REKAYASA EMBRIO

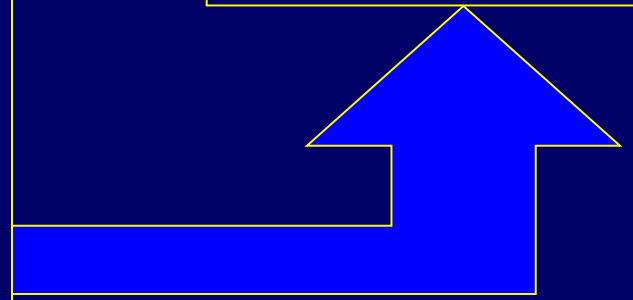
= tindakan atau intervensi yg dilakukan pd embrio berdasarkan kaidah ilmu untuk meningkatkan nilai tambah embrio

EMBRIOLOGI

- Pd mulanya dipelajari scr deskriptif → mencari jawaban: apa, kapan & bagaimana embrio terbentuk
- Embriologi eksperimental & embriologi perbandingan → mencari jawaban mengapa embrio itu terbentuk
- Embriologi eksperimental mrp cikal bakal rekayasa embrio

ALASAN

1. Penyediaan kebutuhan bahan pokok bagi manusia
2. Upaya rehabilitasi fungsi reproduksi (klinis)
3. Pelestarian hayati (konservasi)
4. Pendidikan & penelitian → eksplorasi informasi dasar
5. Menghasilkan teknologi → bioteknologi embrio



Rekayasa spermatozoa

- Testis dipandang tidak efisien → spermatozoa yg dihasilkan jmlnya sgt berlebihan drpd yg diperlukan
- Hal tsb mrp isyarat bahwa spermatozoa perlu utk diberdayakan
- Rekayasa spermatozoa:
  - Inseminasi buatan (IB)
  - Kriopreservasi
  - Mikroinjeksi spermatozoa → utk IVF

Assisted insemination

a procedure where sperm is placed into the reproductive tract of a woman for the purpose of impregnating the woman by using means other than sexual intercourse

Procedure:

- intracervical insemination (ICI)
- Intrauterine insemination (IUI)

KRIOPRESERVASI

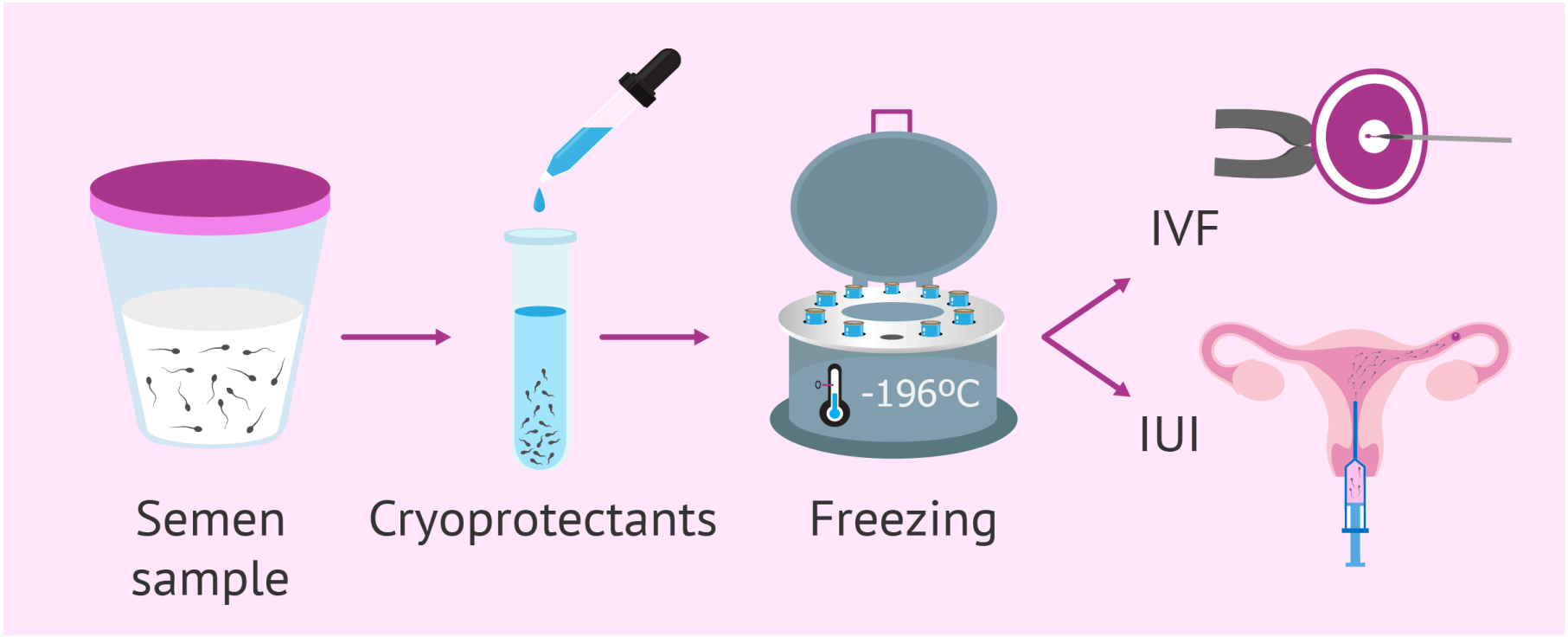
- Kriopreservasi: penyimpanan embrio/sel gamet dlm btk beku (-196^{o}C dlm LN_2)
- Alasan:
  - Embrio/sel gamet mpy daya tahan hidup relatif singkat
  - Aman
  - Bisa dimanfaatkan di masa datang (bank sperma)

Teknik:

1. Slow freezing
2. Rapid freezing
3. Vitrifikasi

- Kendala dlm kriopreservasi → kerusakan/kematian embrio/sel gamet krn:
  - Terbentuknya intracellular ice crystal → pd rapid freezing
  - Peningkatan konsentrasi cairan sitoplasma → pd slow freezing
  - Dehidrasi sel

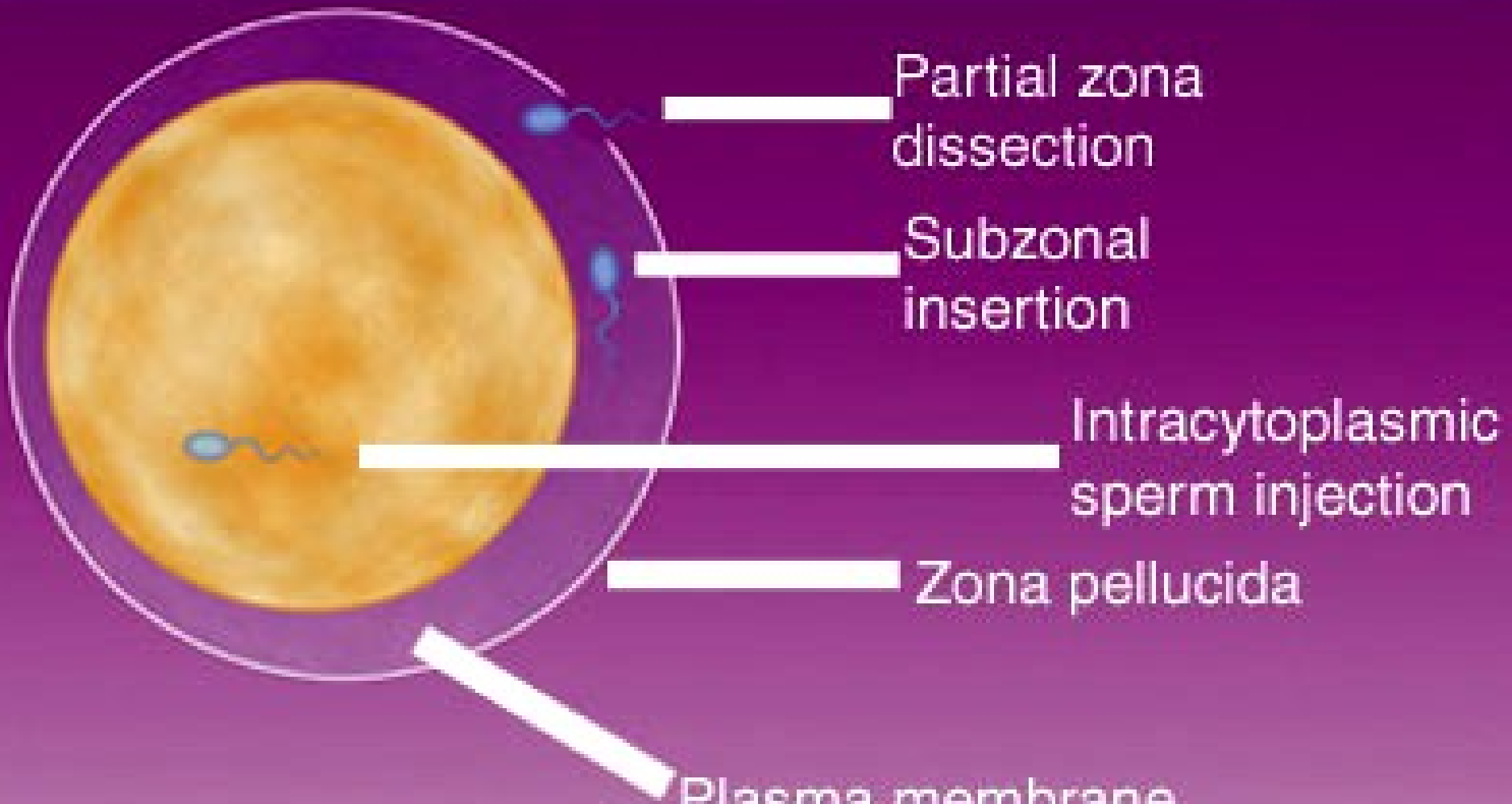
- Utk menanggulangi hal tsb:
  - Penambahan krioprotektan (utk menghambat dehidrasi sel) → gliserol, dimethylsulfoxide (DMSO), etilen glikol, sukrosa, rafinosa

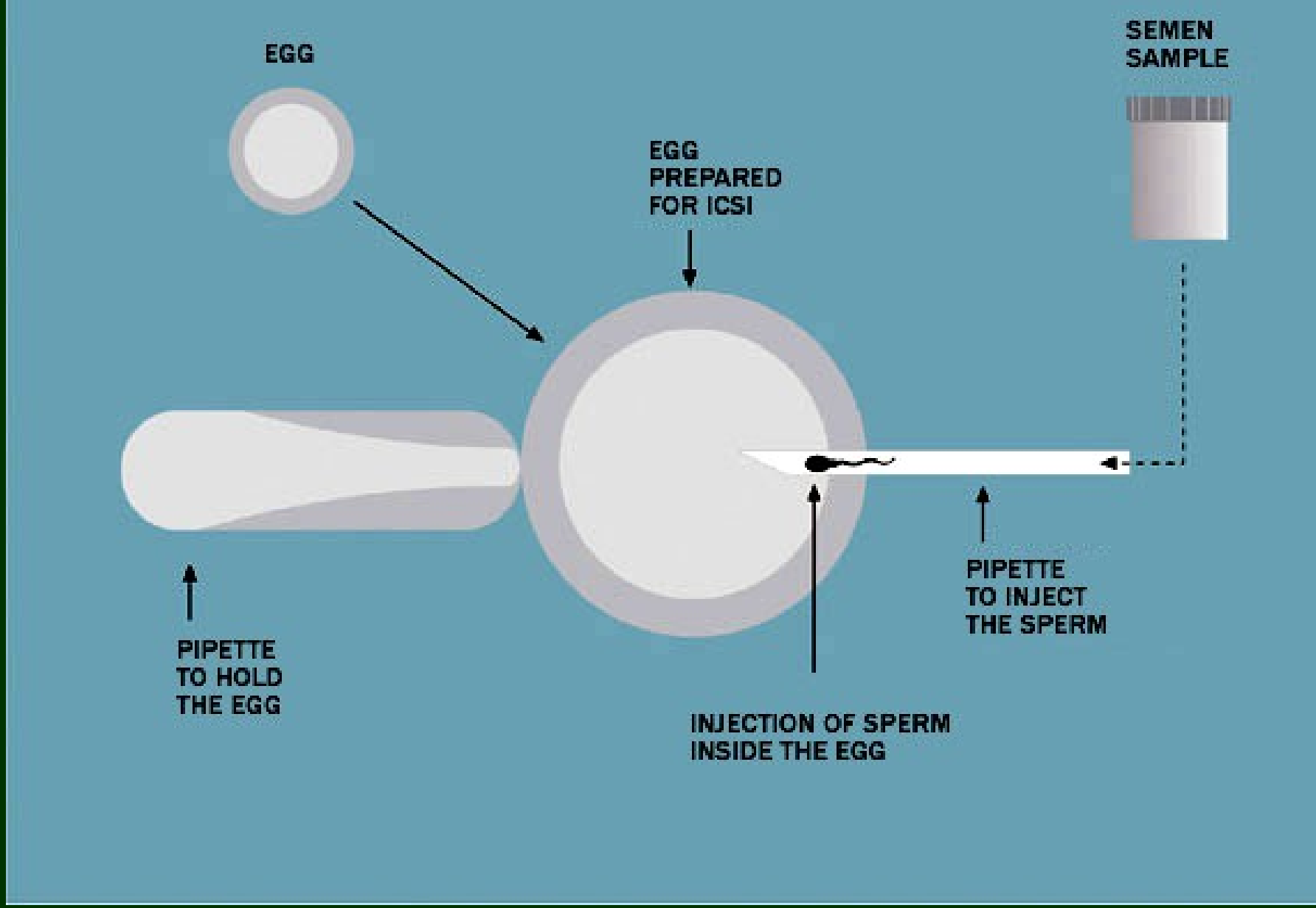

-196ºC
IVF
IUI
Semen sample
Cryoprotectants
Freezing

Mikroinjeksi spermatozoa

* Digunakan dlm IVF
* Dgn cara menyuntikkan satu sel spermatozoa dlm sel telur
* Sperm recovery
  - Pd manusia dgn kasus infertilitas: disfungsi ereksi (kegagalan ejakulasi), kualitas spermatozoa buruk & azoospermi/oligospermi
  - Caranya:
    - ✓ PESA (percutaneus epididymal sperm aspiration)
    - ✓ TESA (testicular sperm aspiration)
    - ✓ TESE (testicular sperm extraction)
    - ✓ MESA (microsurgical epididymal sperm aspiration)
* Cara mikroinjeksi spermatozoa:

1. SUZI (subzonal sperm insertion) → menempatkan spermatozoa ke dlm daerah sub zona pelusida
2. ICSI (intracytoplasmic sperm injection) → injeksi spermatozoa ke dlm sitoplasma sel telur


Partial zona dissection
Subzonal insertion
Intracytoplasmic sperm injection
Zona pellucida
Plasma membrane


EGG
SEMEN SAMPLE
EGG PREPARED FOR ICSI
PIPETTE TO HOLD THE EGG
PIPETTE TO INJECT THE SPERM
INJECTION OF SPERM INSIDE THE EGG

REKAYASA SEL TELUR

- Superovulasi → memungkinkan peningkatan produksi sel telur
- Kriopreservasi
- IVF (in vitro fertilization)

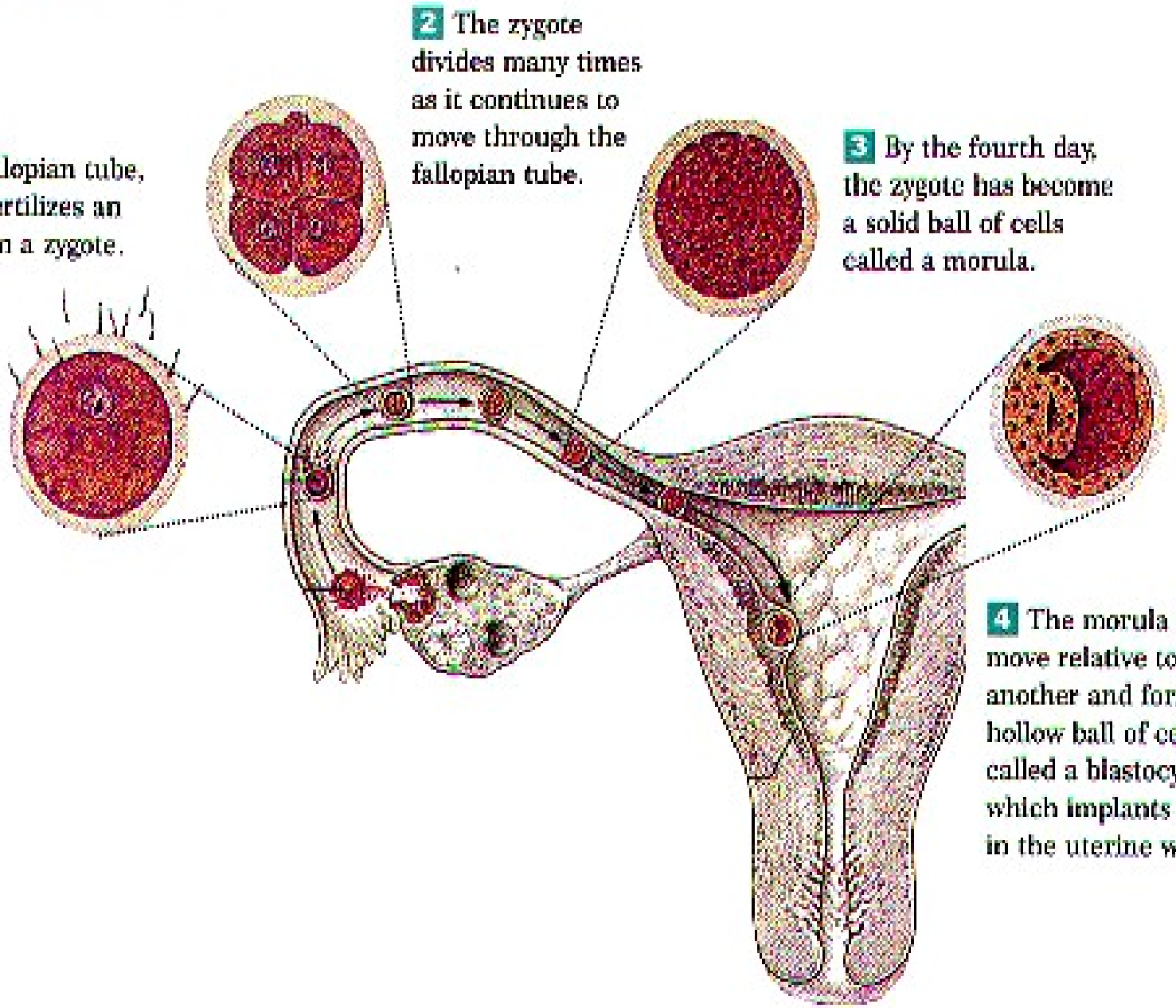
SUPEROVULASI

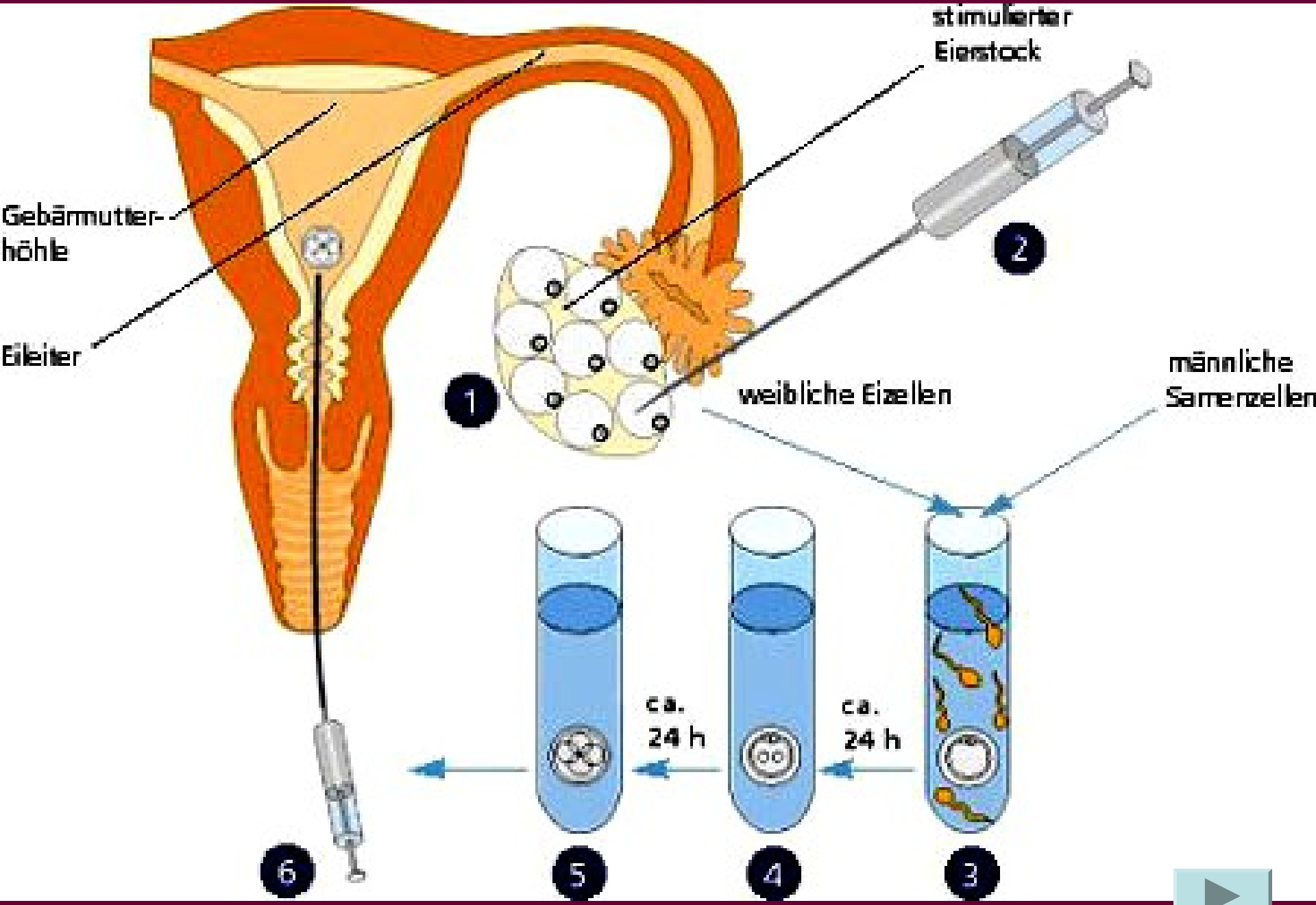
- Superovulasi: peningkatan produksi sel telur pd hewan/manusia yg produksi sel telurnya relatif sedikit
- Induksi superovulasi: dgn gonadotropin
- Mekanisme:
  1. Menggertak pertumbuhan folikel → PMSG ~ FSH
  2. Menggertak ovarium agar mengadakan ovulasi → hCG ~ LH
- Koleksi sel telur
  - Aplikasi IVF
  - Kriopreservasi → bank oosit
  - Model uji dlm penelitian2 → misal utk uji toksikologi

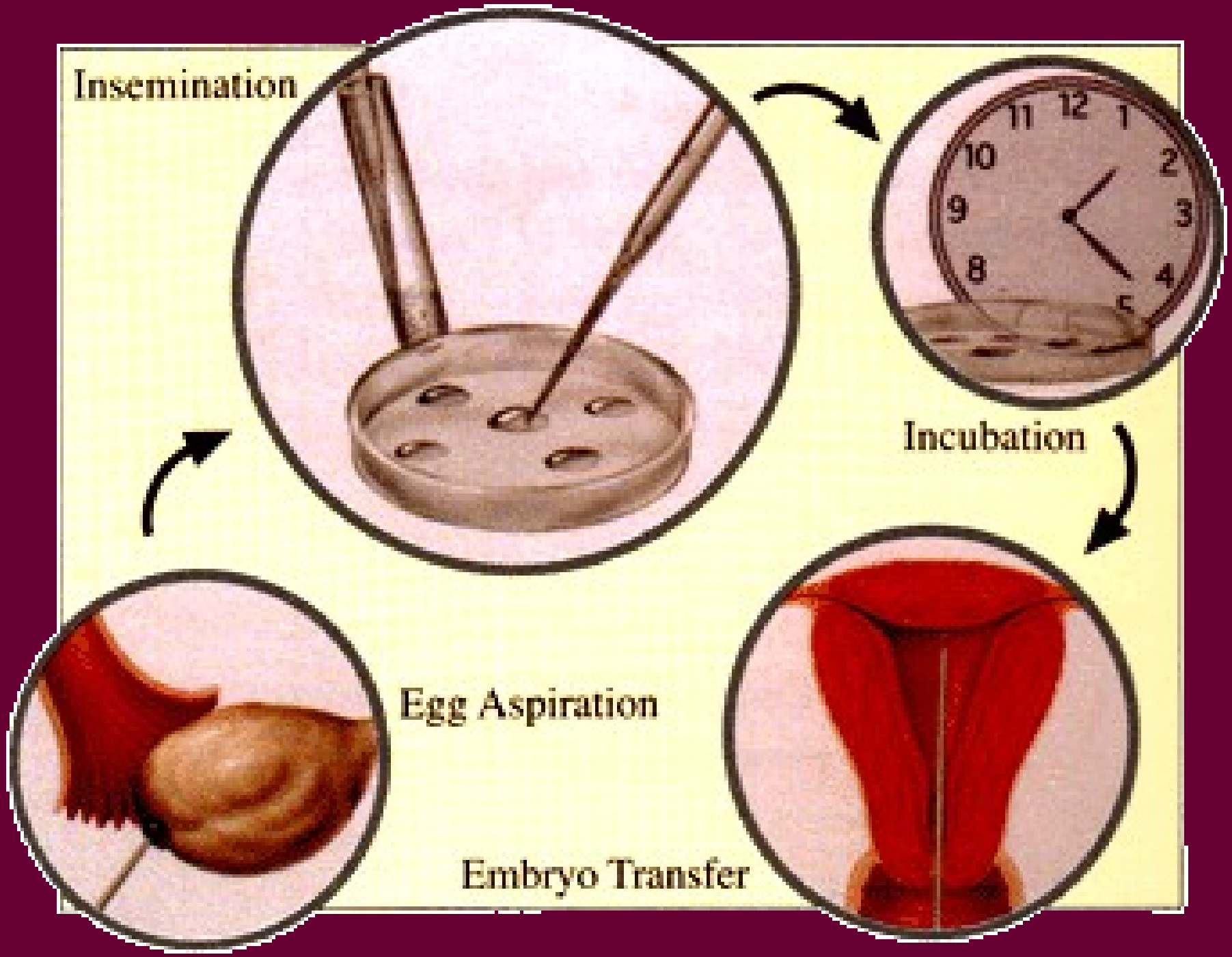
I V F (in vitro fertilization)

- *Man made embryo* → fertilisasi yg tjd di luar tubuh induk (di luar oviduk) yg direkayasa oleh manusia
- Pertama kali:
  - Th 1959 pd kelinci
  - Louis Brown (1978) bayi tabung pertama
- Aplikasi pd manusia telah bermanfaat dlm penanganan infertilitas meski tingkat keberhasilannya hy 35-40%
- Tahapan (pd mns):
  1. Stimulasi ovarium
  2. Oocyte retrieval/pengambilan oosit & sperm recovery
  3. Fertilisasi → scr konvensional atau bisa dgn mikroinjeksi spermatozoa (SUZI / ICSI)
  4. Kultur embrio & embryo transfer
  5. Cryopreservation
  6. Pre-implantation genetic diagnosis (PGD) → utk menyingkirkan penyakit2 yg menurun terkait sex & poliploidi

Embryonic Implantation




stimulierter
Eierstock
Gebärmutter-
höhle
Eileiter
2
1
weibliche Eizellen
männliche
Samenzellen
ca.
24 h
ca.
24 h
6
5
4
3


Insemination
Incubation
Egg Aspiration
Embryo Transfer

Rekayasa embrio

- Embryo sexing
- Kriopreservasi embrio
- Kultur embrio
- Embrio transfer: non surgical & surgical
- Embrio chimera
- Embrio cloning
- Embrio transgenik
- Produksi embryonic stem cell (ESC)

EMBRIO SEXING

- Embrio sexing: penentuan jenis kelamin embrio
- Metode:
  - Analisis kromosom → mendeteksi kromatin kelamin
  - Deteksi imunologis → antigen H-Y
  - Menggunakan Y-specific DNA probes

KULTUR EMBRIO

- Utk kepentingan IVF kultur embrio dilakukan sampai mencapai tahap morula/blastosis → menghasilkan kehamilan yg lebih baik drpd pd tahap sebelumnya
- Faktor yg ptg dlm kultur embrio:
  1. Medium (nutrisi)
  2. Substrat (wadah)
  3. Suhu
  4. Sumber gas CO_2
- Hambatan dlm kultur embrio adlh terjadinya fenomena *cell block* → pertumbuhan & perkembangan embrio terhambat
- Embrio hasil kultur dpt:
  - Segera ditransfer (embryo transfer) ke dlm alat reproduksi induk aslinya maupun bukan (*surrogate mother*) sampai berkembang mjd fetus & dilahirkan
  - Disimpan utk digunakan di kemudian hari

EMBRYO CHIMERA

- Hwn khimera mrp hwn yg mempunyai 2 atau lebih populasi sel dgn kandungan genetik yg berbeda → dpt berasal dr 1 spesies atau gabungan spesies yg berbeda
- Keunikan khimera: keragaman genetik yg tersimpan dlm satu individu shg dpt mjd bank gen hdp yg potensial → mosaic embryo
- Cara produksi embrio khimera:
  - Menginjeksikan inner cell mass ke dlm blastocoel
  - Agregasi/penggabungan embrio pd tahap yg lebih dini
- Hasil embrio khimera → embrio transfer

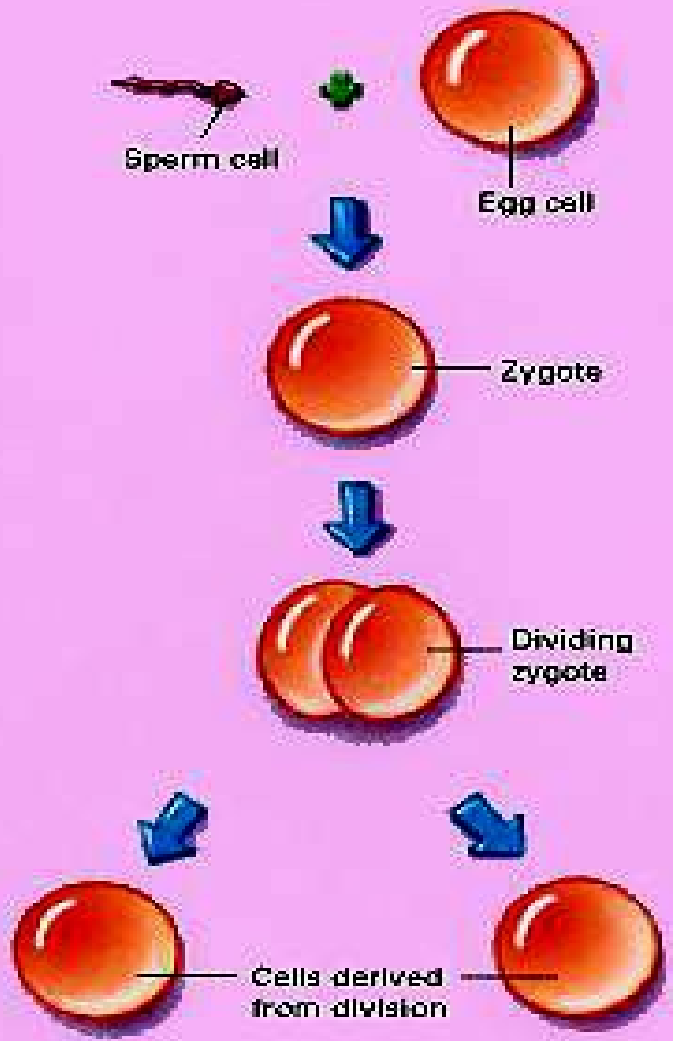
EMBRYO CLONING

- Kloning: upaya duplikasi suatu material biologi yg dilakukan secara aseksual
- Teknologi kloning:
  - Kloning DNA (recombinant DNA technology, molecular cloning, gene cloning) → proses transfer fragmen DNA asing tertentu dr suatu organisme ke dlm suatu elemen genetic lain yg mampu berplikasi sendiri spt plasmid bakteri, selanjutnya DNA asing tsb akan dpt mengalami propagasi dlm sel inang
  - Kloning reproduksi → teknologi yg digunakan utk memproduksi individu (hwn) yg mempunyai inti DNA yg sama
  - Kloning utk kepentingan terapi → memproduksi ESC yg berasal dr upaya proliferasi inner cell mass yg diisolasi dr embrio tahap blastosis

KLONiNG REPRODUKSi

1. Meniru kejadian kembar identik → dgn cara membelah embrio (embryo splitting) pd tahap perkembangan awal, masing2 belahan embrio dpt berkembang mjd individu baru dgn genetik yg sama → TWINNING

2. Transfer nukleus → individu baru dpt terbentuk dgn mengganti nukleus oosit dgn nukleus sel somatis (somatic cell nuclear transfer/SCNT) → lahir domba Dolly (1997)

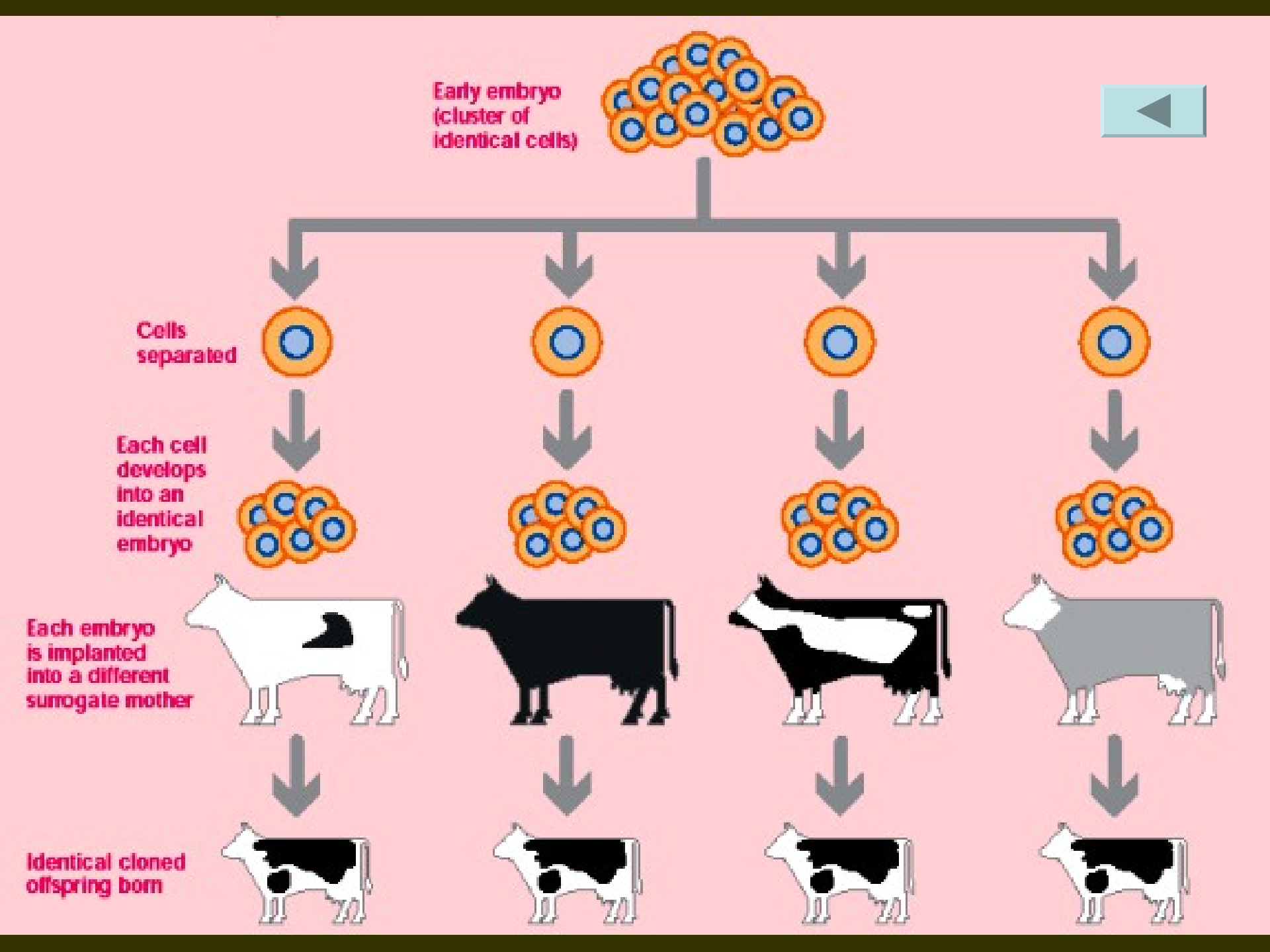


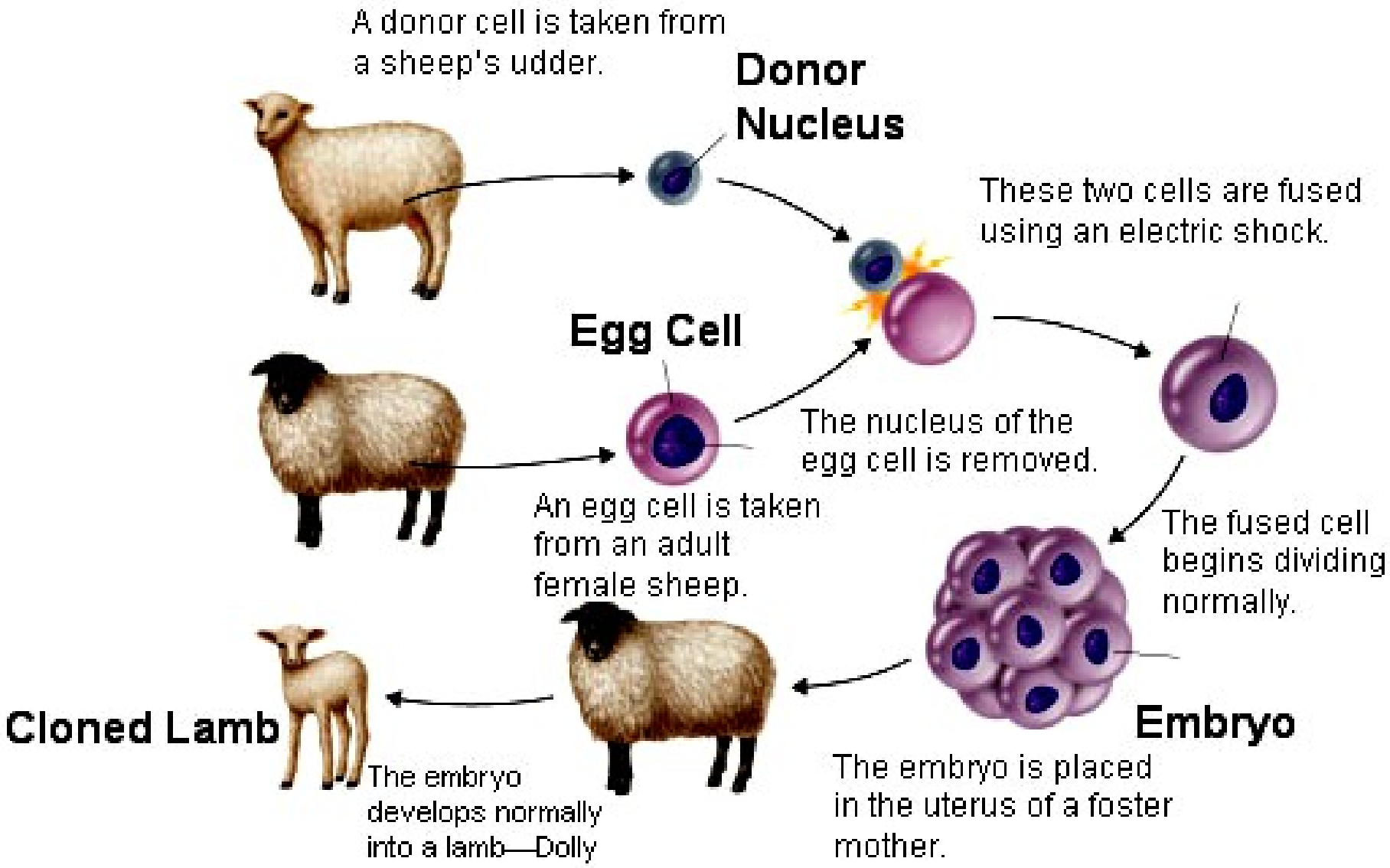
1. A sperm cell combines with an egg cell to form a zygote.

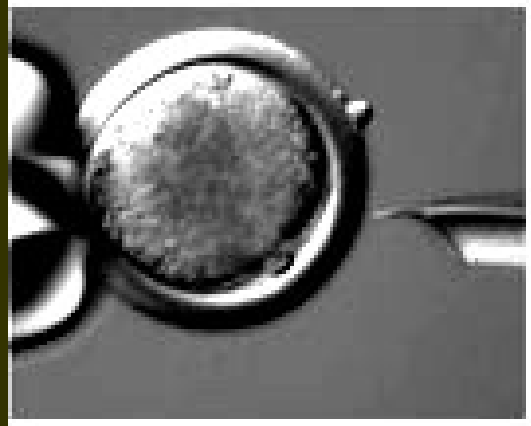
2. The zygote divides into two cells.

3. The cells split apart from each other.

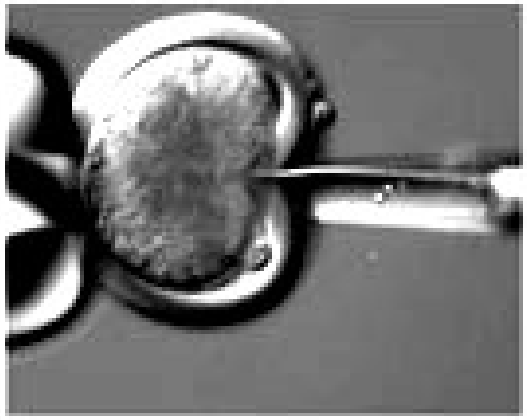
4. The two cells develop into identical embryos, which grow into natural identical twins, who are clones of each other. (When embryos are split artificially, the resulting embryos are placed into the wombs of surrogate mothers to complete their development.)


Early embryo (cluster of identical cells)
Cells separated
Each cell develops into an identical embryo
Each embryo is implanted into a different surrogate mother
Identical cloned offspring born

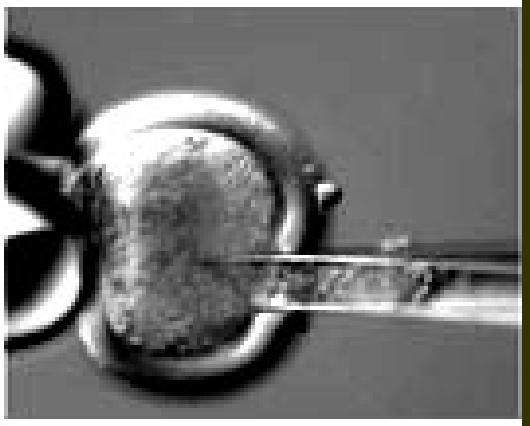

A donor cell is taken from a sheep's udder.
Donor Nucleus
These two cells are fused using an electric shock.
Egg Cell
The nucleus of the egg cell is removed.
An egg cell is taken from an adult female sheep.
The fused cell begins dividing normally.
Embryo
Cloned Lamb
The embryo develops normally into a lamb—Dolly
The embryo is placed in the uterus of a foster mother.



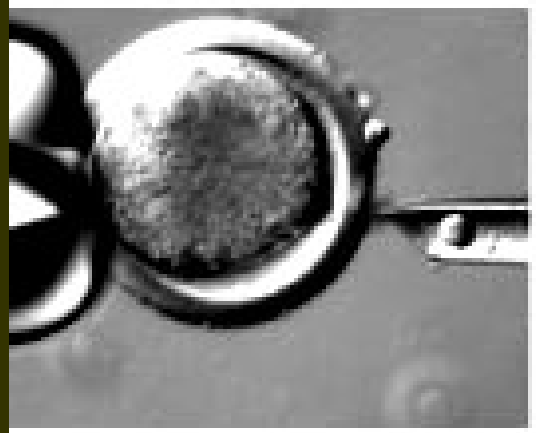
This is a human oocyte (egg).



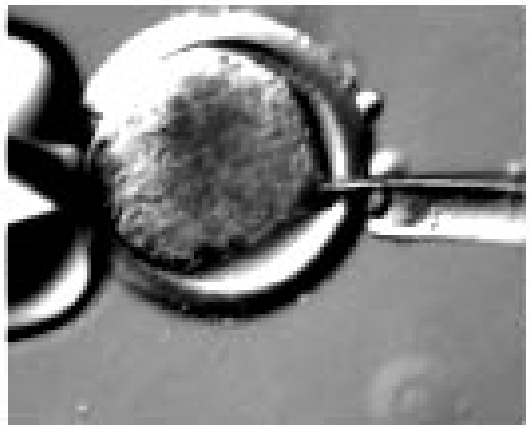
A pipette penetrating the egg.



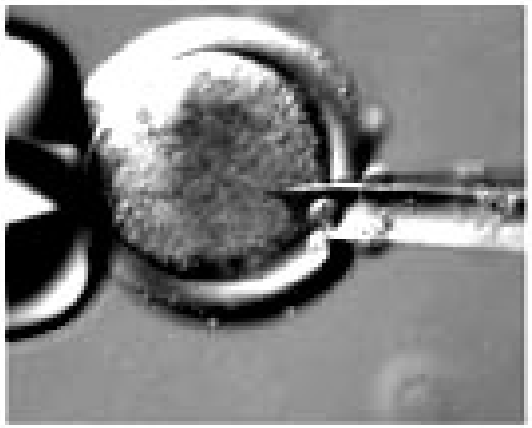
Nucleus removed from the egg.



The enucleated egg.



A pipette penetrating the egg.

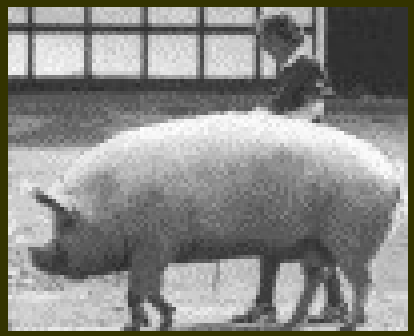


The somatic cell injected into the egg.

EMBRIO TRANSGENIK

- Hewan transgenik: hwn yg genomnya telah mengalami modifikasi krn telah disisipi olh DNA asing (dr spesies yg berbeda) melalui teknologi rekombinan DNA
- Alasan melakukan modifikasi gen:
  - Identifikasi, isolasi & karakterisasi gen utk memahami begaimanan fungsi gen & pengaturannya
  - Mdpt *diseases model* utk pengembangan gen therapy
  - Memproduksi protein2 bioaktif utk kepentingan terapeutik → misal hormon & growth factor, hemoglobin, laktoferin, antibodi dll
  - Merangsang peningkatan hasil peternakan dgn kualitas yg diinginkan → misal susu mgd sedikit kolesterol, wol lebih tebal, resisten thdp penyakit tertentu

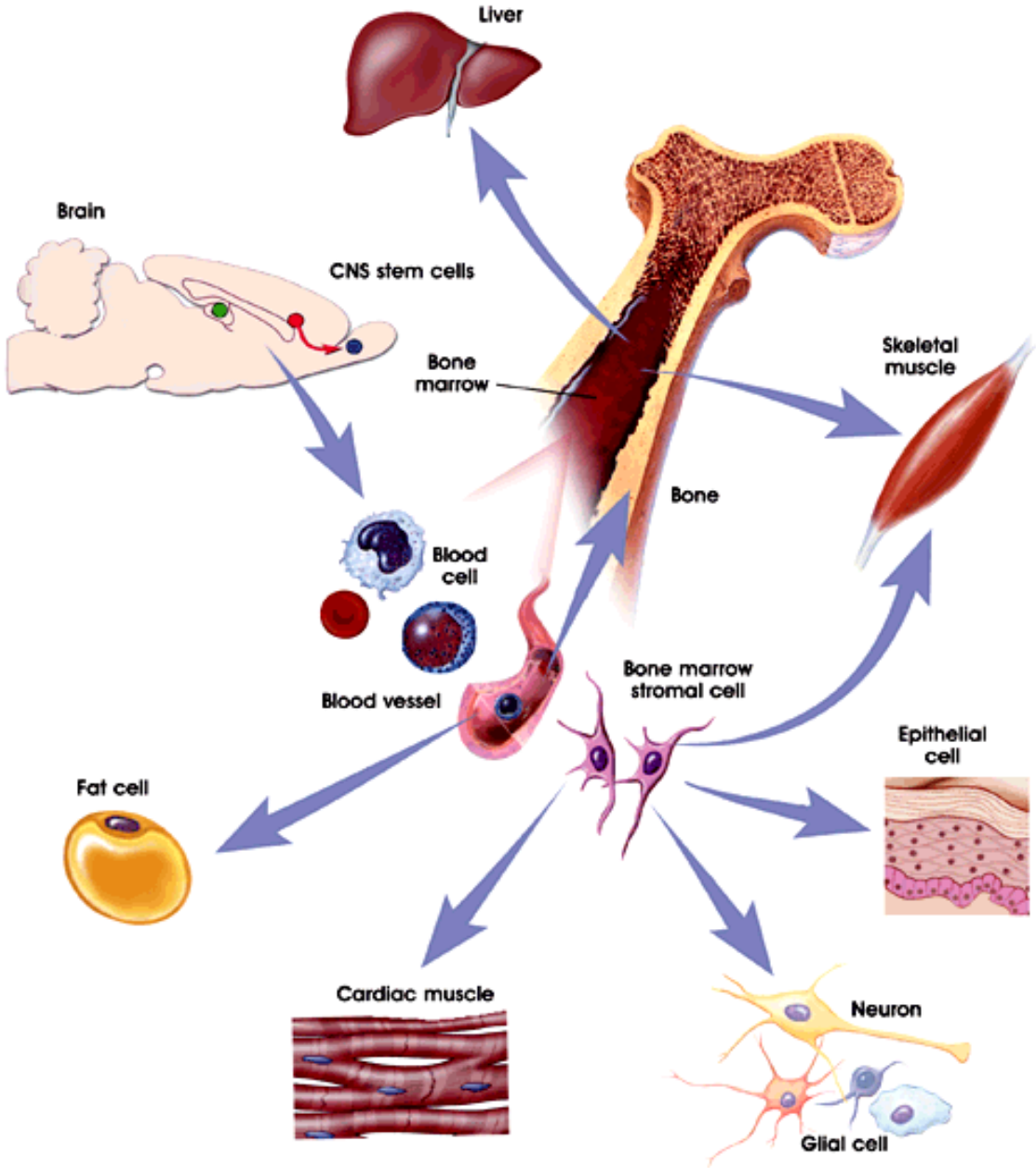
- Hwn transgenik pertama lahir pd th 1976 → mencit transgenik
- Populer mulai th 1982 dgn lahirnya mencit transgenik yg genomnya telah disisipi olh gen hormon pertumbuhan dr mns
- Mammalia transgenic:
  - Tdk semua jenis protein bisa dihasilkan dr bakteri
  - Produksinya dlm jml besar (jk pd bakteri terbatas)
  - Bisa direproduksi, mudah dimaintenance & easily delivery method
- Metode memproduksi embrio transgenik:
  - Mikroinjeksi DNA pd pronukleus zigot
  - Transfer gen malalui embryonic stem cells
  - Transfer gen melalui retrovirus

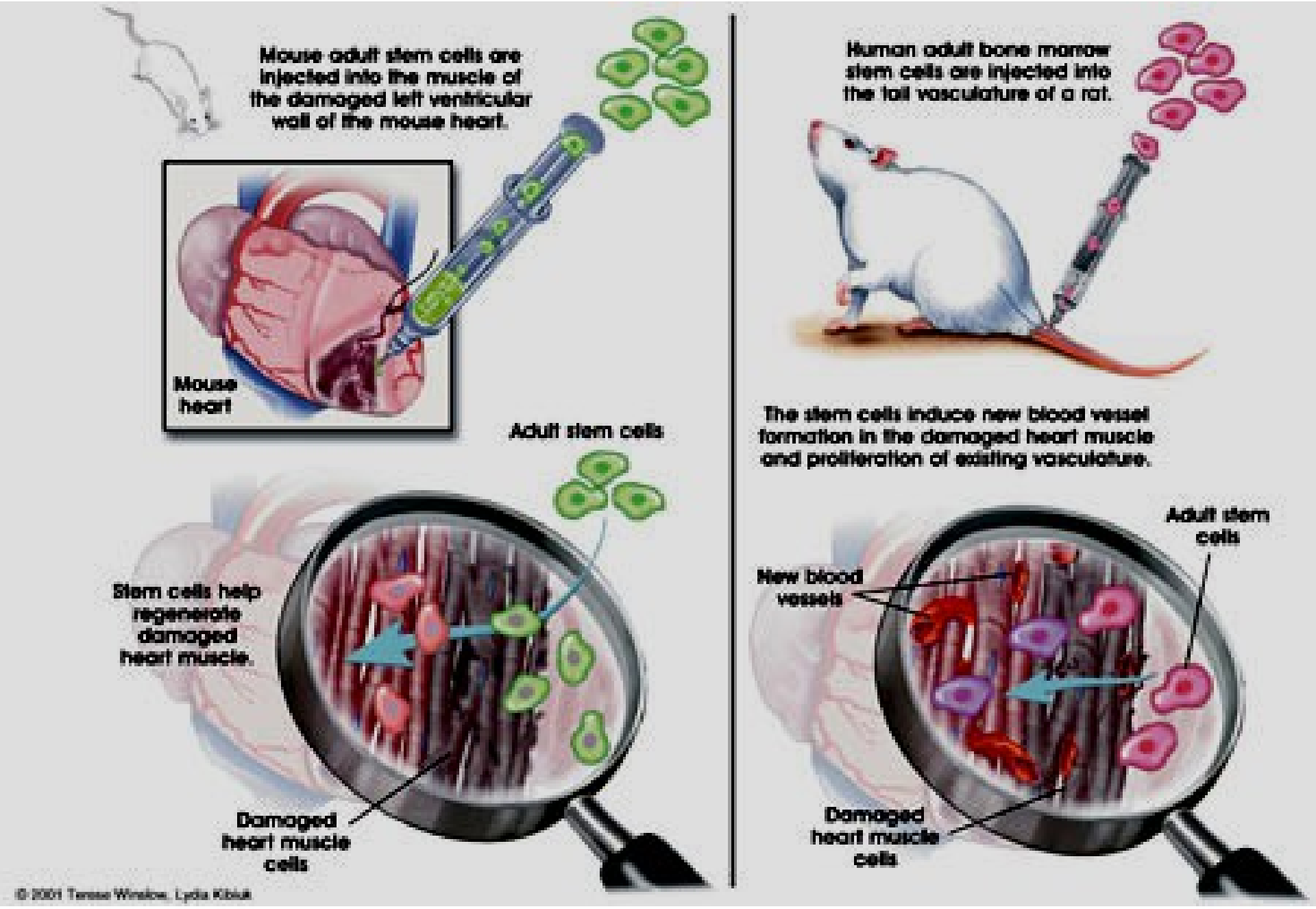


Embryonic stem cells (ESC)

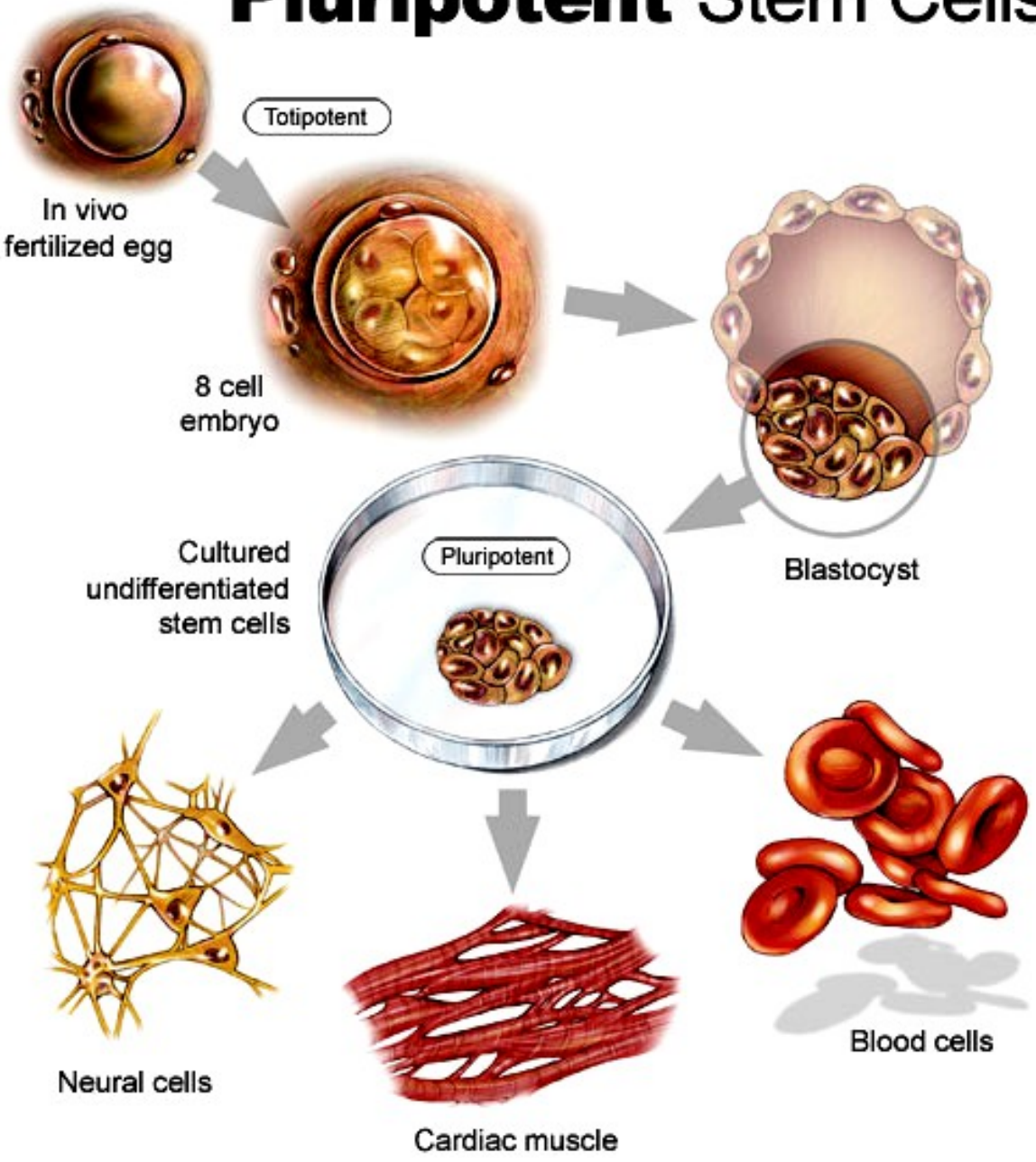
- Stem cell: sel2 yg blm berdiferensiasi yg dpt berproliferasi dlm jangka wkt tdk terbatas & dpt jg diinduksi utk berdiferensiasi mjd berbagai tipe sel dgn fungsi khusus
- Jenis stem cell: Inner cell mass (ICM) blastosis, umbilical cord, plasenta, organ dewasa (jarang kecuali pd sumsum tulang, darah, mata, hati, kulit & otak)
- Stem cell berpotensi utk digunakan dlm penyembuhan berbagai penyakit degeneratif (Parkinson, diabetes, peny jantung, Alzheimer & perlukaan spinal cord)
- Embryonic stem cell tdp pd inner cell mass blastosis

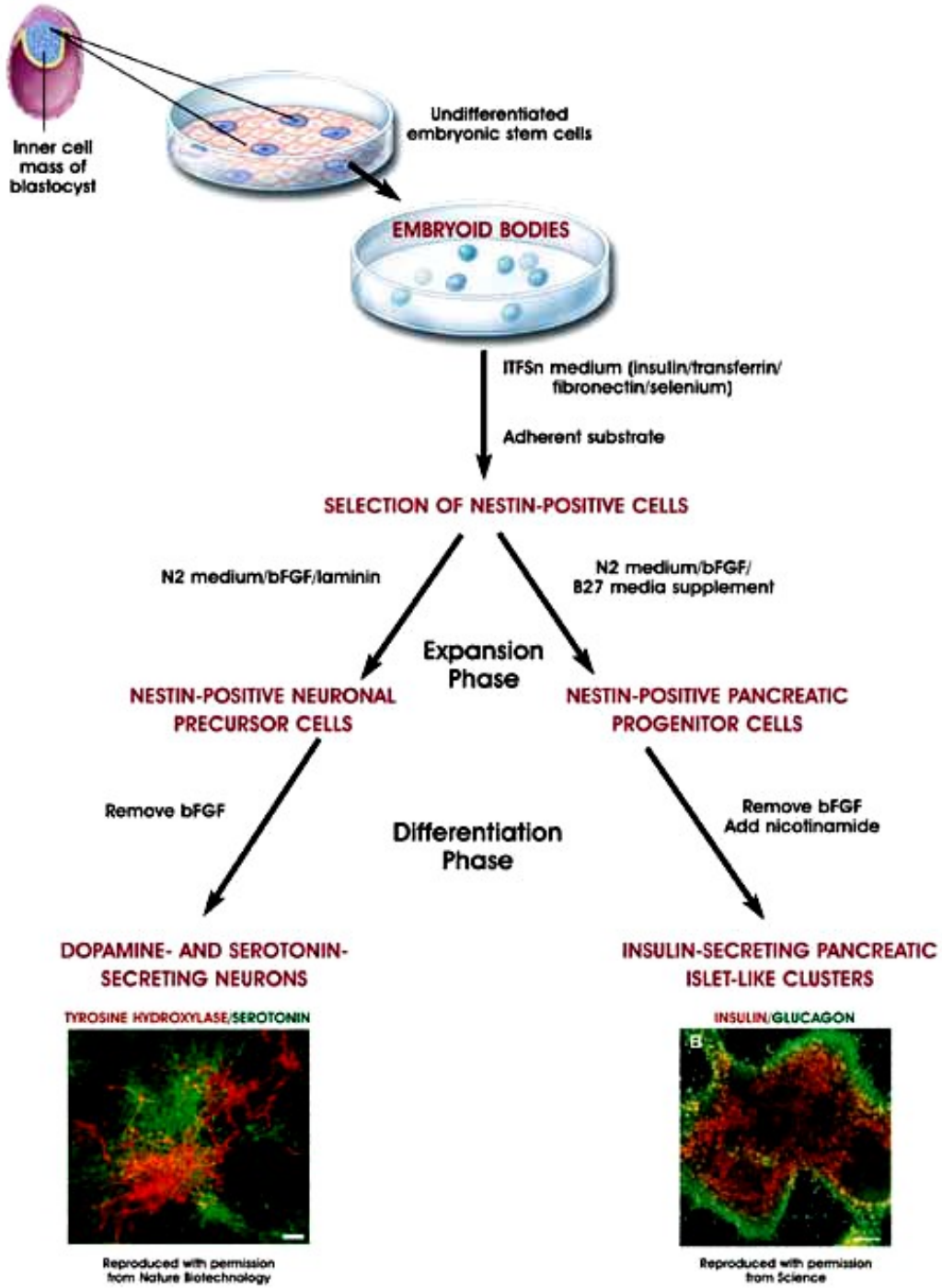
- Sifat ESC:
  - Pluripoten → dpt dipertahankan ketika dikultur dlm media yg mgd faktor2 penghambat diferensiasi
  - Dpt berproliferasi membentuk populasi sel yg homogen selama periode wkt yg hampir tdk terbatas tanpa kehilangan sifat pluripotensinya
  - Dpt mempertahankan komplemen kromosom yg stabil & normal stlh dikultur
  - Dpt berdiferensiasi mjd berbagai tipe sel
  - Dpt mempertahankan morfologi & ekspresi gen yg konsisten dgn sel2 embrionik asalnya
- ESC dpt menghasilkan: sel2 lemak, sel otak & sel syaraf, sel pankreas penghasil insulin, sel2 tulang, sel2 hematopoietic, sel2 endothelial, sel2 otot polos, otot seran lintang & otot jantung
- Aplikasinya: ESC ditransplantasikan pd organ yg rusak




Mouse adult stem cells are injected into the muscle of the damaged left ventricular wall of the mouse heart.
Mouse heart
Adult stem cells
Stem cells help regenerate damaged heart muscle.
Damaged heart muscle cells
Human adult bone marrow stem cells are injected into the tail vasculature of a rat.
The stem cells induce new blood vessel formation in the damaged heart muscle and proliferation of existing vasculature.
Adult stem cells
New blood vessels
Damaged heart muscle cells
© 2001 Teresa Winslow, Lydia Kibiuk

Pluripotent Stem Cells




Inner cell mass of blastocyst
Undifferentiated embryonic stem cells
EMBRYOID BODIES
ITFSn medium (insulin/transferrin/ fibronectin/selenium)
Adherent substrate
SELECTION OF NESTIN-POSITIVE CELLS
N2 medium/bFGF/laminin
N2 medium/bFGF/ B27 media supplement
Expansion Phase
NESTIN-POSITIVE NEURONAL PRECURSOR CELLS
NESTIN-POSITIVE PANCREATIC PROGENITOR CELLS
Remove bFGF
Differentiation Phase
Remove bFGF Add nicotinamide
DOPAMINE- AND SEROTONIN-SECRETING NEURONS
TYROSINE HYDROXYLASE/SEROTONIN
INSULIN-SECRETING PANCREATIC ISLET-LIKE CLUSTERS
INSULIN/GLUCAGON
B
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