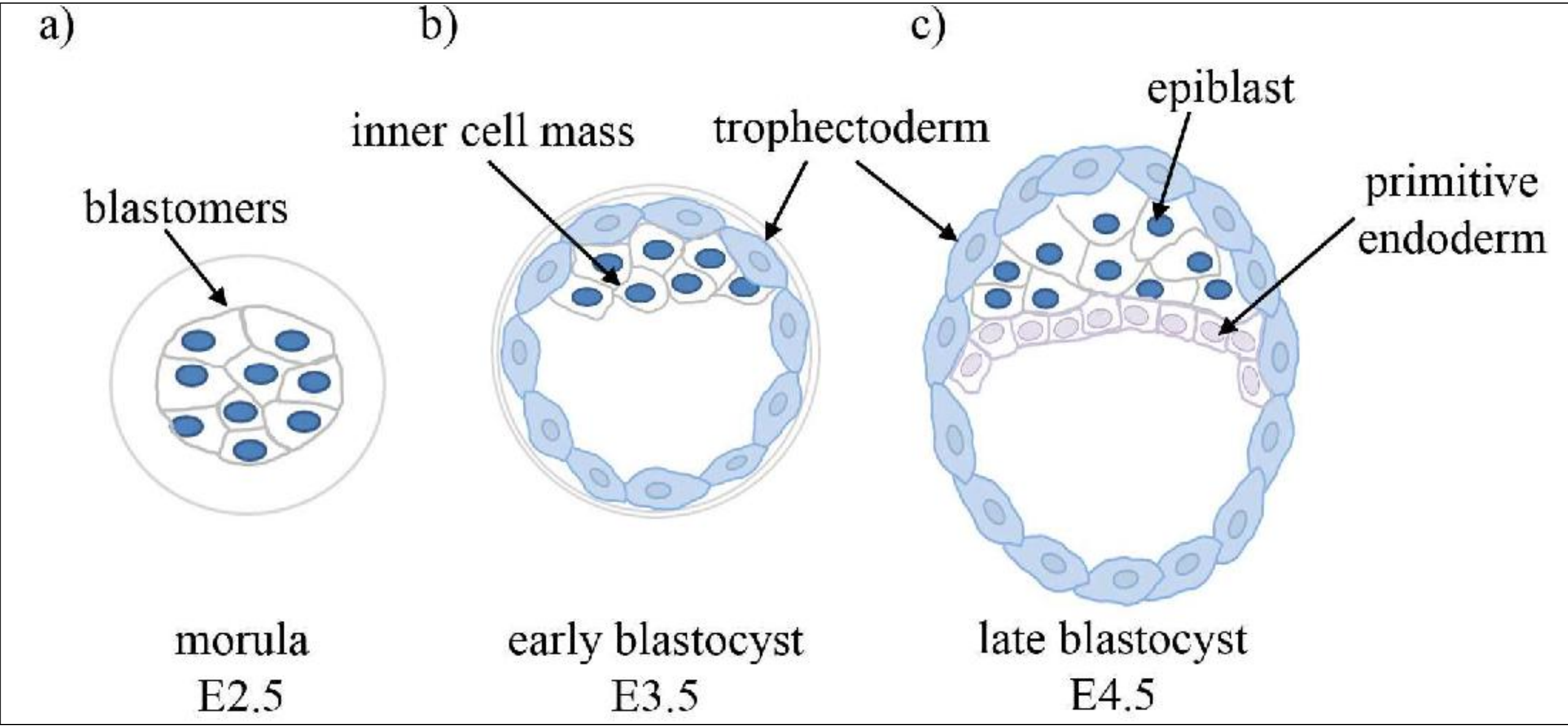


Epiblast Stem Cells (EpiSCs)

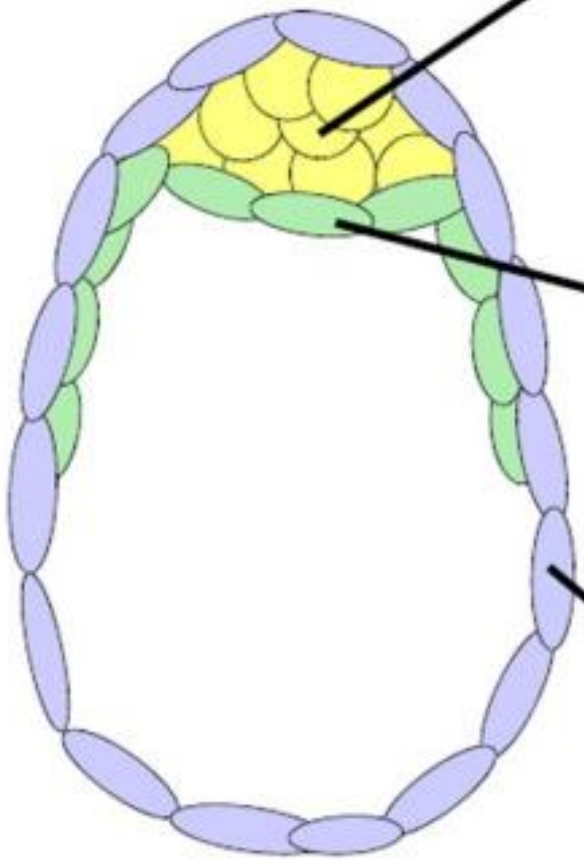
- Epiblast stem cells are derived from the postimplantation mouse embryo just after implantation and prior to gastrulation (Chenoweth et al., 2010).

- Compared to mouse ES cells, they have a distinct gene expression profile (Pluripotency markers: Oct4, Sox2, Nanog expressed but no expression of Klf2, Klf4, Rex1, Nrb04, Fgf4; Specification markers Fgf5, T are expressed) and different mechanisms to regulate pluripotency and differentiation, but they are still pluripotent and hence can differentiate into all three germ layers both in vivo and in vitro (Brons et al., 2007; Tesar et al., 2007).

- They resemble much more closely to human ES cells than mouse ES cells (Brons et al., 2007; Tesar et al., 2007).


a)
b)
c)
blastomers
inner cell mass
trophectoderm
epiblast
primitive
endoderm
morula
E2.5
early blastocyst
E3.5
late blastocyst
E4.5

late
blastocyst



epiblast
(pluripotent)

embryo

primitive
endoderm

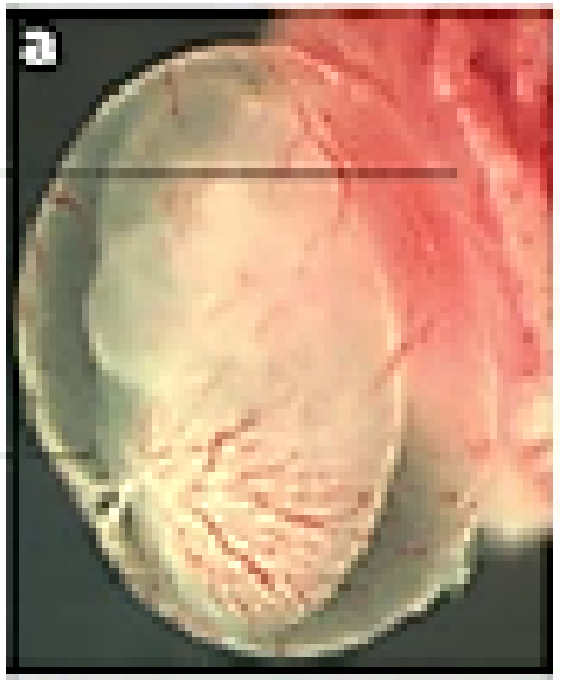
trophectoderm

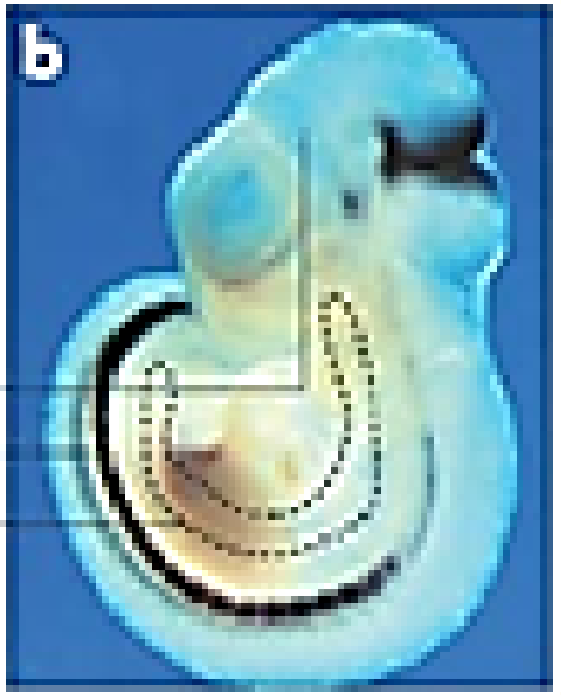
Extra-embryonic tissues,
e.g. placenta etc.

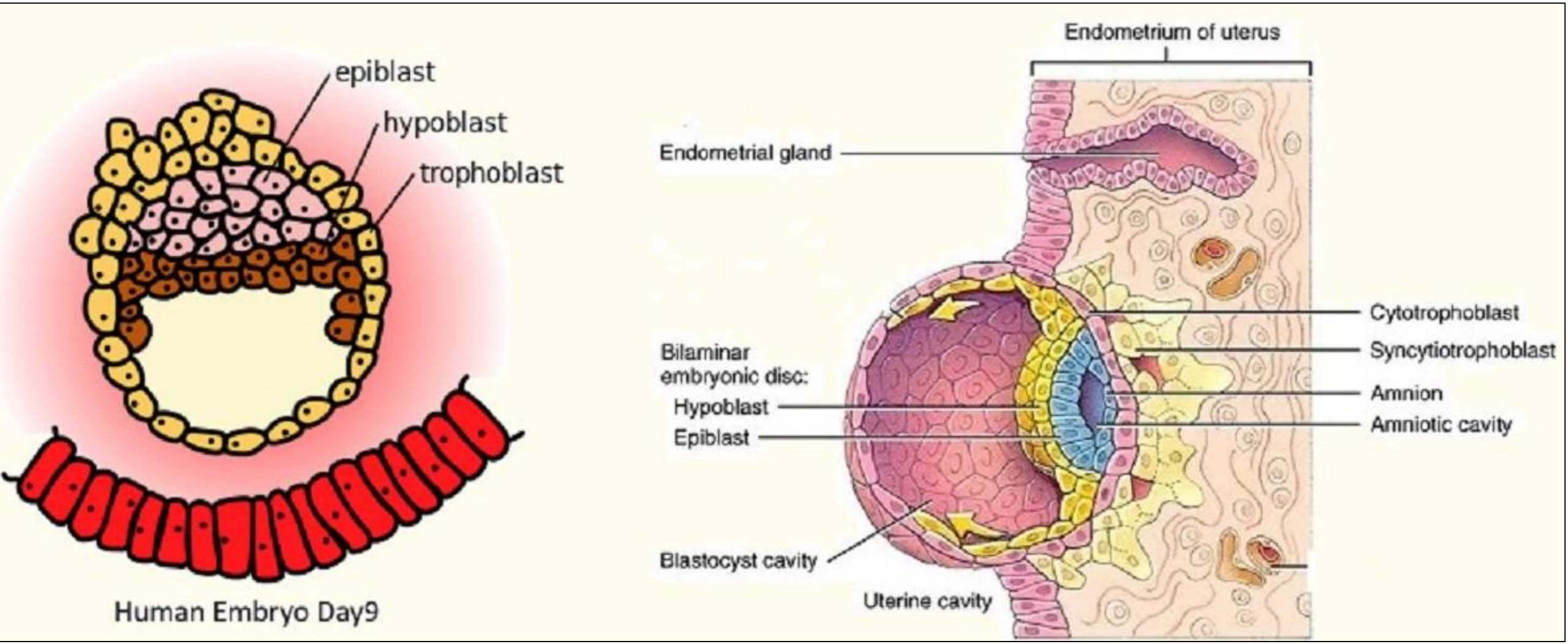
Trophectoderm → Ectoplacental cone
Extraembryonic ectoderm

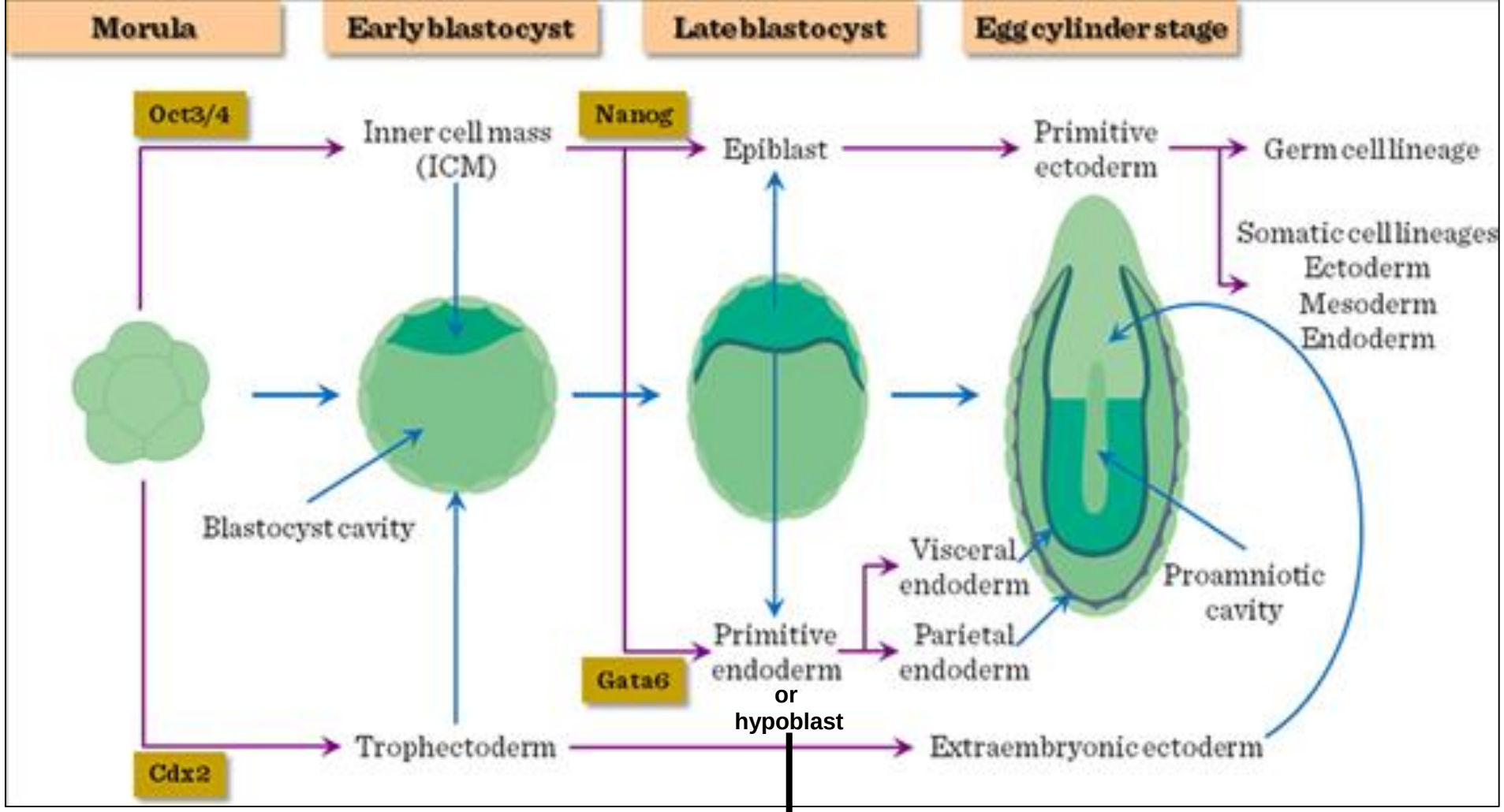
Primitive endoderm → Visceral endoderm
Extraembryonic mesoderm

Inner cell mass → Epiblast → Ectoderm
Mesoderm
Endoderm






epiblast
hypoblast
trophoblast
Human Embryo Day9
Endometrium of uterus
Endometrial gland
Cytotrophoblast
Syncytiotrophoblast
Amnion
Amniotic cavity
Bilaminar
embryonic disc:
Hypoblast
Epiblast
Blastocyst cavity
Uterine cavity



primitive endoderm has major function in proper organogenesis of the fetus and efficient exchange of nutrients, gases and wastes.

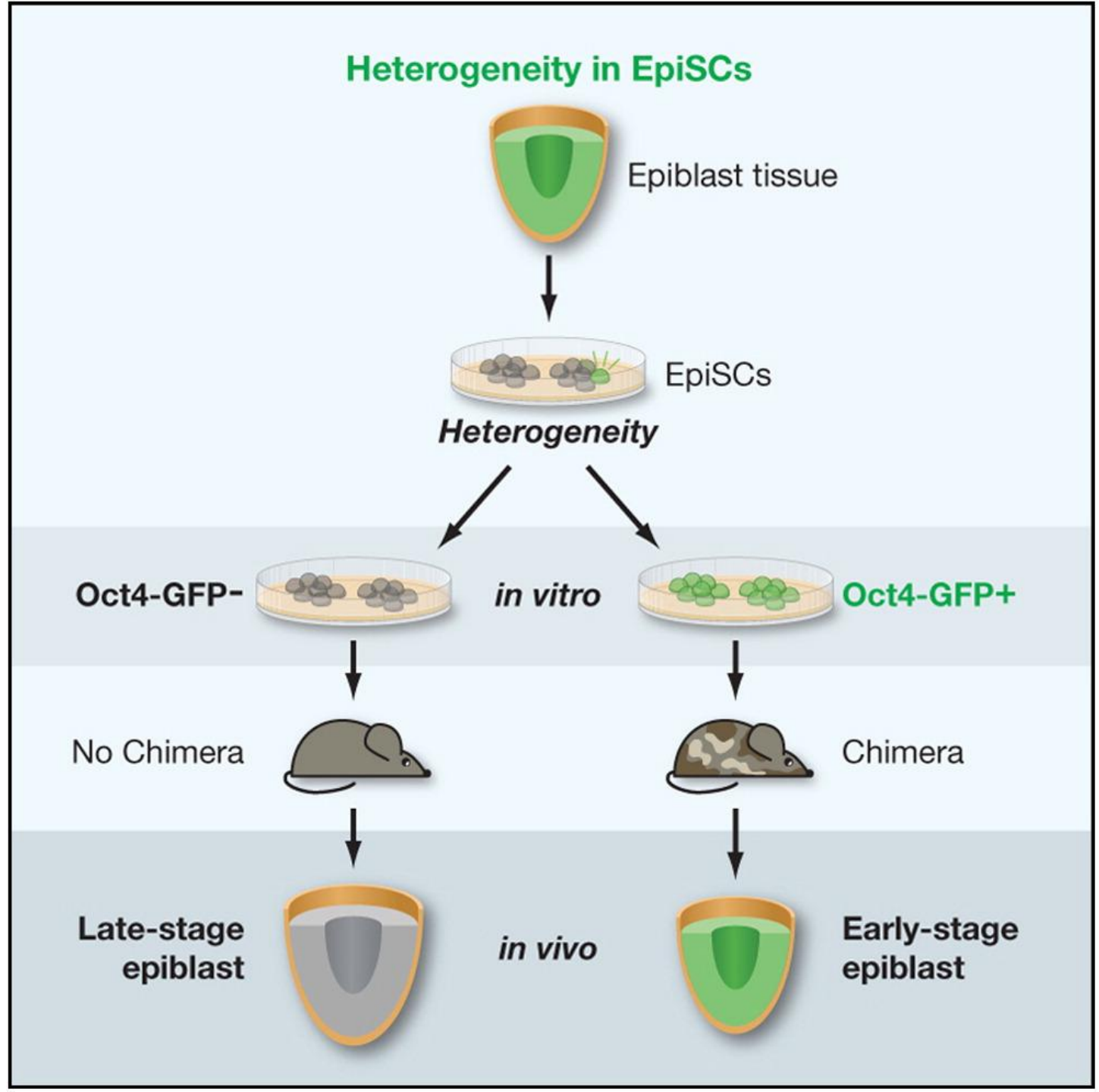
- **Key transcriptional regulators have been identified for lineage specification during early embryo development.**

- Oct4 and Cdx2 have been identified as key regulators of the first embryo differentiation steps. Oct4-deficient embryos develop to the blastocyst stage, but the inner cell mass cells are not pluripotent and only trophectoderm cells can be observed (Nichols et al., 1998).

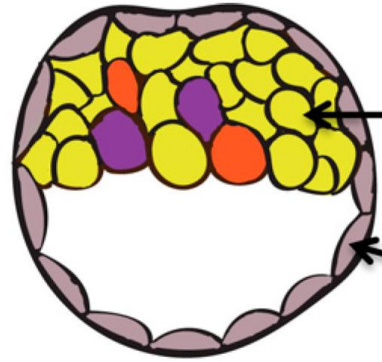
- Cdx2 deficient mice fail to implant due to the lack of trophectoderm development (Strumpf et al., 2005).

- Therefore, during early development, interaction between these two regulators antagonizes each other and is required for segregation of the ICM and trophectoderm fate (Niwa et al., 2005).

- Recently, it has been reported that Tead4 is a key upstream transcription factor required for specification of trophectoderm in pre-implantation mouse embryos (Nishioka et al., 2008).


Heterogeneity in EpiSCs
Epiblast tissue
EpiSCs
Heterogeneity
Oct4-GFP−
in vitro
Oct4-GFP+
No Chimera
Chimera
Late-stage epiblast
in vivo
Early-stage epiblast

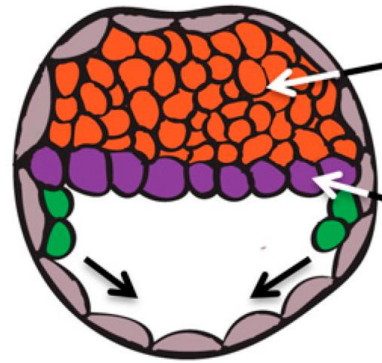
E3.5 Blastocyst
(Early)



Inner Cell Mass
Oct4, Gata6, Nanog

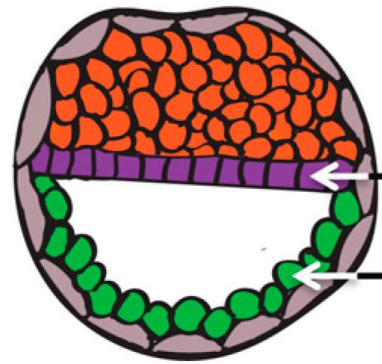
Trophectoderm
Cdx2

E4.5 Blastocyst
(Late)



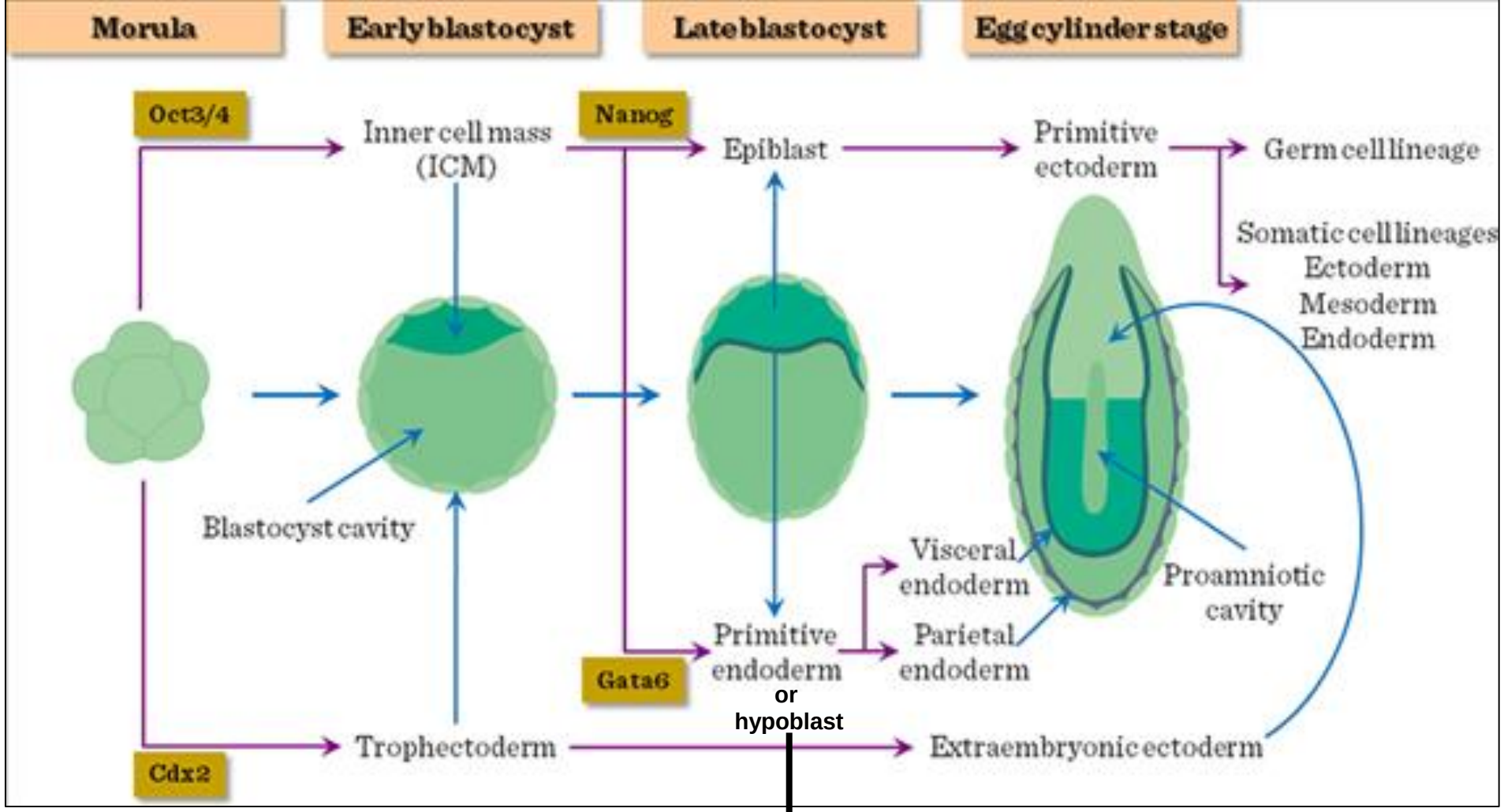
Epiblast
Nanog

Primitive Endoderm
Gata 4,6, Sox7, 17, Dab2, FoxA2



Visceral Endoderm

Parietal Endoderm
Thrombomodulin

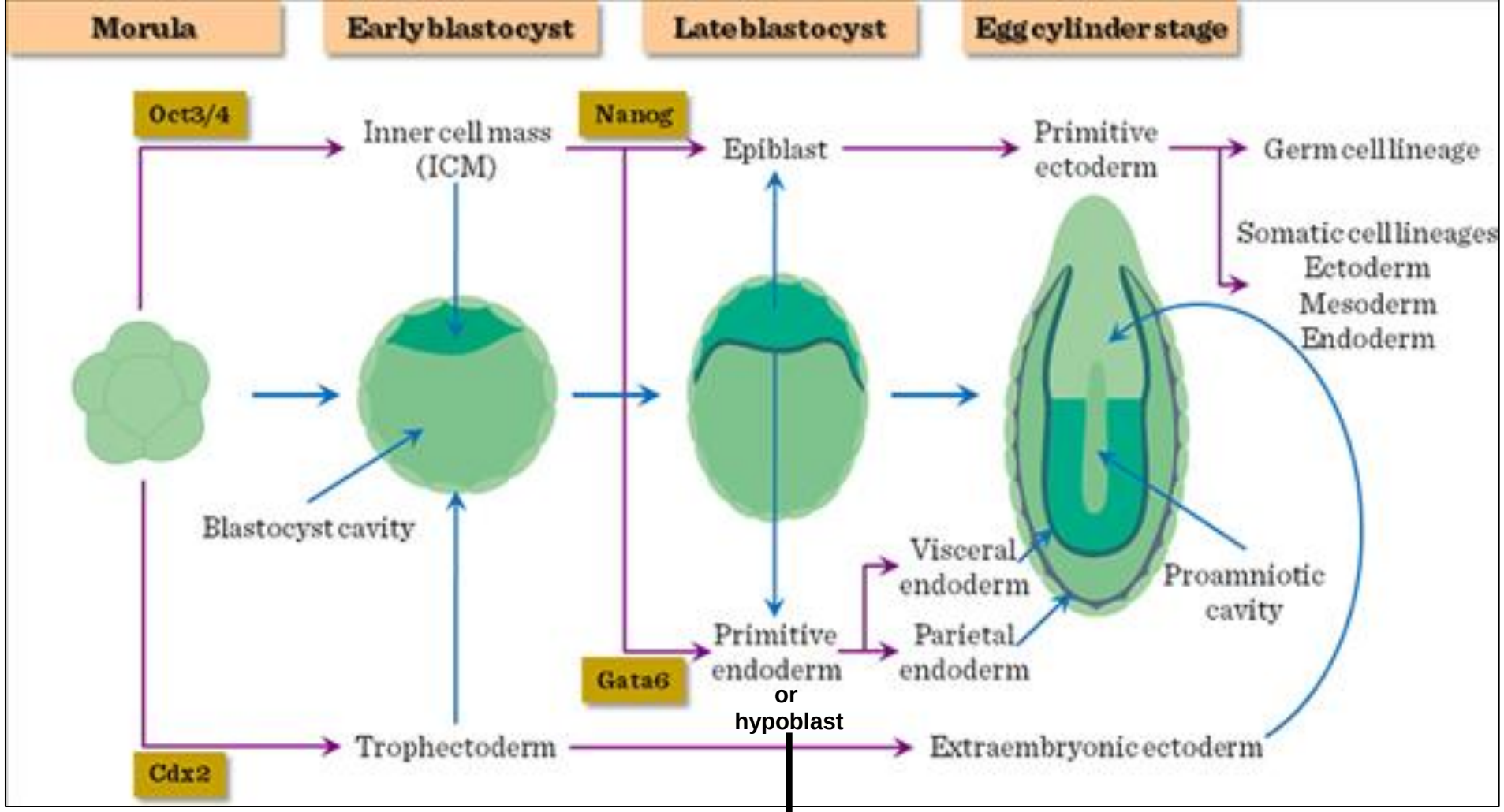


primitive endoderm has major function in proper organogenesis of the fetus and efficient exchange of nutrients, gases and wastes.

- In absence of Nanog, mouse embryos develop trophectoderm and primitive endoderm normally, but lack epiblast cells (Chambers et al., 2003; Mitsui et al., 2003).

- GATA4 or GATA6 is expressed only in the primitive endoderm and extraembryonic endoderm cells and not in epiblast cells. Forced expression of GATA4 or GATA6 in ES cells causes them to differentiate into primitive endoderm (Fujikura et al., 2002). This result is similar to that caused by loss of Nanog function (Mitsui et al., 2003).

- In addition, these two factors get upregulated in absence of Nanog (Mitsui et al., 2003) thus antagonizing each other to define epiblast and primitive endoderm lineages (Chazaud et al., 2006).



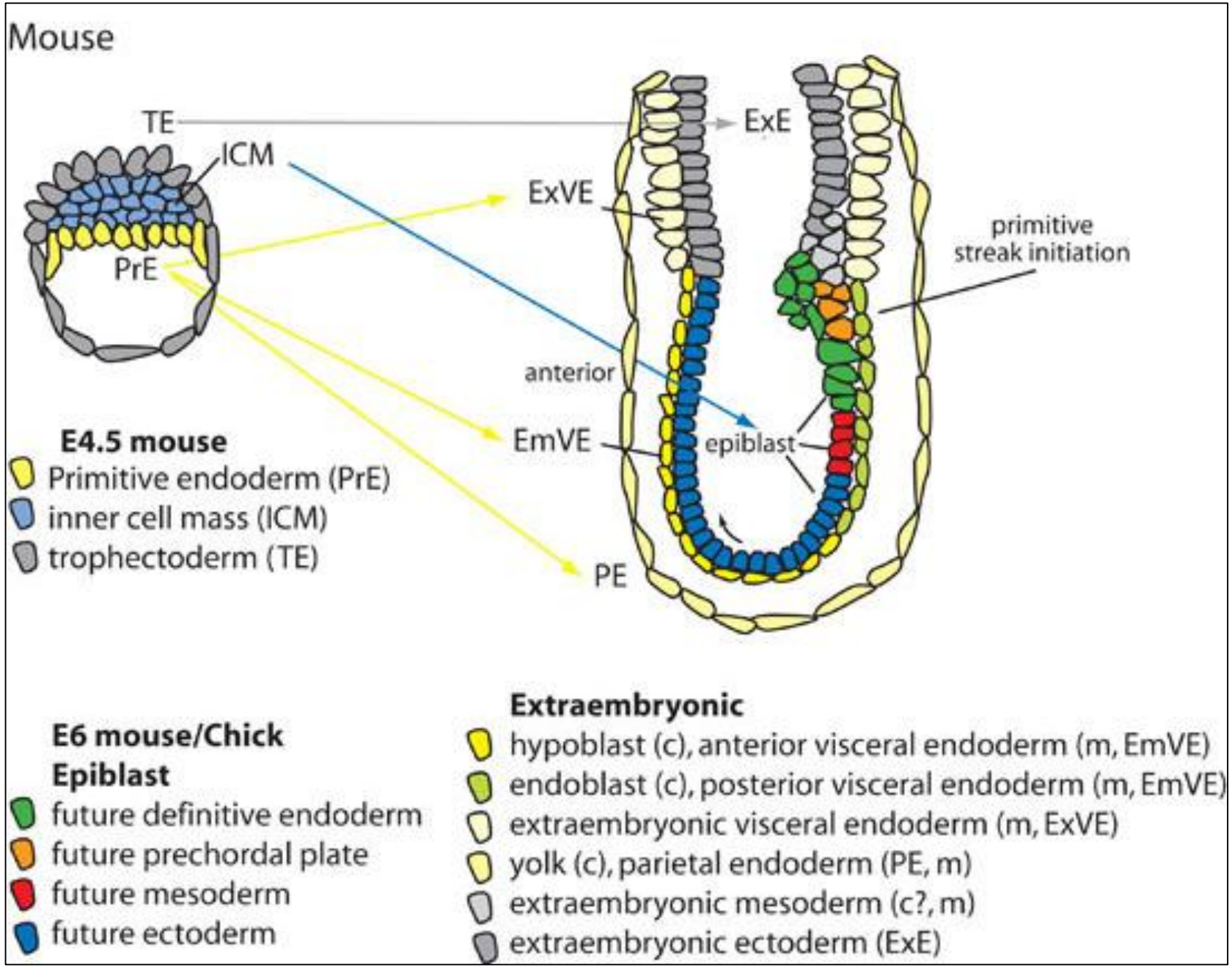
primitive endoderm has major function in proper organogenesis of the fetus and efficient exchange of nutrients, gases and wastes.

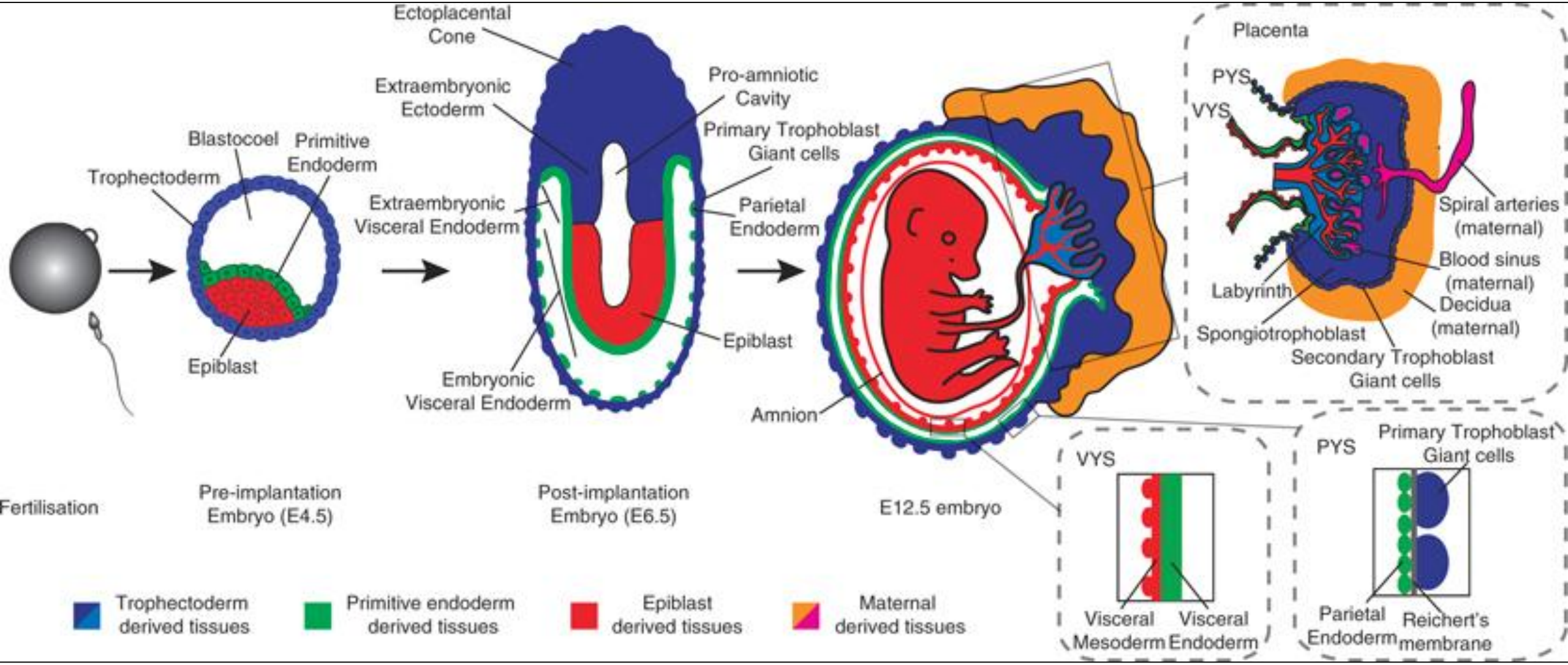
- The hypoblast does not contribute to the embryo, but it has great influence on the orientation and patterning of the embryonic axis. **Hypoblast (or primitive endoderm)** at the time of implantation forms **parietal and visceral endoderm.**

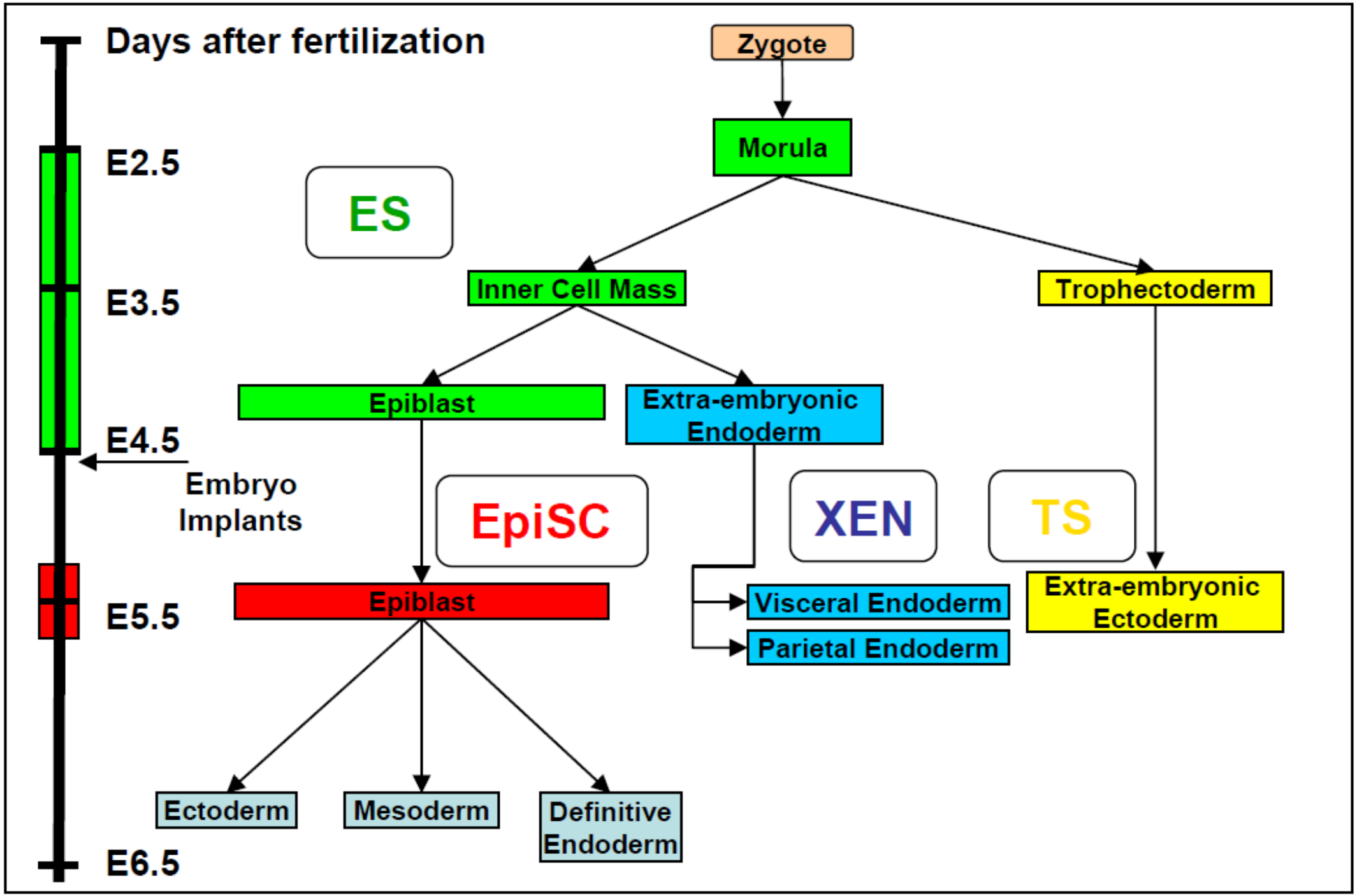
- Hypoblast no longer in contact with the epiblast becomes the parietal endoderm and hypoblast adjacent to the epiblast forms the visceral endoderm.

- The **parietal endoderm** associates with the giant trophoblastic cells and secretes a thick basement membrane converting the blastcoel into the primary yolk sac. The yolk sac becomes filled with maternal plasma proteins.

- In mouse embryo, the **visceral endoderm** develop from the primitive endoderm of the blastocyst during the implantation stage covering the epiblast cells and elongates to become an egg cylinder. The **visceral endoderm digests these proteins to provide nutrients for the rapidly dividing epiblast cells and also induces apoptosis of central cells in the epiblast forming the proamniotic cavity and holds an important instructive role later in development when it aids in patterning of the embryo** (Rossant and Tam, 2009).


Mouse
TE
ICM
PrE
ExE
ExVE
primitive
streak initiation
anterior
EmVE
epiblast
PE
E4.5 mouse
Primitive endoderm (PrE)
inner cell mass (ICM)
trophectoderm (TE)
E6 mouse/Chick
Epiblast
future definitive endoderm
future prechordal plate
future mesoderm
future ectoderm
Extraembryonic
hypoblast (c), anterior visceral endoderm (m, EmVE)
endoblast (c), posterior visceral endoderm (m, EmVE)
extraembryonic visceral endoderm (m, ExVE)
yolk (c), parietal endoderm (PE, m)
extraembryonic mesoderm (c?, m)
extraembryonic ectoderm (ExE)


Ectoplacental Cone
Extraembryonic Ectoderm
Pro-amniotic Cavity
Primary Trophoblast Giant cells
Blastocoel
Primitive Endoderm
Trophectoderm
Extraembryonic Visceral Endoderm
Parietal Endoderm
Epiblast
Embryonic Visceral Endoderm
Amnion
Placenta
PYS
VYS
Spiral arteries (maternal)
Blood sinus (maternal)
Labyrinth
Decidua (maternal)
Spongiotrophoblast
Secondary Trophoblast Giant cells
Fertilisation
Pre-implantation Embryo (E4.5)
Post-implantation Embryo (E6.5)
E12.5 embryo
VYS
Visceral Mesoderm
Visceral Endoderm
PYS
Primary Trophoblast Giant cells
Parietal Endoderm
Reichert's membrane
Trophectoderm derived tissues
Primitive endoderm derived tissues
Epiblast derived tissues
Maternal derived tissues


Days after fertilization
E2.5
E3.5
E4.5
Embryo Implants
E5.5
E6.5
Zygote
Morula
ES
Inner Cell Mass
Trophectoderm
Epiblast
Extra-embryonic Endoderm
EpiSC
XEN
TS
Epiblast
Visceral Endoderm
Parietal Endoderm
Extra-embryonic Ectoderm
Ectoderm
Mesoderm
Definitive Endoderm

Embryonic Germ Cells (EGCs)

Embryonic Germ Cells (EGCs)

- Embryonic germ (EG) cells, derived from primordial germ cells found in the gonadal ridge of a late embryo, have many of the properties of embryonic stem cells. The primordial germ cells in an embryo develop into stem cells that in an adult generate the reproductive gametes (sperm or eggs).

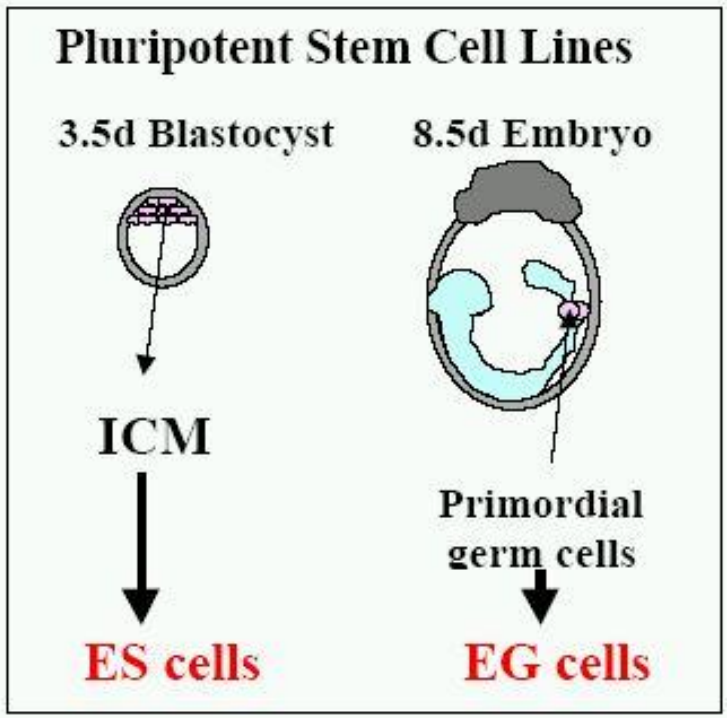
- Embryonic germ cells are the cells in the embryo that give rise to the reproductive cells – gametes – of sexually reproducing organisms. In animals, male gametes are sperm cells and female gametes are egg cells, also known as ova.

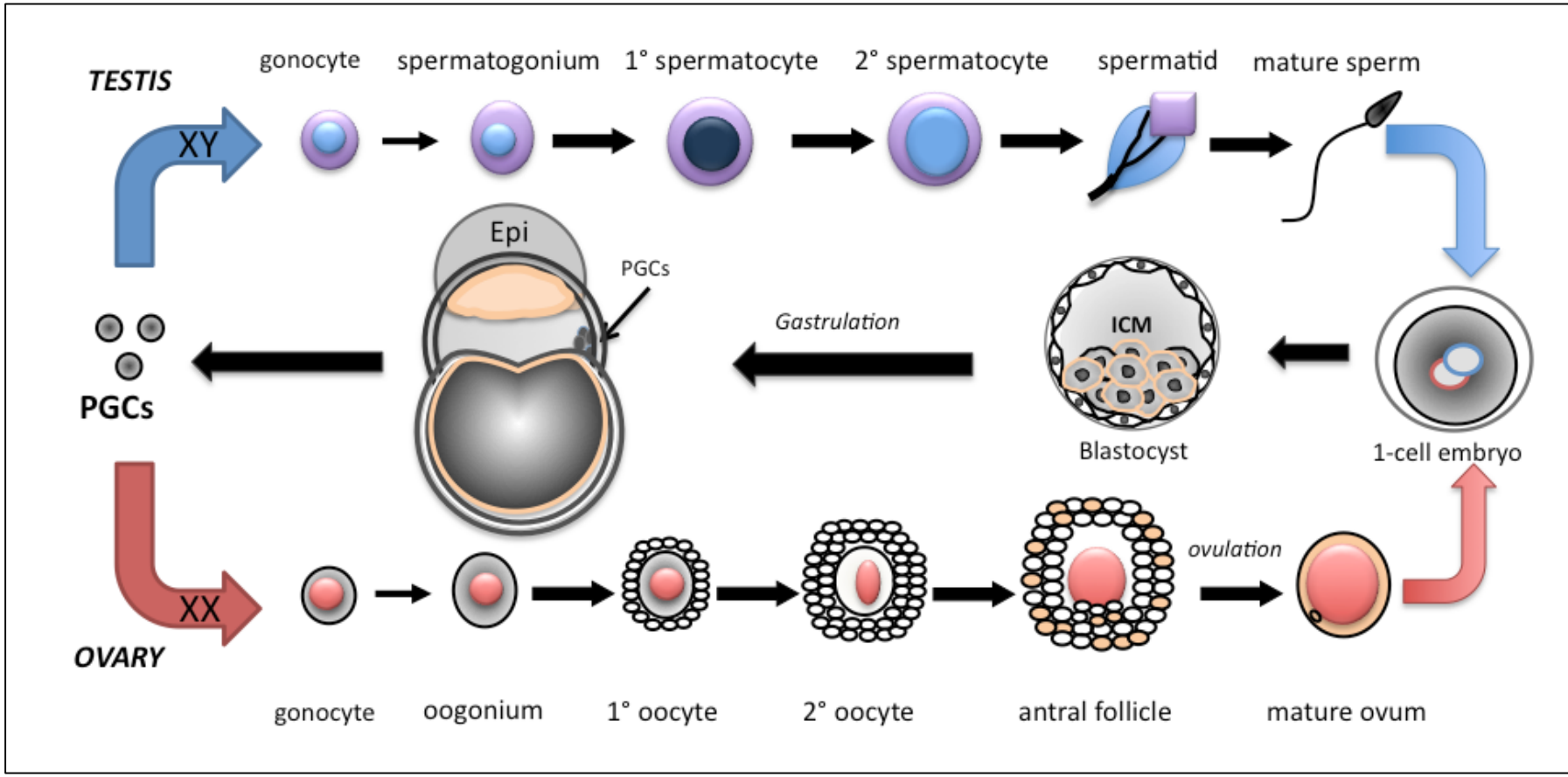
- Embryonic germ cells (EGs) are derived from primordial germ cells or diploid germ cell precursors of fetal genital ridges.

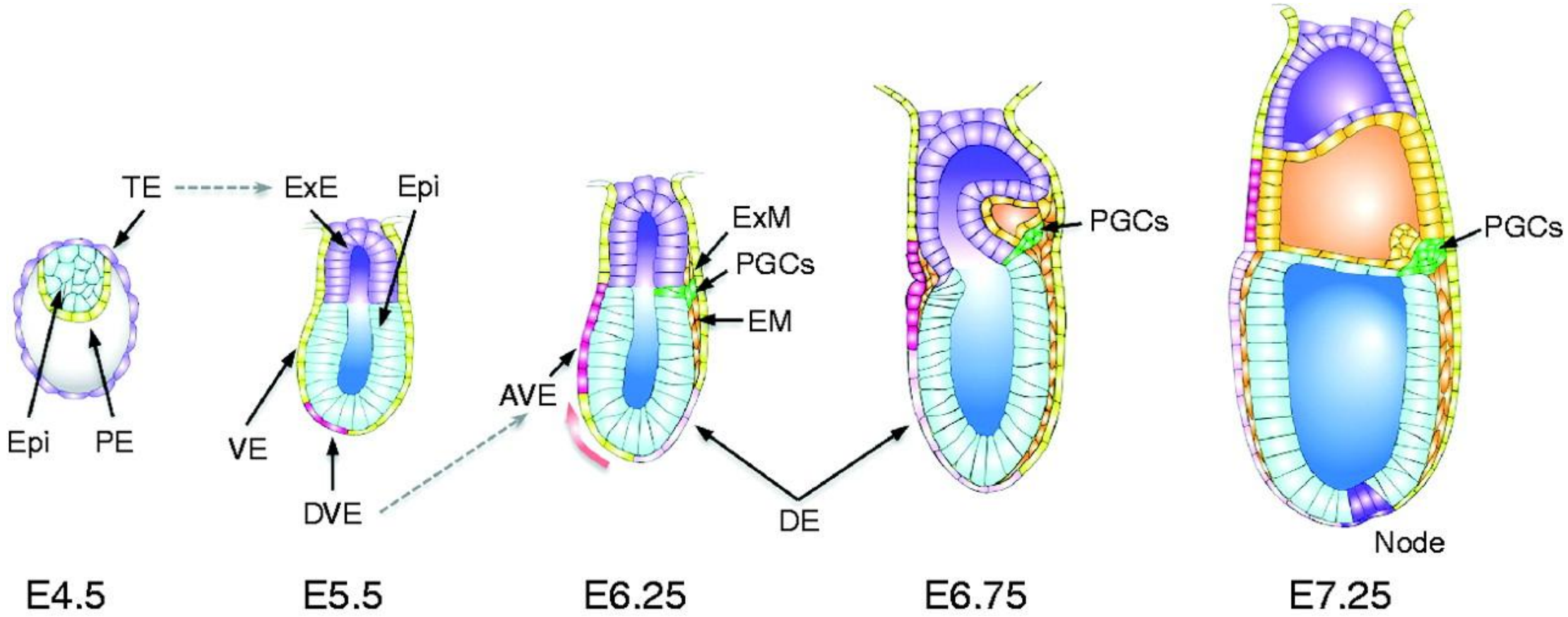
- These cells exist transiently in the embryo before they closely associate with somatic cells of the gonads and then become committed as germ cells (Liu et al., 2004; Matsui et al., 1992; Shamblott et al., 1998).

- They are cultured on feeder layers to give rise to cells that resemble embryonic stem cells.

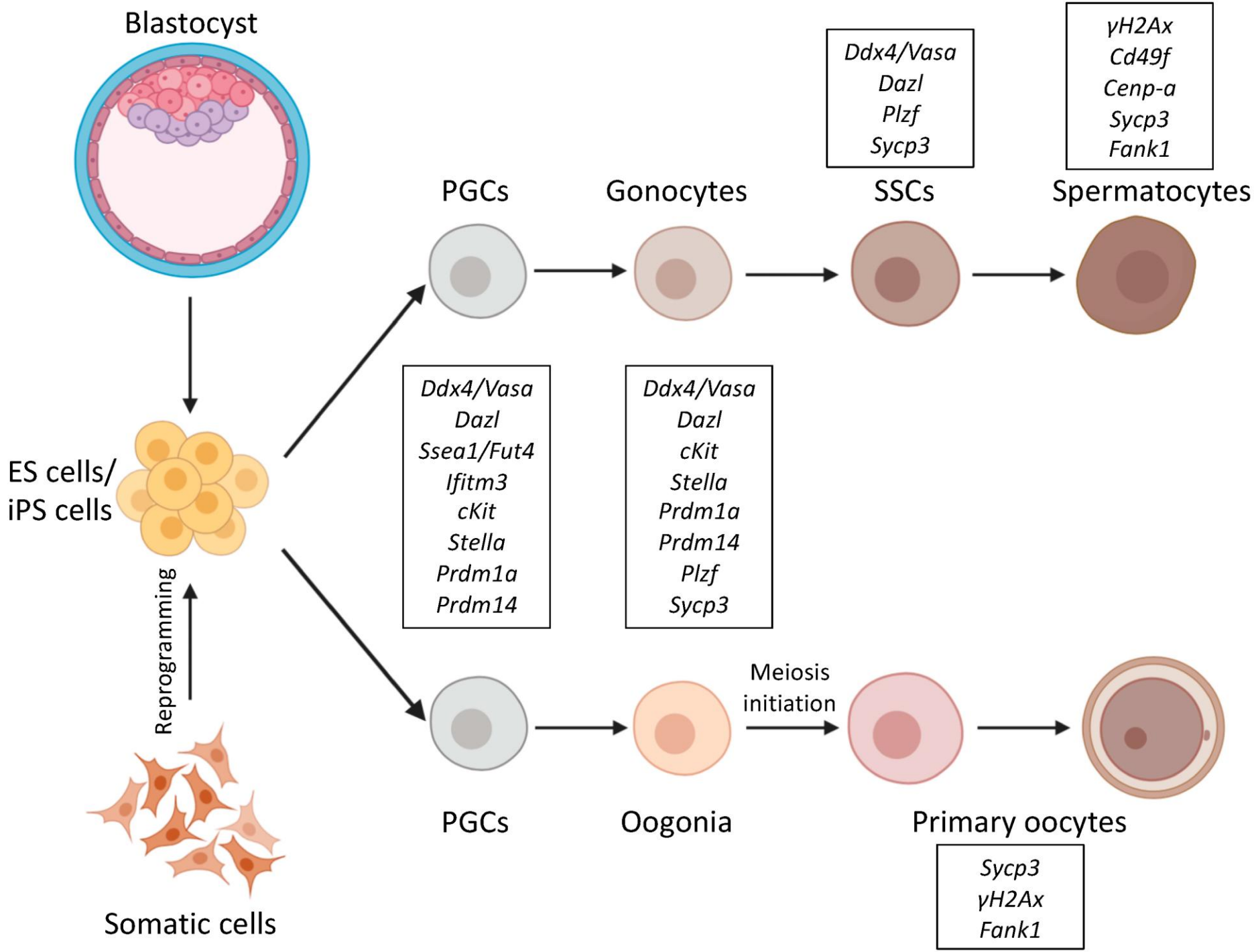
- These EG cells are pluripotent and form teratocarcinomas when injected into nude mice and contribute to chimeras (Stewart et al., 1994).


Pluripotent Stem Cell Lines
3.5d Blastocyst
8.5d Embryo
ICM
Primordial germ cells
ES cells
EG cells


TESTIS
gonocyte
spermatogonium
1° spermatocyte
2° spermatocyte
spermatid
mature sperm
XY
Epi
PGCs
Gastrulation
ICM
Blastocyst
1-cell embryo
PGCs
XX
OVARY
ovulation
gonocyte
oogonium
1° oocyte
2° oocyte
antral follicle
mature ovum



Early development of the mouse embryo. Six days after fertilization (E6.25), the mouse embryo consists of three layers. Primordial germ cells (PGCs, red dots) arise from a cell population in the proximal epiblast adjacent to the extra-embryonic ectoderm. These cells then pass through the primitive streak and give rise to several extra-embryonic mesodermal lineages and to germ cells. By E7.25, a distinct cluster of ~45 tissue non-specific, alkaline phosphatase (Tnap)-positive PGCs is present within the extra-embryonic mesoderm (red dots).


Blastocyst
ES cells/
iPS cells
Reprogramming
Somatic cells
PGCs
Gonocytes
SSCs
Spermatocytes
Ddx4/Vasa
Dazl
Plzf
Sycp3
γH2Ax
Cd49f
Cenp-a
Sycp3
Fank1
Ddx4/Vasa
Dazl
Ssea1/Fut4
Ifitm3
cKit
Stella
Prdm1a
Prdm14
Ddx4/Vasa
Dazl
cKit
Stella
Prdm1a
Prdm14
Plzf
Sycp3
PGCs
Oogonia
Meiosis
initiation
Primary oocytes
Sycp3
γH2Ax
Fank1

Embryonic Carcinoma Cells (ECCs)

Embryonic Carcinoma Cells (ECCs)

- Embryonal carcinoma is a relatively uncommon type of germ cell tumour that occurs in the ovaries and testes.

In the ovary, embryonal carcinoma is quite rare, amounting to approximately three percent of ovarian germ cell tumours.

In the testis, pure embryonal carcinoma is also uncommon, and accounts for approximately ten percent of testicular germ cell tumours.

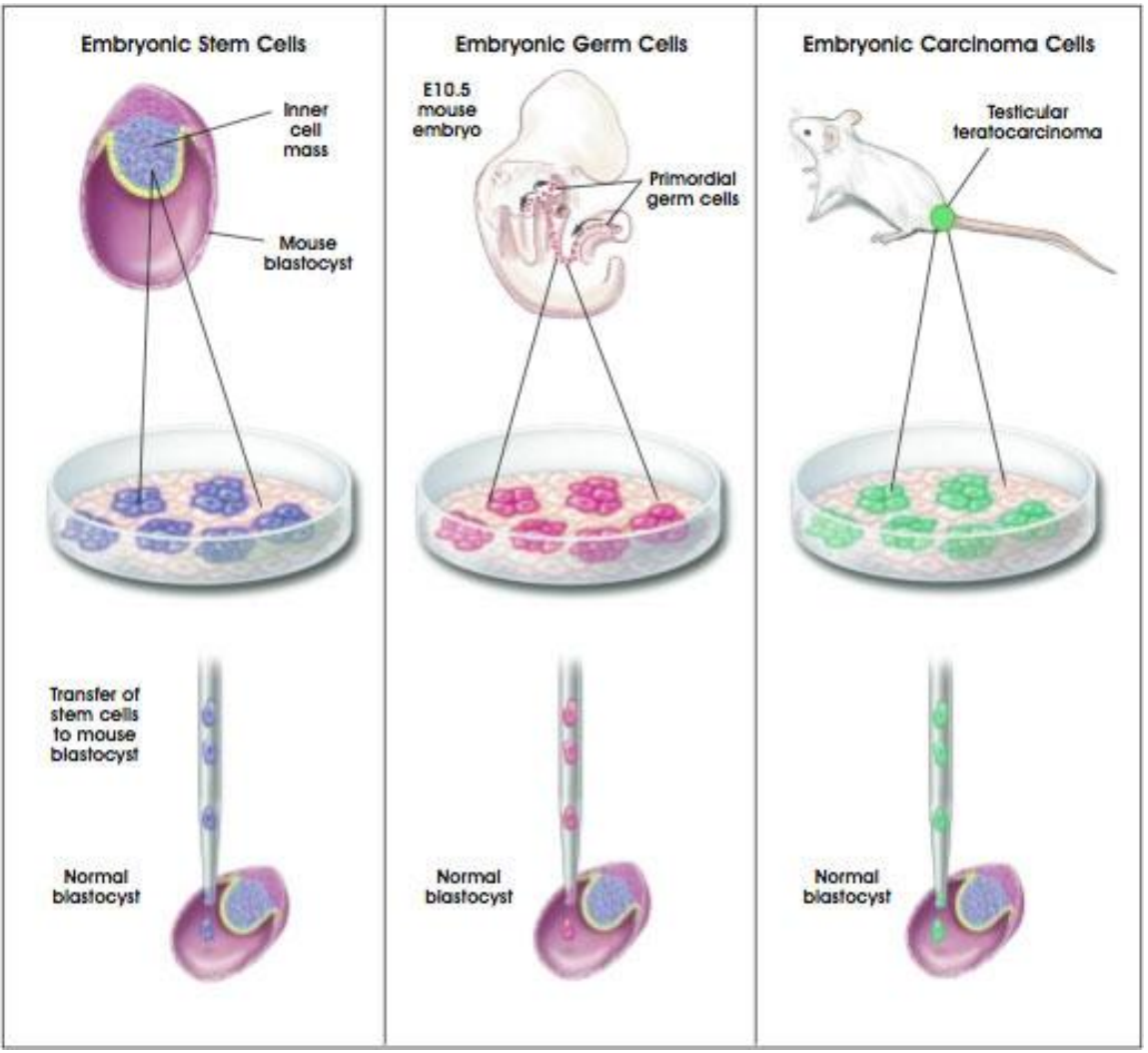
- Prior to the derivation of embryonic stem cells, a pluripotent embryonic cell line derived from germ line tumors was established and extensively used in research. These germ line tumors are stem cells of embryonic teratocarcinoma.

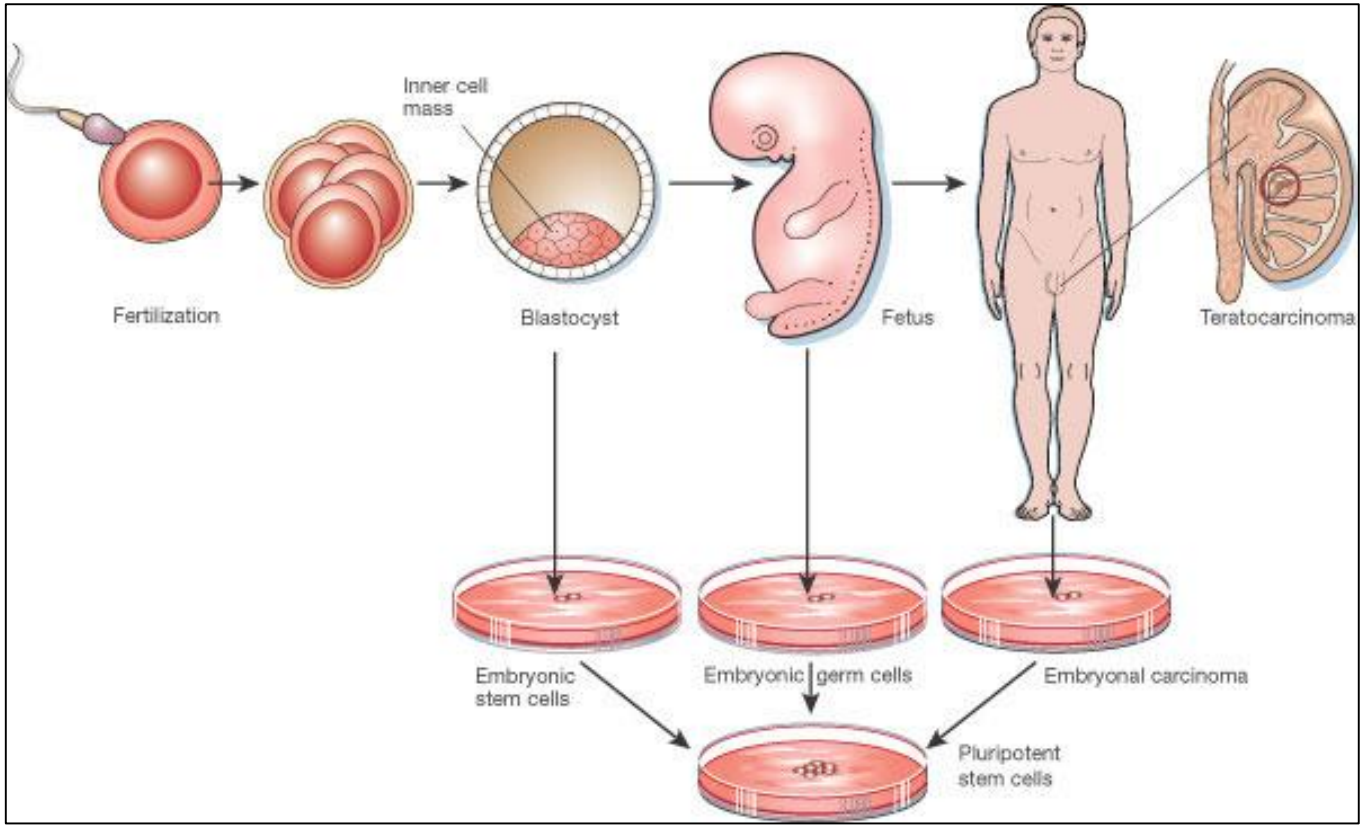
- These stem cells exhibit the same characteristics as ES cells.

- They can be maintained in culture for long periods of time and they can differentiate into each of the three embryonic germ layers, endoderm, mesoderm and ectoderm (Solter, 2006).

- They can contribute to all the cell lineages when they are injected into blastocyst for the generation of chimeric mice (Bradley et al., 1984).

- However the tumor identity of these cell lines does not make them a good tool for in vivo work or studies into embryonic development (Solter, 2006).


Embryonic Stem Cells
Inner cell mass
Mouse blastocyst
Transfer of stem cells to mouse blastocyst
Normal blastocyst
Embryonic Germ Cells
E10.5 mouse embryo
Primordial germ cells
Normal blastocyst
Embryonic Carcinoma Cells
Testicular teratocarcinoma
Normal blastocyst


Inner cell mass
Fertilization
Blastocyst
Fetus
Teratocarcinoma
Embryonic stem cells
Embryonic germ cells
Embryonal carcinoma
Pluripotent stem cells

Embryo-derived pluripotent stem cells

- All these embryo-derived stem cells can differentiate into multiple cell types of a human body. These cells contain the genetic information for all cell types. They can be easily cultured in large quantities. These cells could be used to study early events in human development in vitro to avoid birth defects and placental abnormalities that lead to spontaneous abortion.

- When transplanted into target tissues, pluripotent cells may form tumors (Amariglio et al., 2009; Erdo et al., 2003) and hence evaluation of possible oncogenic properties needs to be looked into.

- Furthermore, ES cell-derived target cell types may also cause an immune reaction in the recipient, causing the rejection of transplanted cells and hence there is a need to overcome the immune rejection barrier. Only autologous transplants were appropriate and patient matched ES cells need to be generated.

- Apart from the scientific limitations, there are ethical and regulatory concerns of destruction of human embryos to isolate these embryo-derived stem cells are also a critical factor. Thus, at this stage, any therapies based on the use of these cells are still hypothetical.