Embryology III

PERIMPLANTATION DEVELOPMENT

autumn 2024

Embryo preparation for implantation

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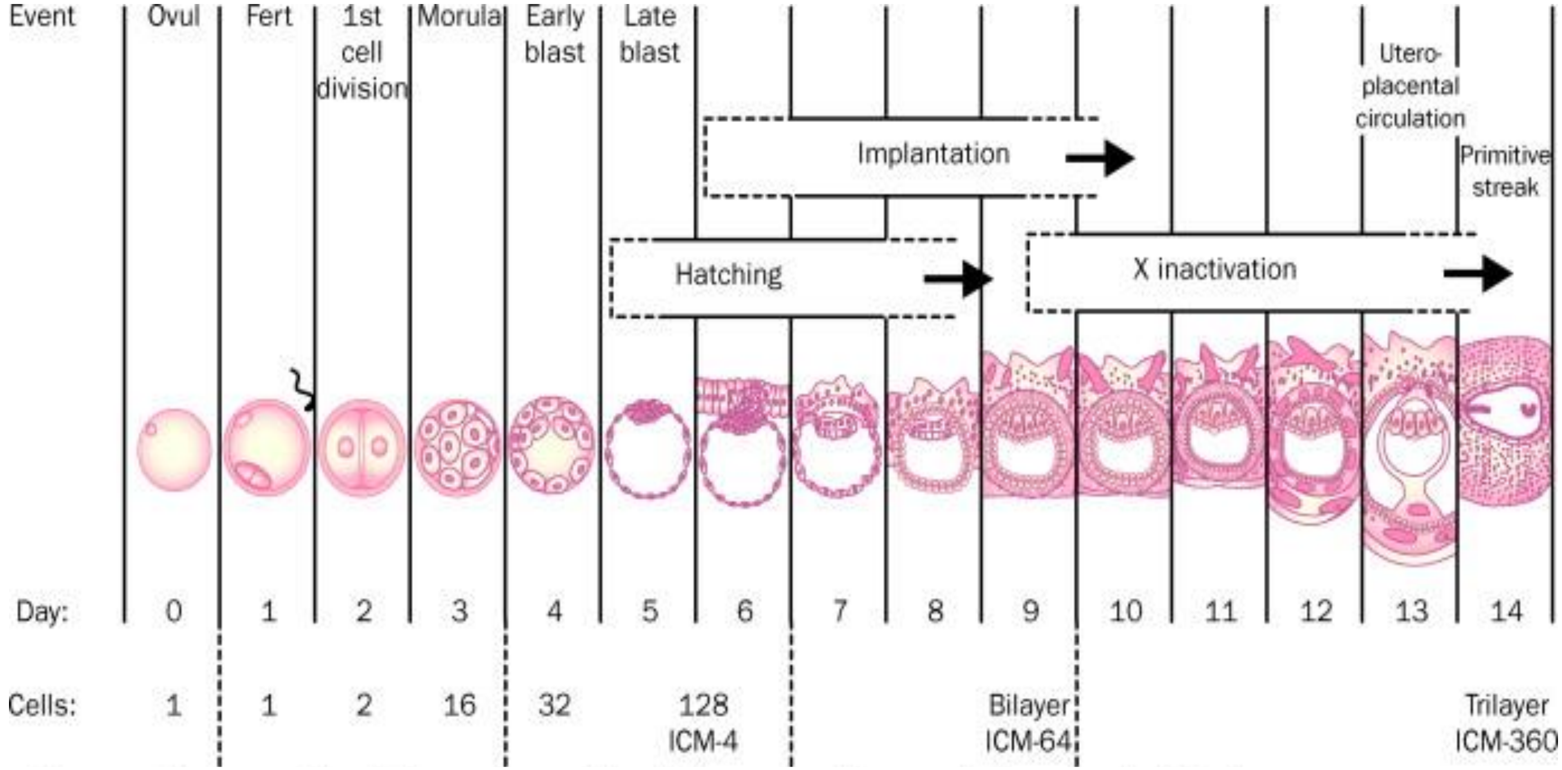
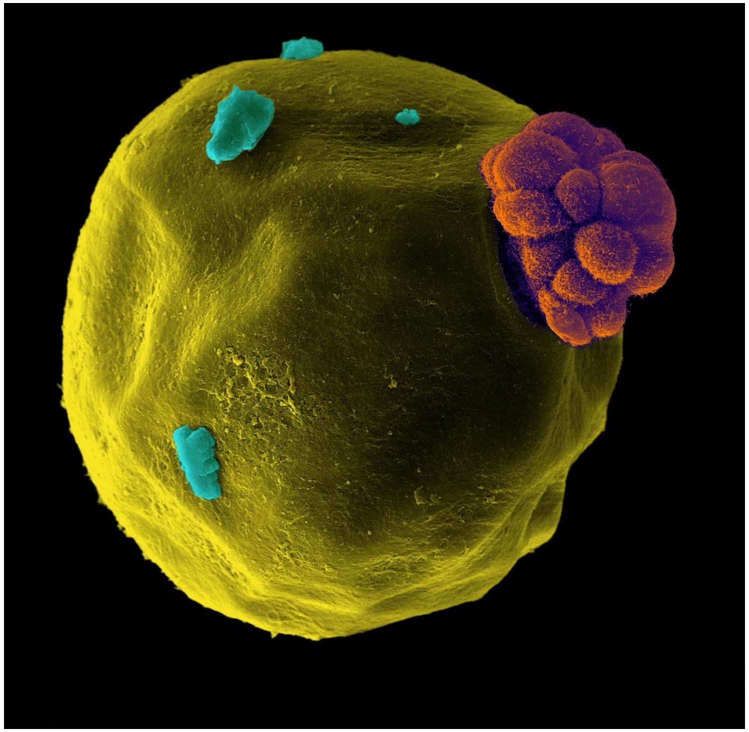
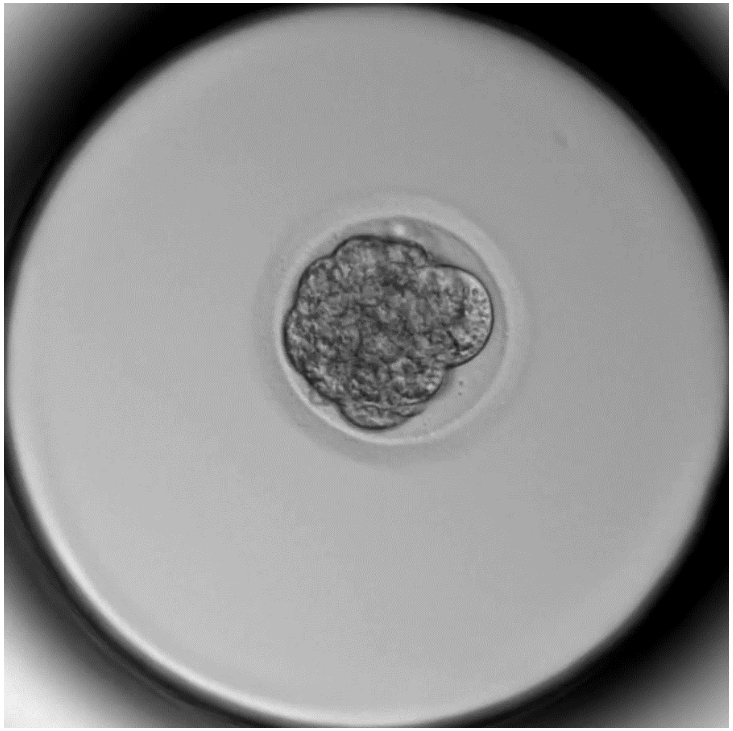
Pre- and periimplantation embryo development



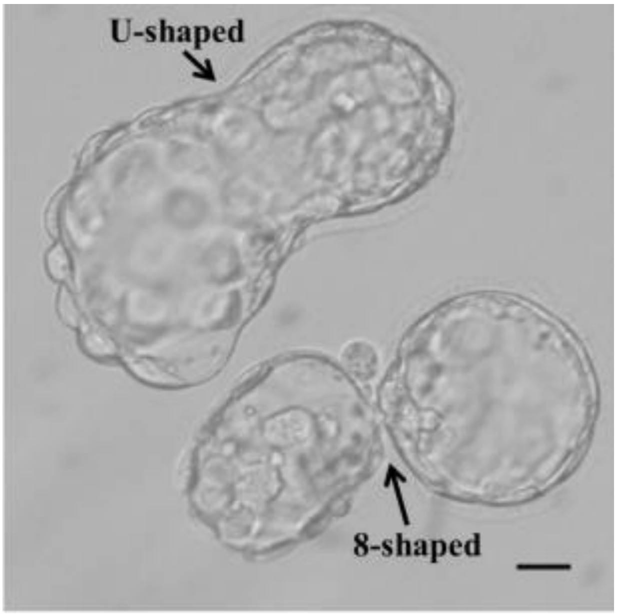
Embryo hatching

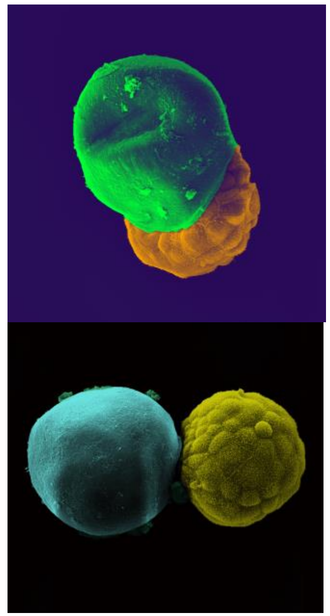
- blastocyst´s escape from a porous glycoprotein coat – Zona pellucida
- a key requirement for successful embryo implantation and establishing pregnancy
- D6-D7 in humans, in the uterus *in vivo,* but can happen spontaneously *in vitro* (uterine effectors not crically required)
- preceded by blastocyst expansion and followed by embryo attachment to the endometrium
- failure to hatch suggests compromised embryo fitness
- hatching delay might cause the embryo to miss the uterine receptive window

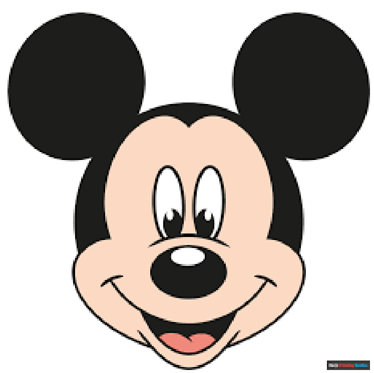


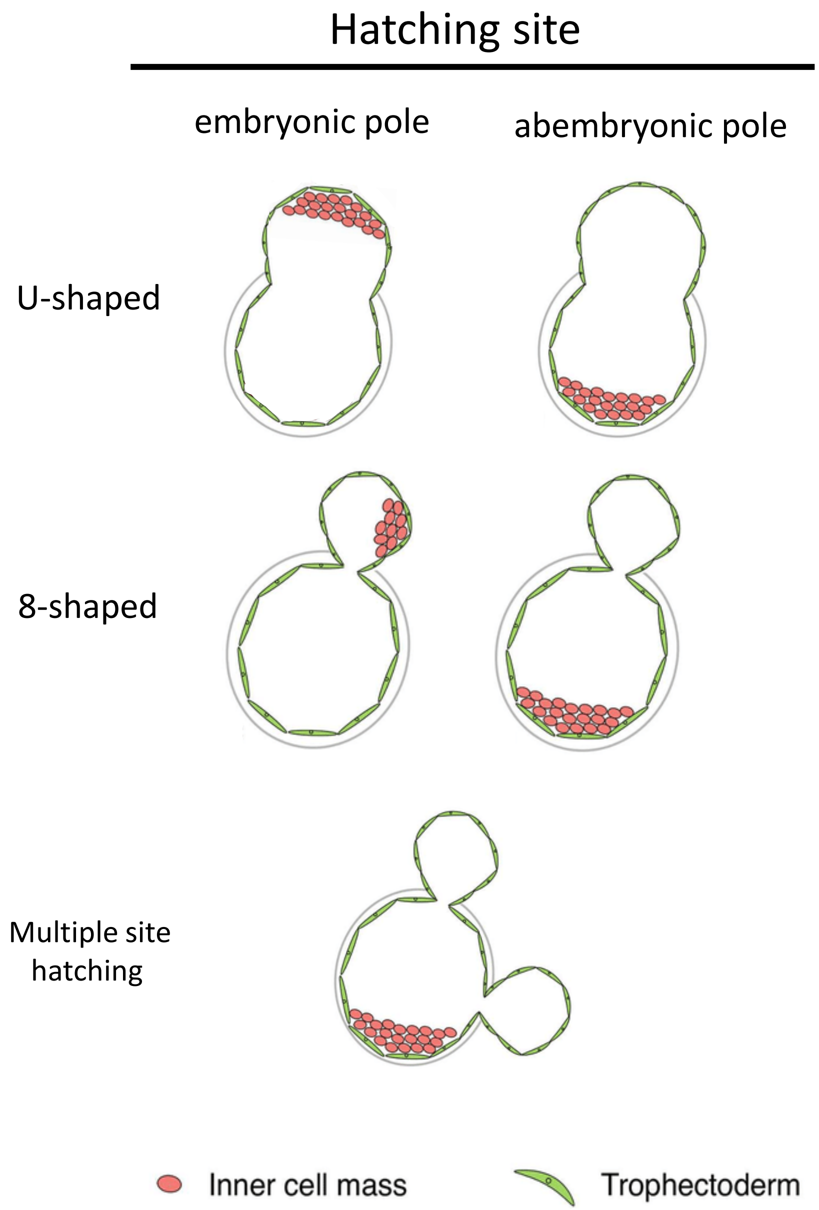


Embryo hatching site









Embryo hatching mechanism

- relatively unknown
- 2 forces
  - 1) physical = mechanical pressure exerted on ZP by blastocyst expansion
  - 2) chemical = enzymatic digestion of ZP

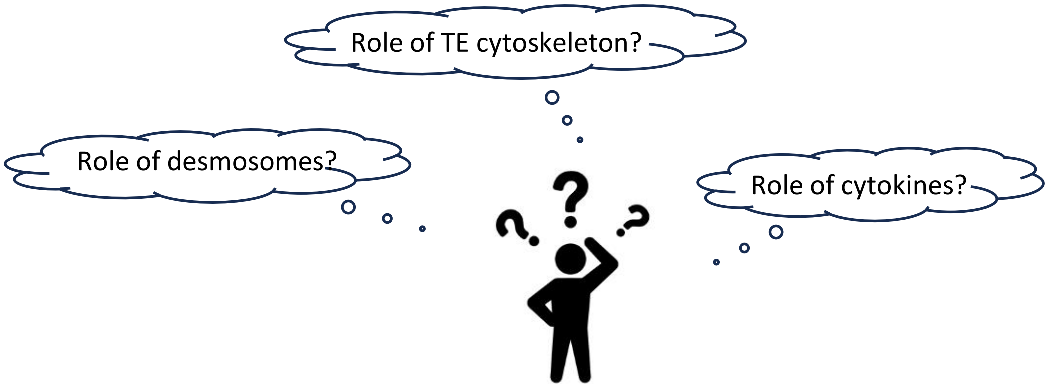
    ←lytic enzymes secreted by embryo´s mural TE

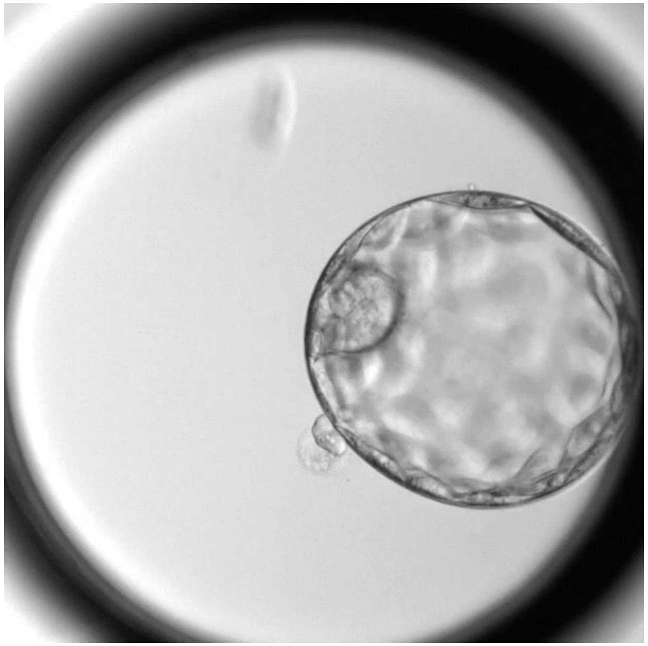
      e.g. trypsin-like Ser-proteases, Cys-proteases, matrix metalloproteinases (MMPs), tissue plasminogen activators (tPAs), cathepsins...

    ←proteases from uterine milieu

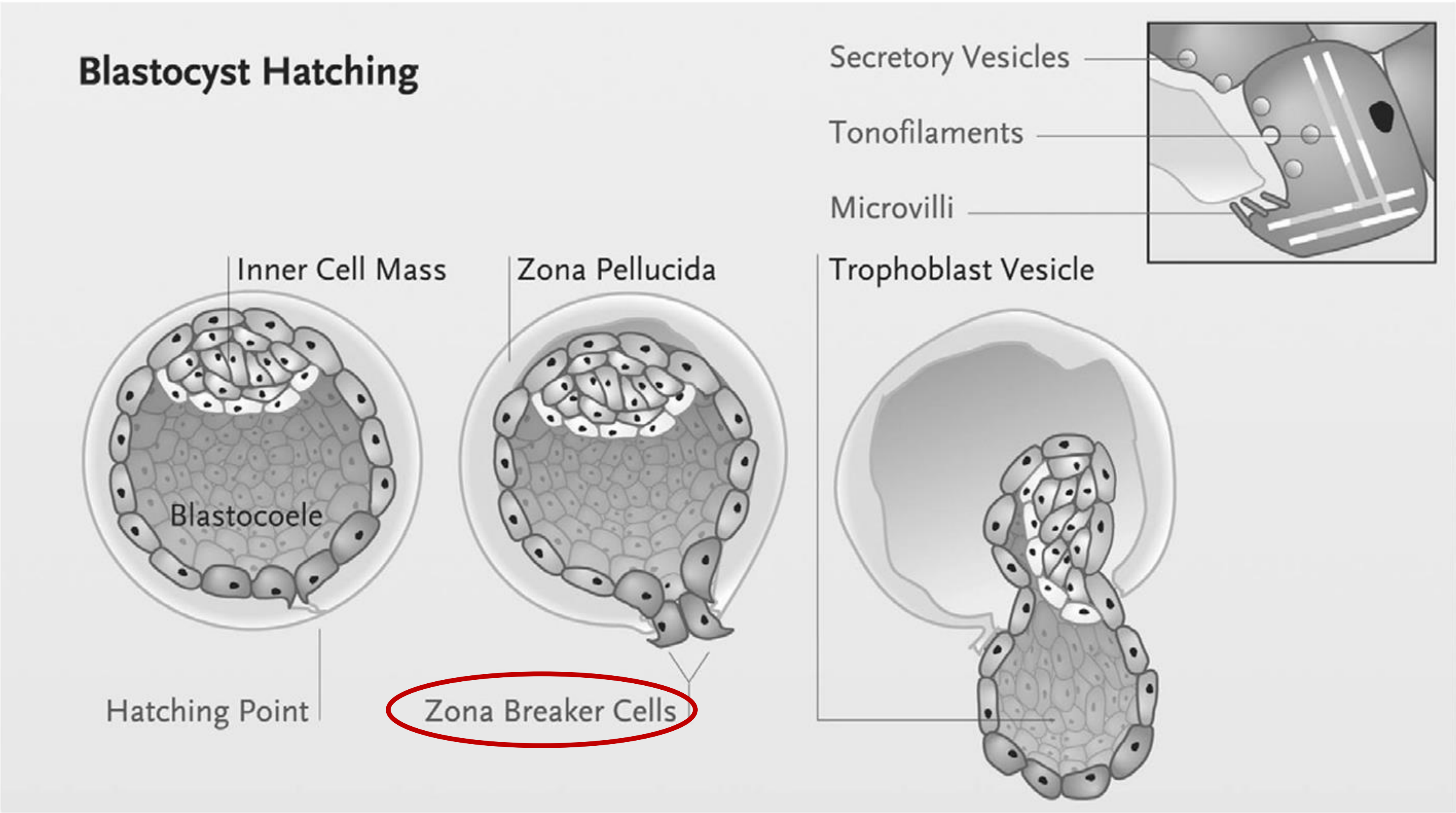
      - strypsine, lysine

- Stages:
  1) ZP softening, thinning, and local rupture
  2) gradual widening of ZP perforation
  3) penetration of leading TE cells through ZP
  4) active movement of cellular mass outside of the ZP





Mechanism of embryo hatching

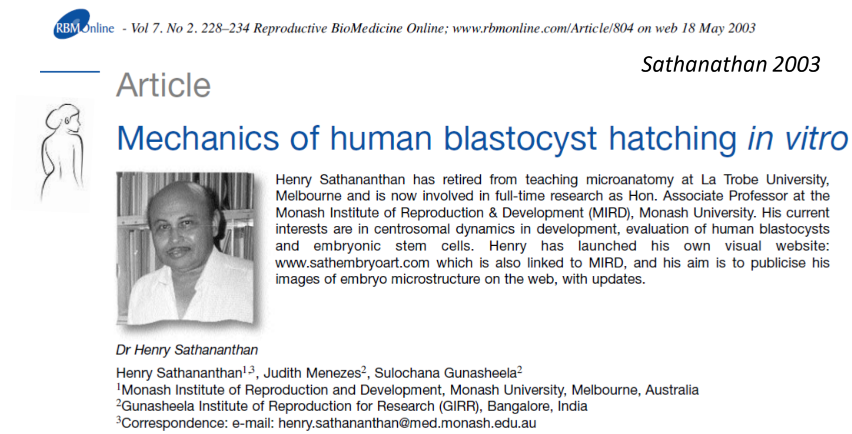


Mechanism of embryo hatching

❖ „Zona-breaker cells"

- TE cells located at points of hatching lining and protruding through ZP
- not epithelial-like morphology but a "plump" appearance
- specialized TE projections that first invade ZP ("hatching pioneers")

Sathanathan 2003


RBMOnline - Vol 7. No 2. 228–234 Reproductive BioMedicine Online; www.rbmonline.com/Article/804 on web 18 May 2003

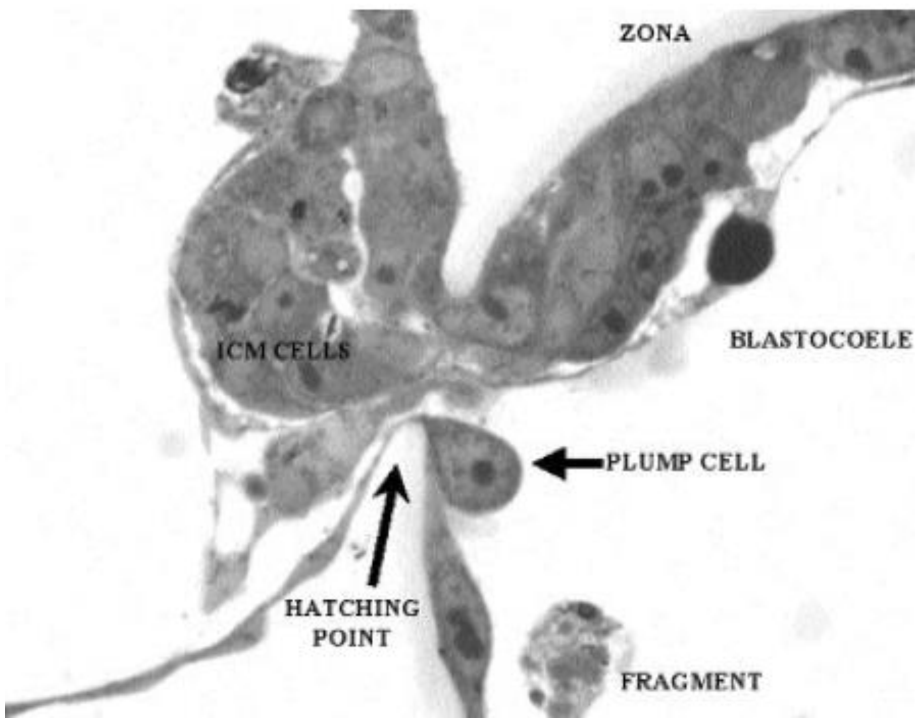
Article

Mechanics of human blastocyst hatching *in vitro*

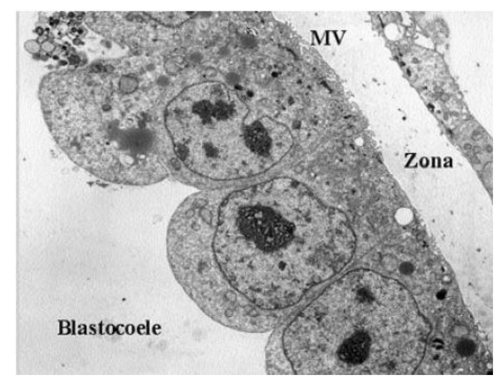
Henry Sathananthan has retired from teaching microanatomy at La Trobe University, Melbourne and is now involved in full-time research as Hon. Associate Professor at the Monash Institute of Reproduction & Development (MIRD), Monash University. His current interests are in centrosomal dynamics in development, evaluation of human blastocysts and embryonic stem cells. Henry has launched his own visual website: www.sathembryoart.com which is also linked to MIRD, and his aim is to publicise his images of embryo microstructure on the web, with updates.

Dr Henry Sathananthan

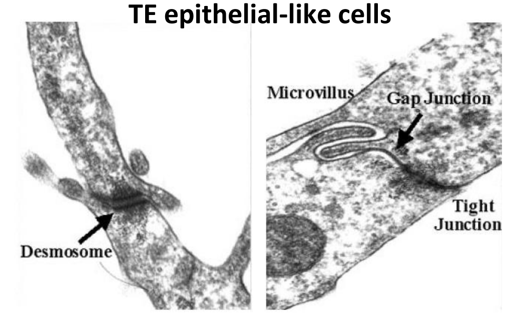
Henry Sathananthan[1,3], Judith Menezes[2], Sulochana Gunasheela[2]
[1]Monash Institute of Reproduction and Development, Monash University, Melbourne, Australia
[2]Gunasheela Institute of Reproduction for Research (GIRR), Bangalore, India
[3]Correspondence: e-mail: henry.sathananthan@med.monash.edu.au



ZP-breaker cells



TE epithelial-like cells



Mechanism of embryo hatching

❖ „Zona-breaker“ cells

- have surface microvilli interacting with the ZP and large bundles of contractile tonofilaments
- contain lysosomes and secrete vesicles that interact with the ZP

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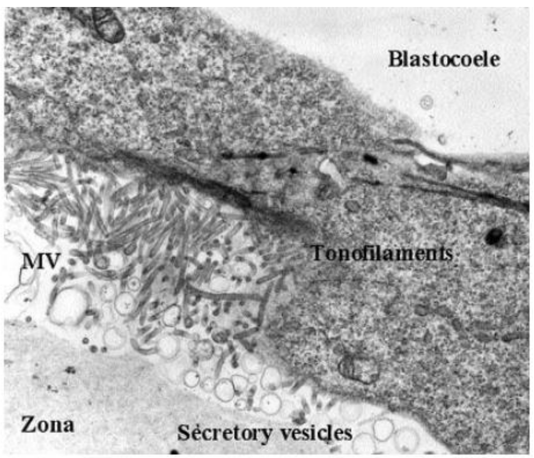
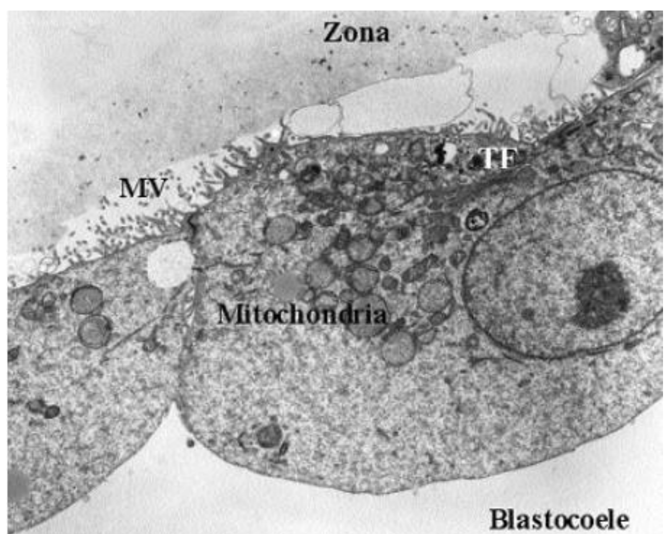
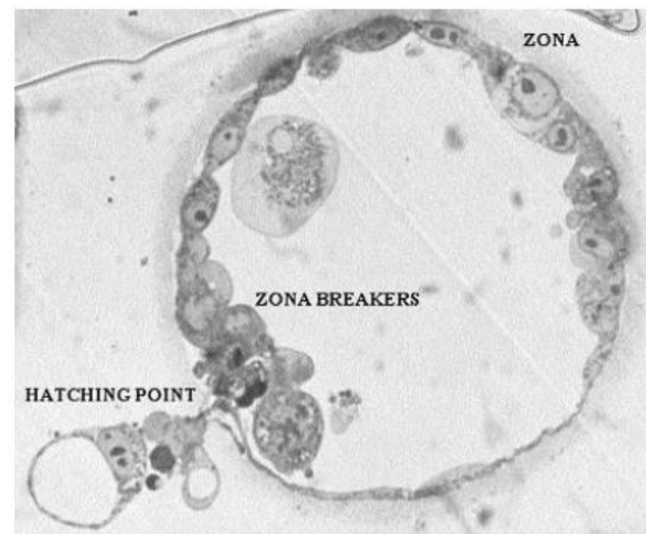


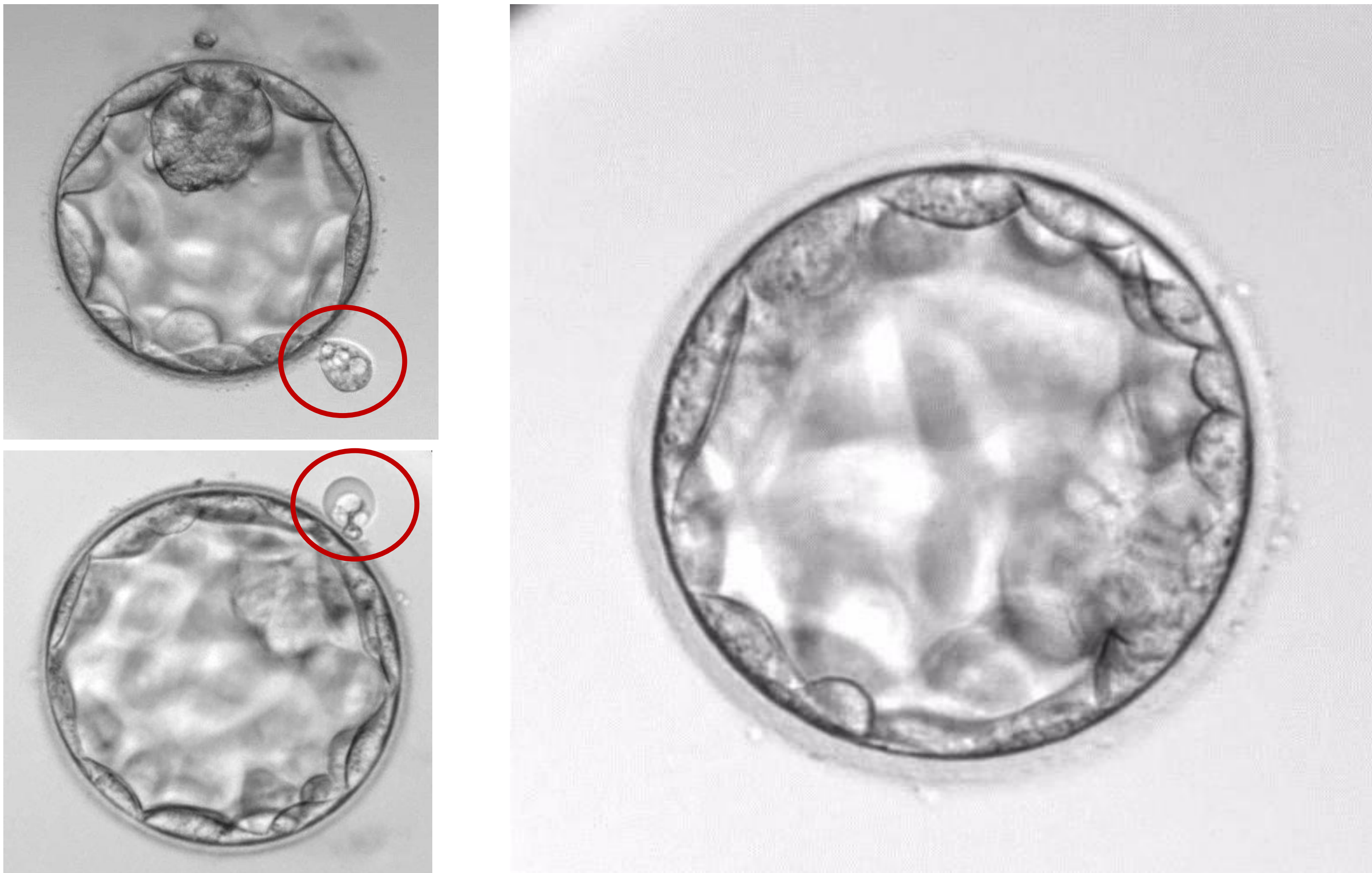




- intense vacuolisation related to secretolytic activity

Mechanism of embryo hatching

- „Zona-breaker“ cells



Mechanism of embryo hatching

❖ „Zona-breaker“ cells

- proteomics (LC-MS)
- ZP breakers (first hatching cells) compared to TE cells inside ZP
- upregulation of specific proteins in the zona breaching cells

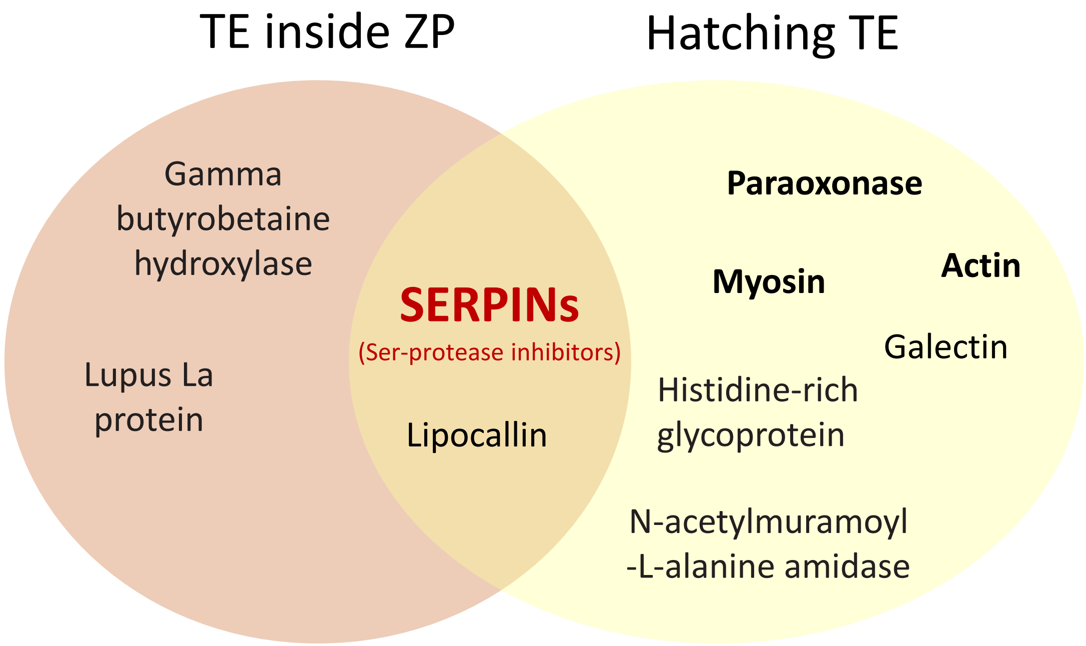
Almagor et al 2020

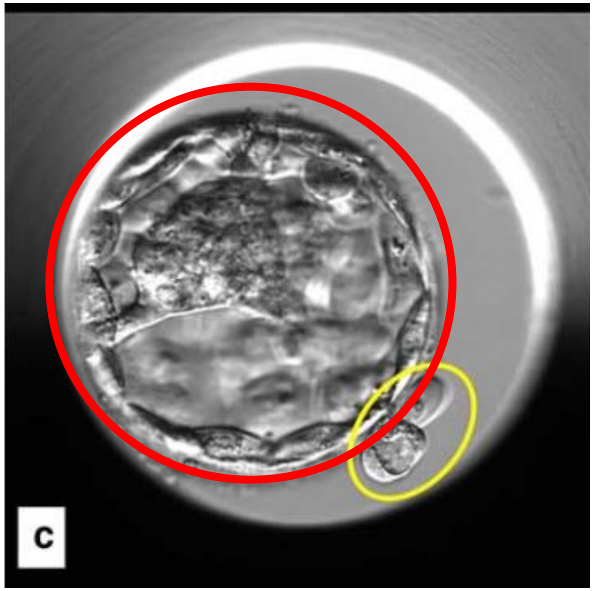
In Vitro Cellular & Developmental Biology - Animal
https://doi.org/10.1007/s11626-020-00522-w

Spontaneous in vitro hatching of the human blastocyst: the proteomics of initially hatching cells

Miriam Almagor[1] · Yishai Levin[2] · Rona Halevy Amiran[1] · Sheila Fieldust[1] · Yael Harir[1] · Yuval Or[1] · Zeev Shoham[1]

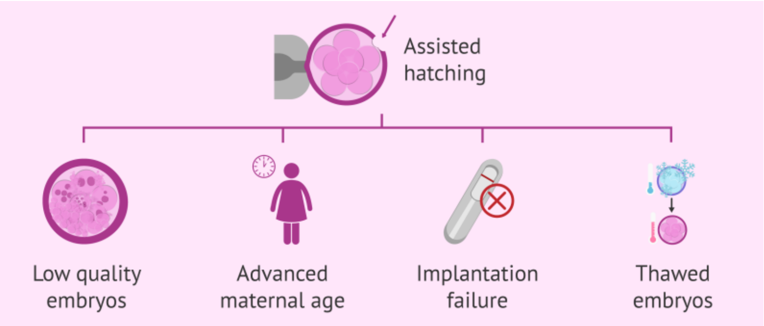
Received: 24 August 2020 / Accepted: 19 October 2020 / Editor: Tetsuji Okamoto



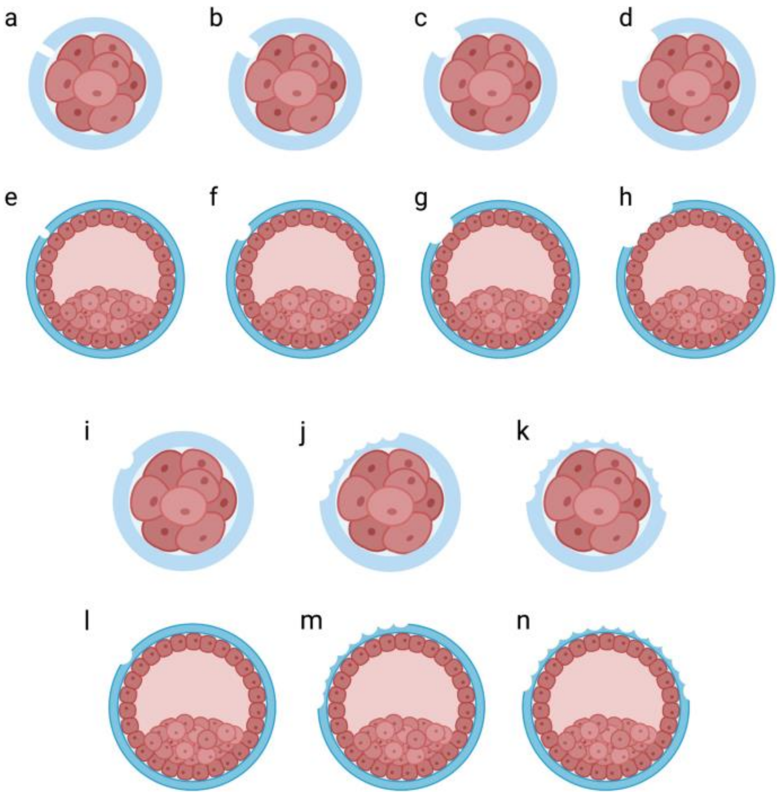


Assisted hatching

- ZP hardening occurring during in vitro culture and freezing/thawing reduces the chance of spontaneous hatching
- lower hatching success in conventional IVF cycles (50%) than after ICSI which leaves small openning in ZP
- assisted hatching (AH) technique used to soften, open, or remove ZP to rescue blastocyst with hatching problems

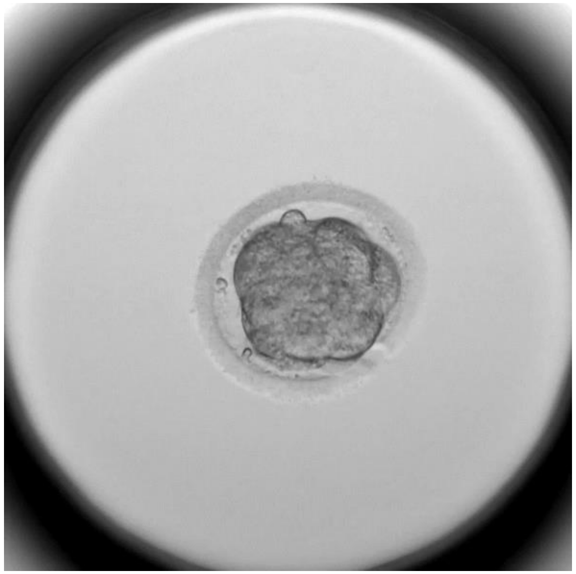


- AH:
  a) Mechanical b) Chemical c) Laser-assisted



- D3/D5-6

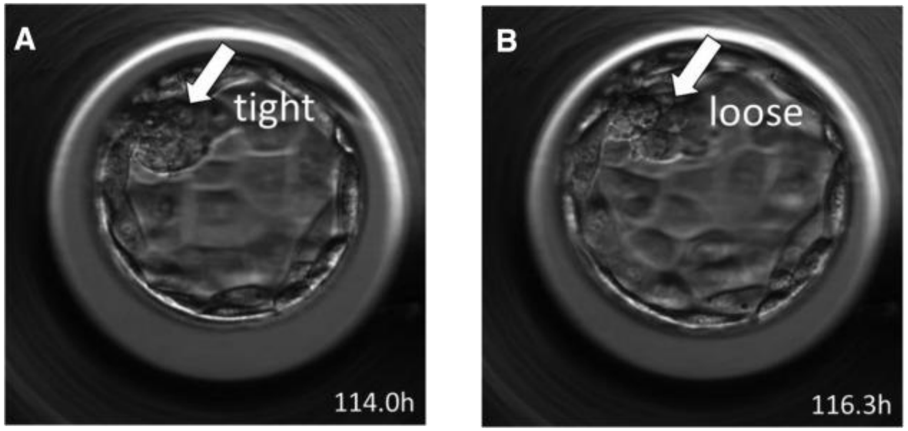
- AH D3 prevents blastocyst expansion!



- controversial (↑implantation rate but not LBR)

Monozygotic twinning

ICM



„compacted ICM“

ICM splitting

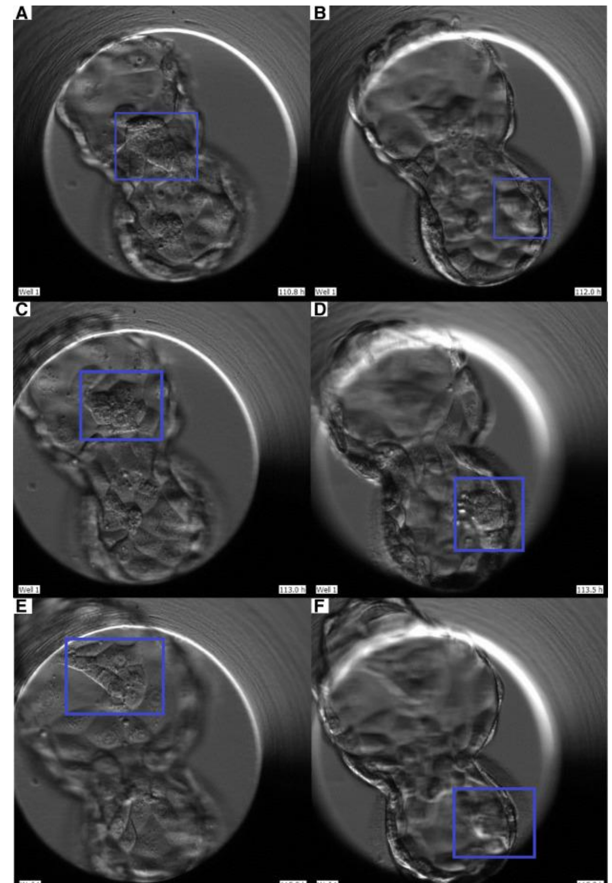
Sutherland et al 2019

RBMO

ARTICLE

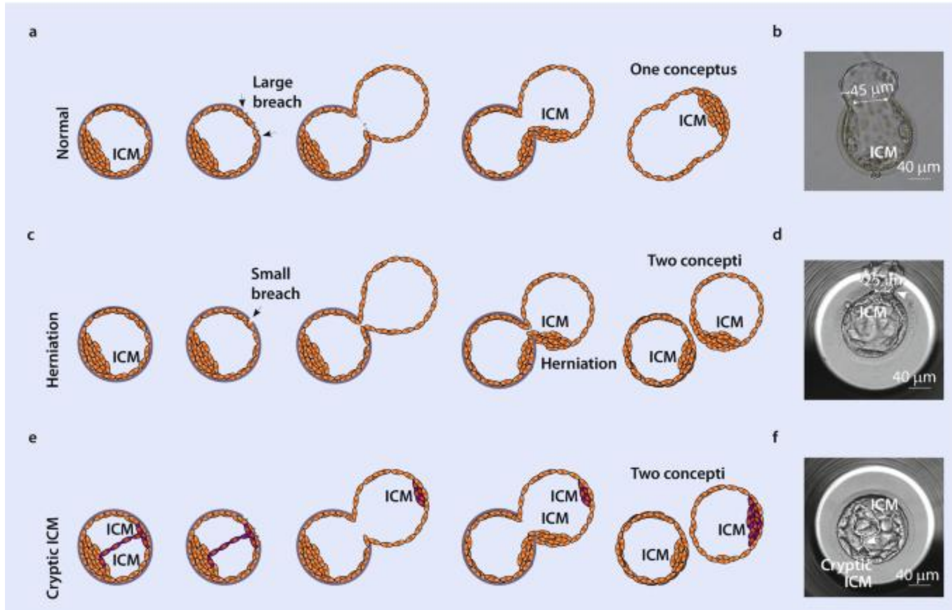
Time-lapse imaging of inner cell mass splitting with monochorionic triamniotic triplets after elective single embryo transfer: a case report

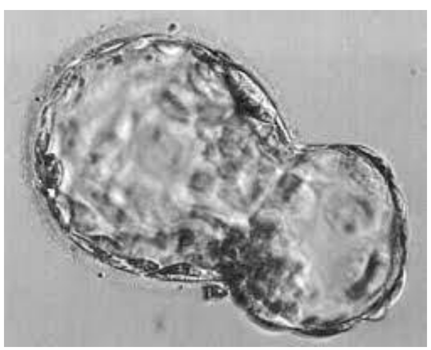
Karina Sutherland*, Joanne Leitch, Helen Lyall, Bryan J Woodward



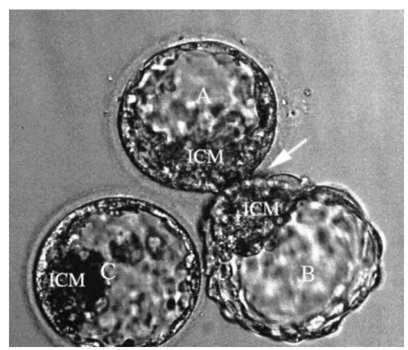
- different focal planes

Monozygotic twinning





Expanded blastocyst
natural hatching



Herniation of non-expanded blastocyst
in assisted hatching and FET cycles

Hatching through multiple openings

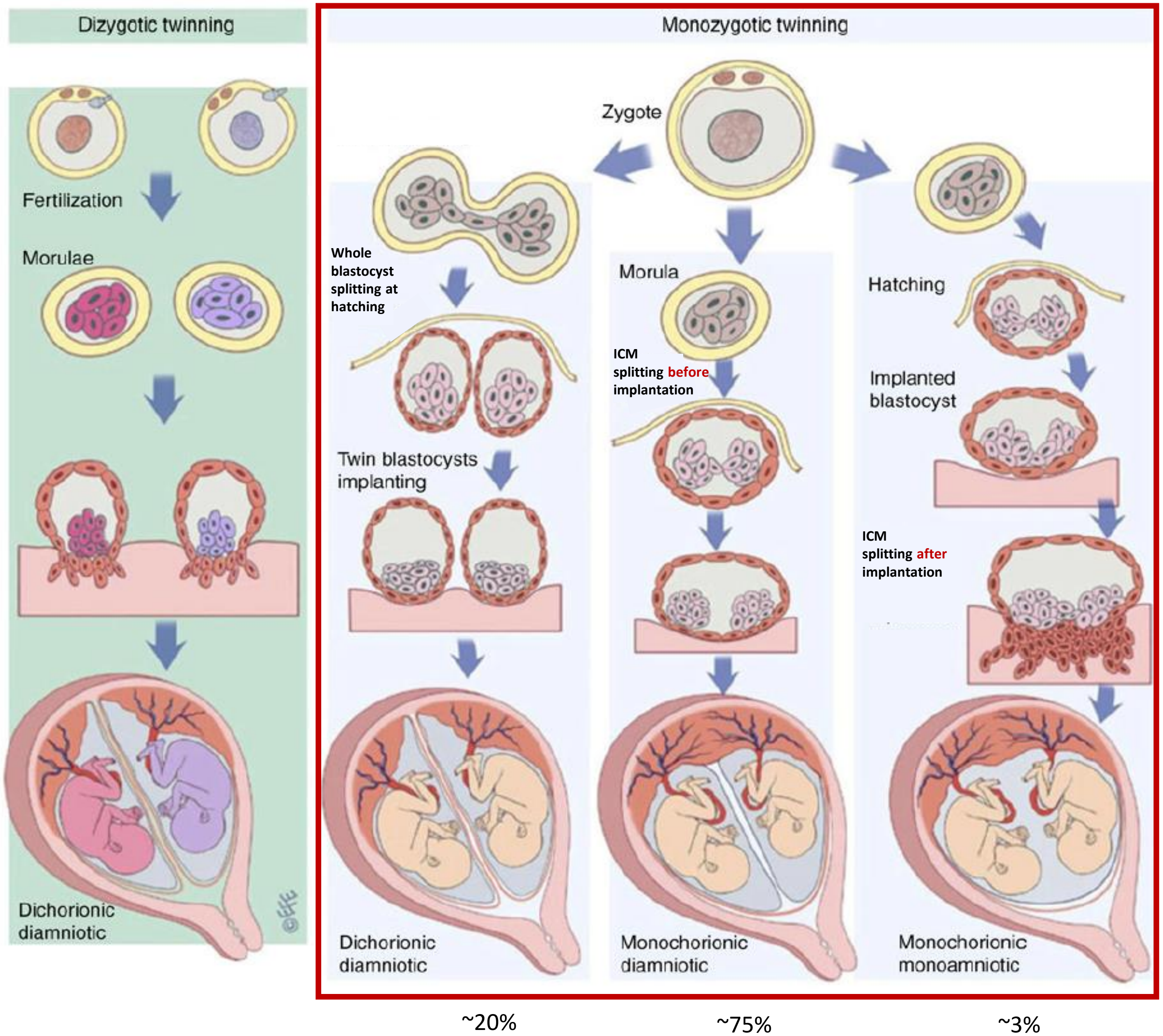
risk of ICM splitting

Monozygotic twinning

- genetically identical same sex twins

- epigenetic marks account for phenotypic discordance

- associated with ART!



Craig et al 2020 (adapted)

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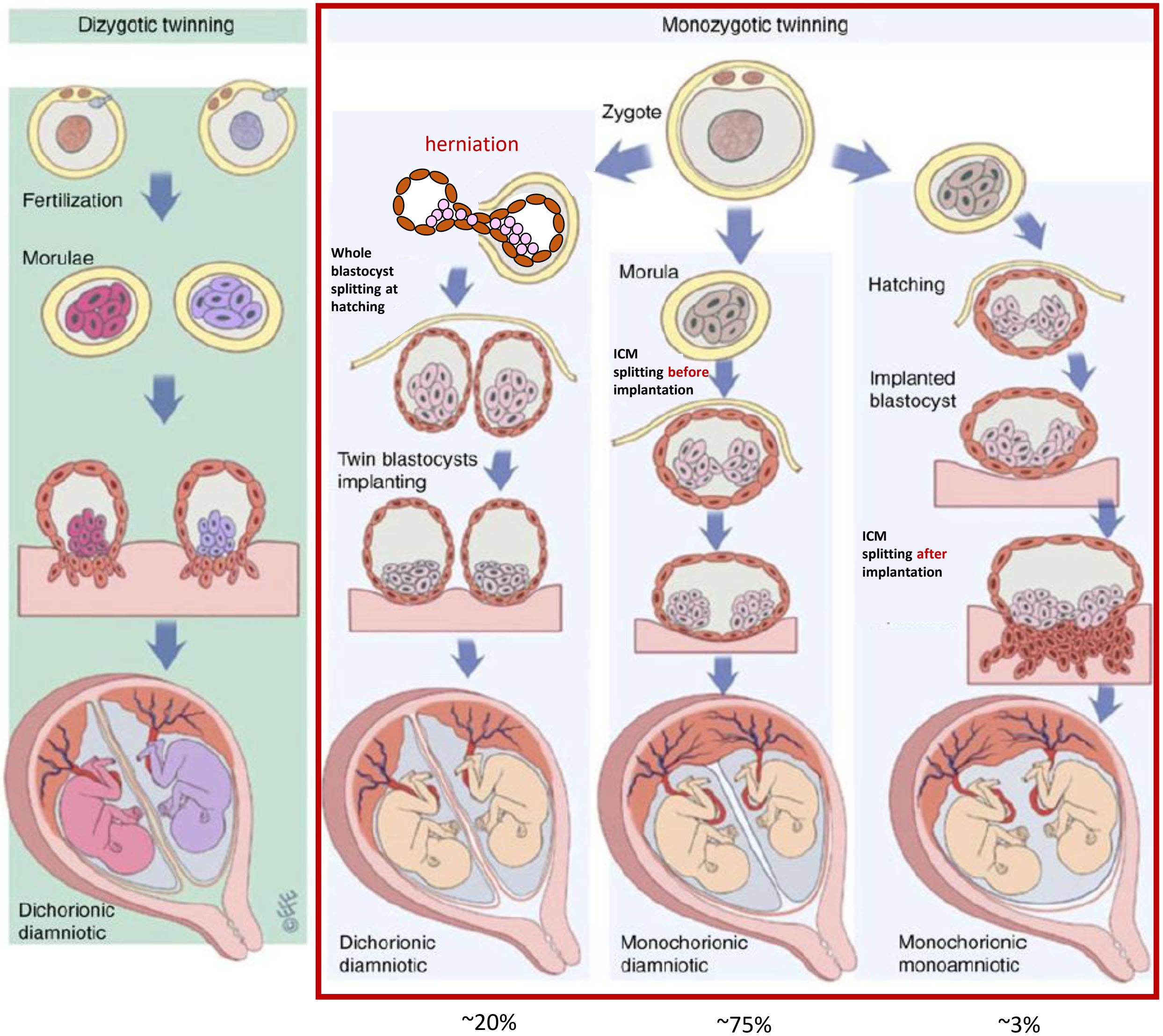
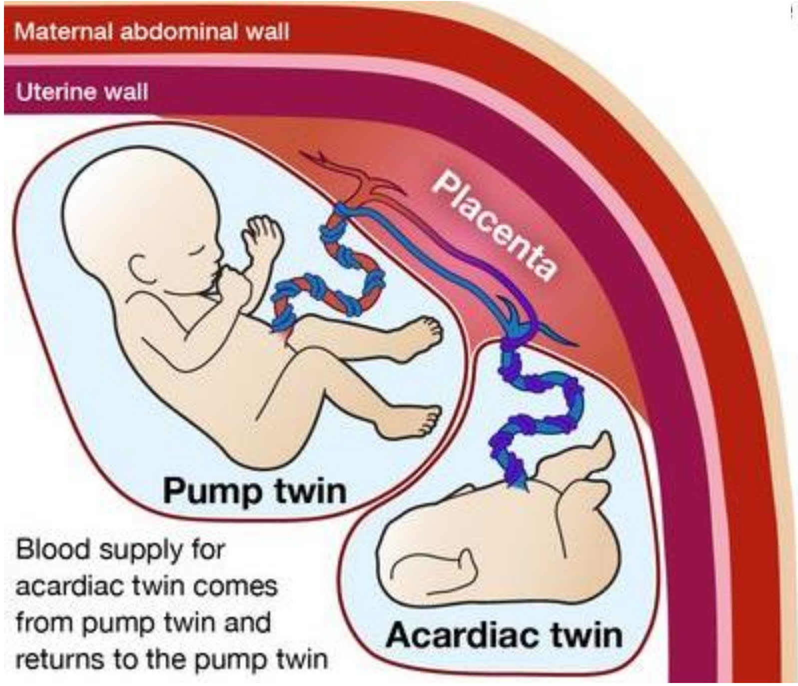


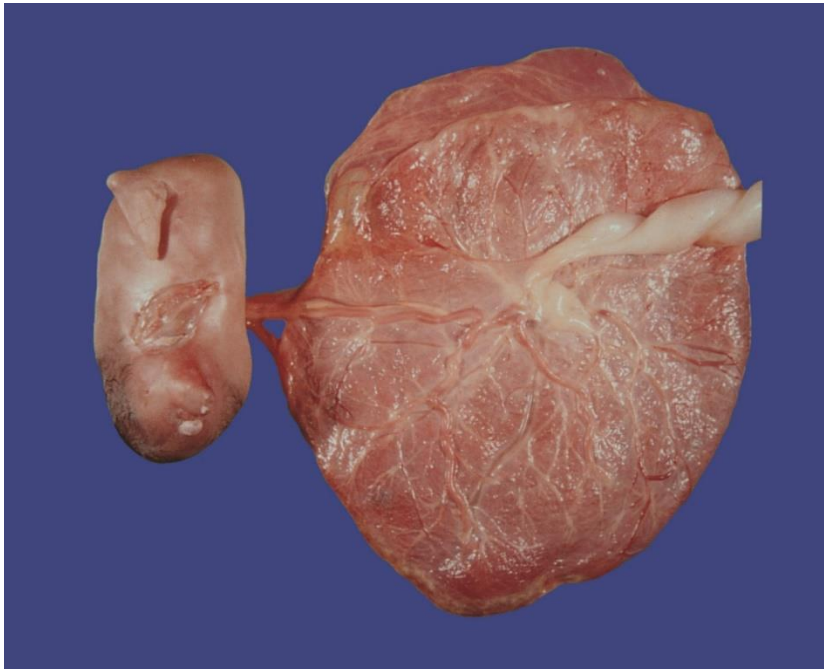
Craig et al 2020 (adapted)

Twinning pathologies

❖ Acardiac twin

- atrophic acardiac twin may compromise development of a healthy twin



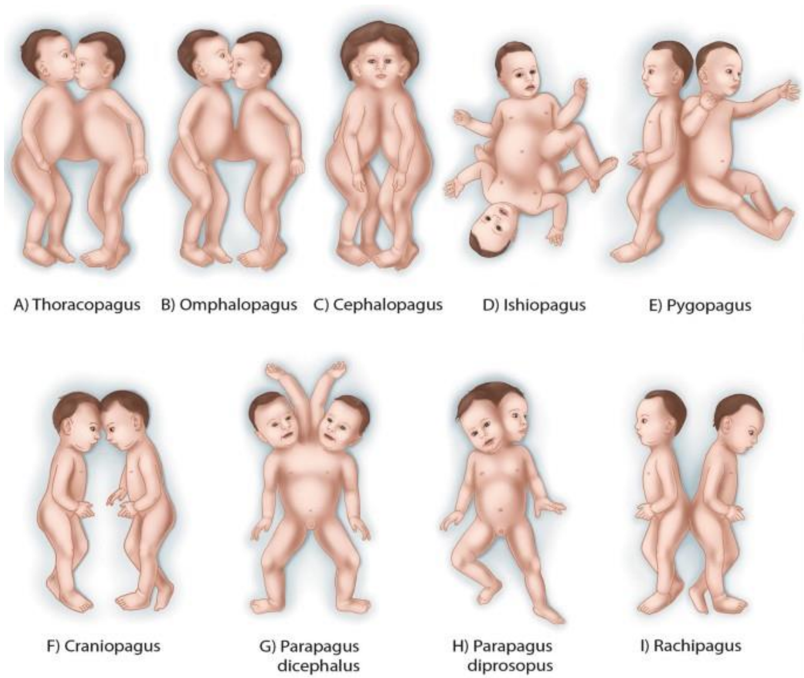


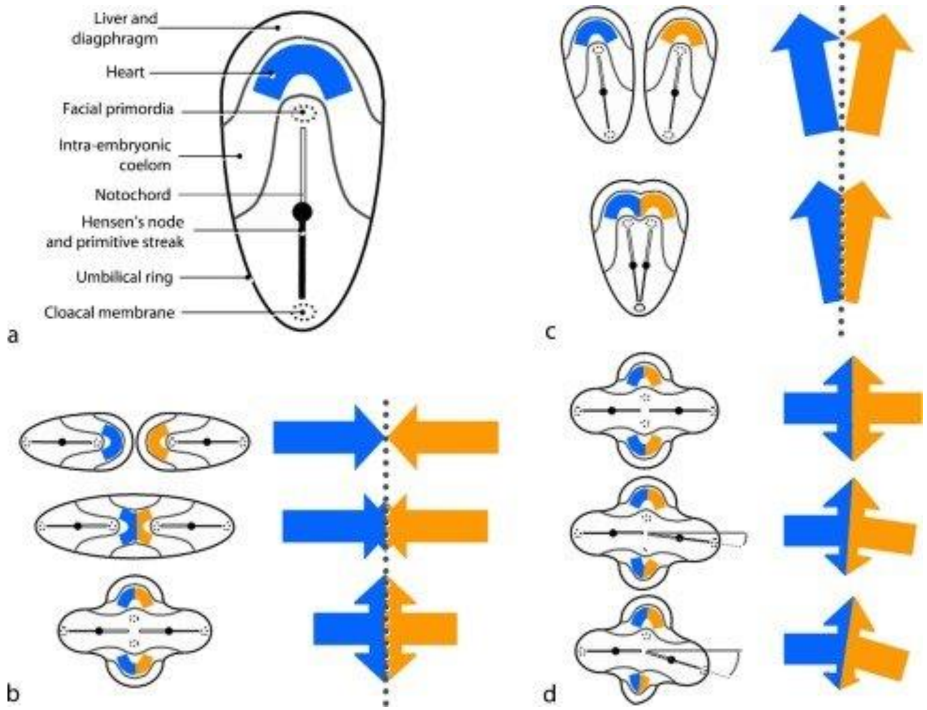
Monozygotic twinning

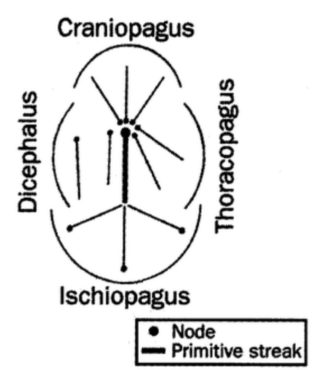
❖ Conjoined („Siamese“) twins

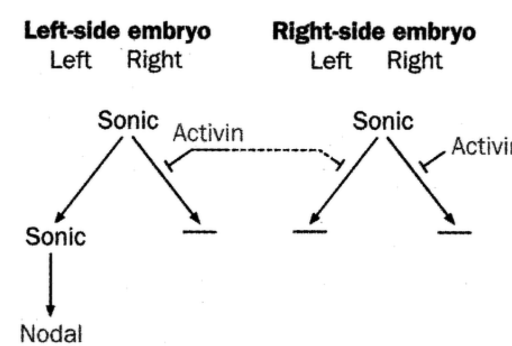
← partial splitting of primitive node/streak

- monochorionic, monoamniotic (2-5% MZ twins)
- aberrant axial (right-left/ craniocaudal) asymmetric patterning
- impaired expression of signaling molecules leads to laterally defects
- nature/degree of union variants









Monozygotic twinning

❖ Parasitic twin

= asymmetrical twin/unequal conjoined twin

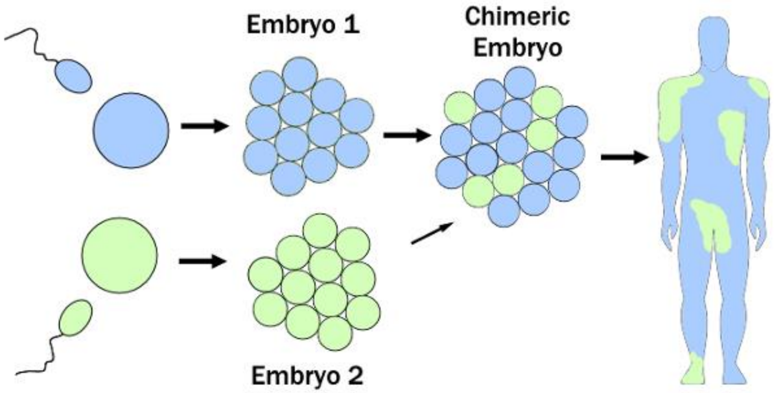
- one twin underdeveloped and attached to its healthy twin

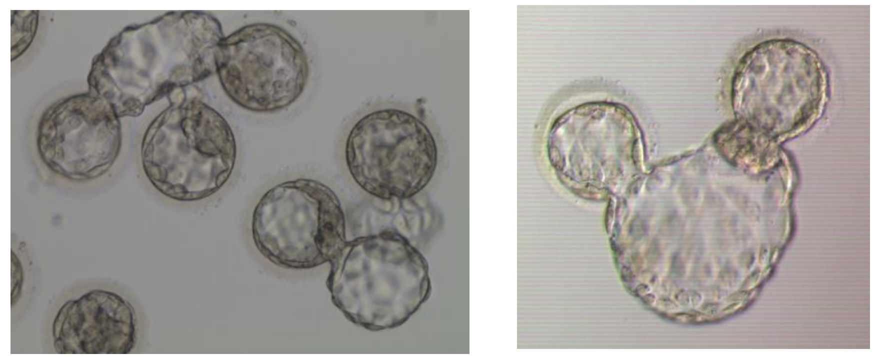
❖ Vanishing twin

- 1 in 8 multigestation
- loss of a twin before 12 wg
- fetal competion for space and nutrition
- non-developing embryo dies in utero and is partially or completely reabsorbed

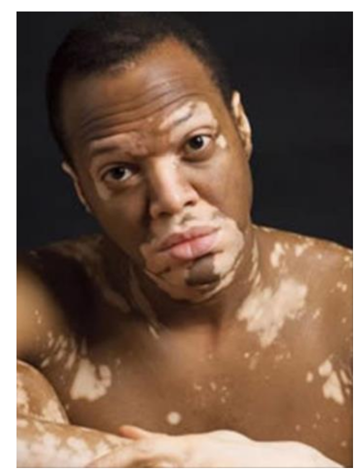
Twinning pathologies

- **genetic chimerism**
  - genetically distinct cell with one organism
  - early embryo fusion?





Blastocyst *in vitro* fusion



Twinning pathologies

❖ Seskvizygotic twins

Case report: Gabett et al, NEJM 2019.

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

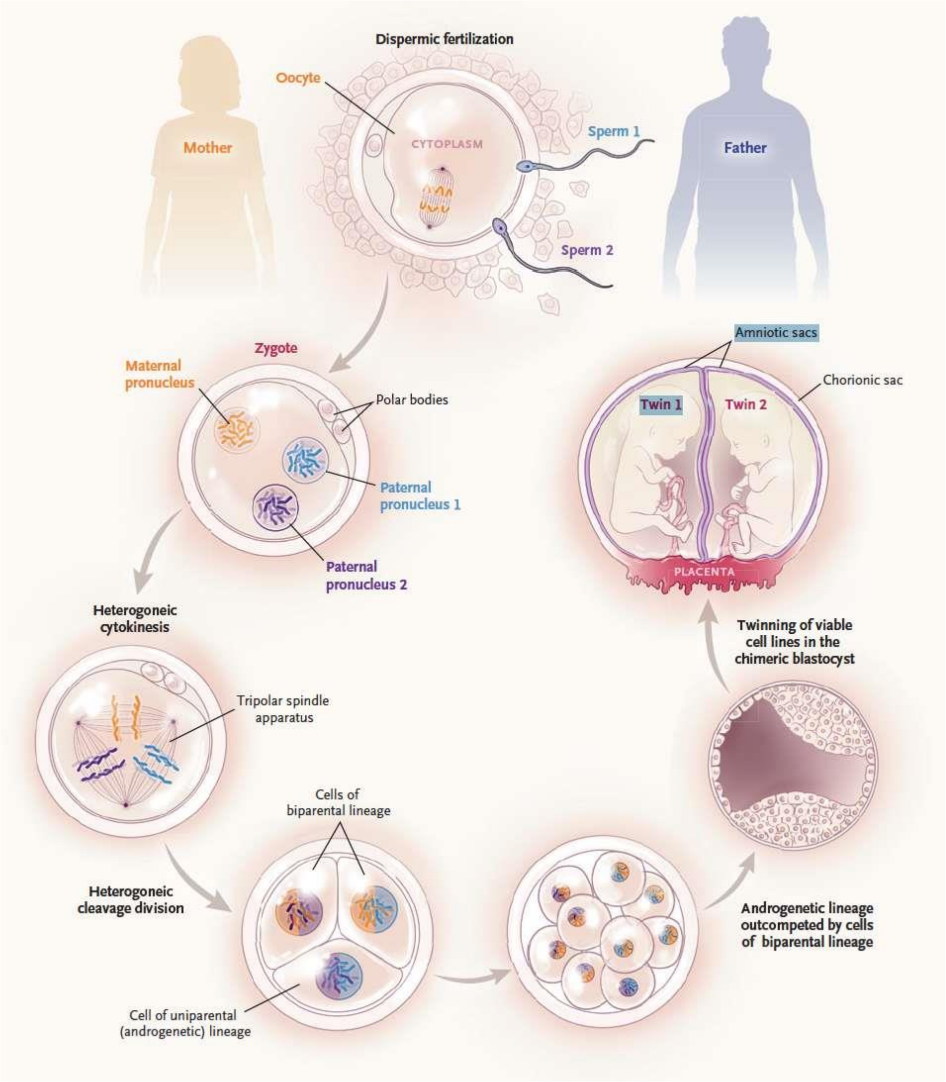
Molecular Support for Heterogonesis Resulting in Sesquizygotic Twinning

Michael T. Gabbett, M.B., B.S., M.Med.Sc., M.H.M., Johanna Laporte, M.D., Renuka Sekar, M.D., Adayapalam Nandini, Ph.D., Pauline McGrath, M.Nurs.Lead., Yadav Sapkota, Ph.D., Peiyong Jiang, Ph.D., Haiqiang Zhang, M.Phil., Trent Burgess, B.Sc., Grant W. Montgomery, Ph.D., Rossa Chiu, M.B., B.S., Ph.D., and Nicholas M. Fisk, M.B., B.S., Ph.D.

SUMMARY

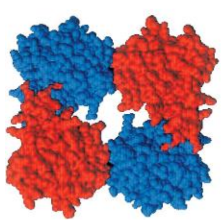
Sesquizygotic multiple pregnancy is an exceptional intermediate between monozygotic and dizygotic twinning. We report a monochorionic twin pregnancy with fetal sex discordance. Genotyping of amniotic fluid from each sac showed that the twins were maternally identical but chimerically shared 78% of their paternal genome, which makes them genetically in between monozygotic and dizygotic; they are sesquizygotic. We observed no evidence of sesquizygosis in 968 dizygotic twin pairs whom we screened by means of pangenome single-nucleotide polymorphism genotyping. Data from published repositories also show that sesquizygosis is a rare event. Detailed genotyping implicates chimerism arising at the juncture of zygotic division, termed heterogonesis, as the likely initial step in the causation of sesquizygosis.

- monochorionic diamniotic twins with discordant sex
- 46XX/46XY chimerism in both children
- share 78% of paternal DNA
- caused by dispermic fertilization?



Maternal zonolytic factors

- proteases and protease inhibitors secreted by the oviduct and uterine lining
- **facilitate embryo hatching *in vivo***
- protease activity is suppressed in the oviduct to prevent ectopic pregnancy
- proteinase **strypsin (ISP-1) and lysis (ISP-2)**
  - co-expressed in uterine glands during the perimplantation period
  - form homo-/hetero-tetramer complexes with ZP-lysis activity
  - human homologous not yet identified !



  - uterine secretion regulated by P4 (↑) and E2 (↓,) during menstrual cycle

MOLECULAR REPRODUCTION AND DEVELOPMENT 62:328–334 (2002)

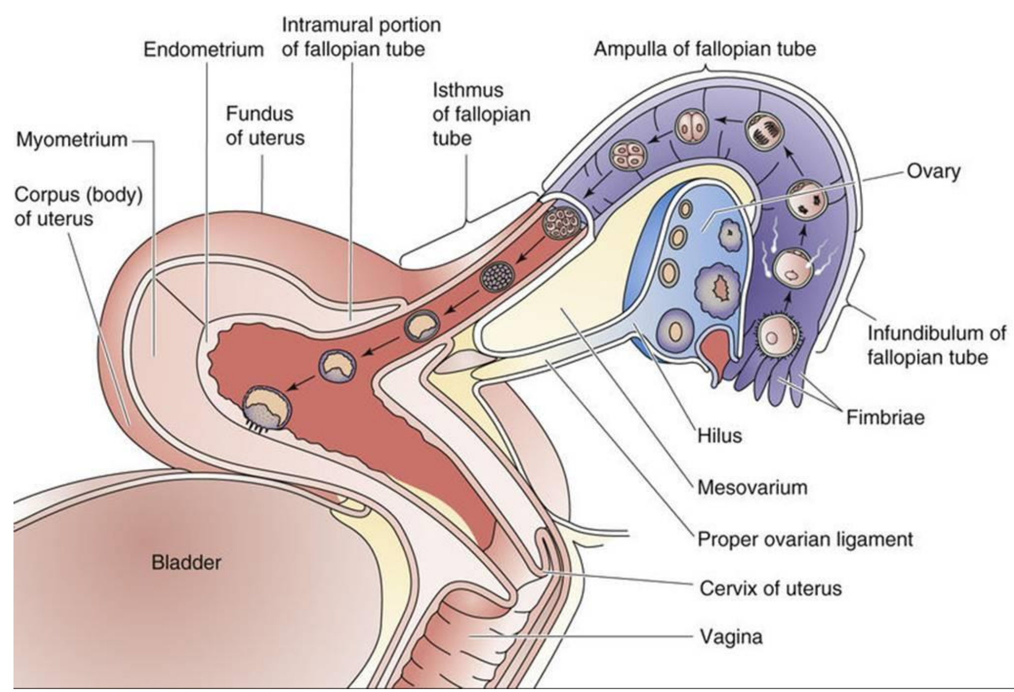
Embryonic Hatching Enzyme Strypsin/ISP1 Is Expressed With ISP2 in Endometrial Glands During Implantation

COLLEEN M. O'SULLIVAN, SHI YING LIU, J. BRADLEY KARPINKA, AND DERRICK E. RANCOURT*
Department of Biochemistry and Molecular Biology, Southern Alberta Cancer Research Centre, University of Calgary, Alberta, Canada

MOLECULAR REPRODUCTION AND DEVELOPMENT 69:252–259 (2004)

Uterine Secretion of ISP1 & 2 Tryptases Is Regulated by Progesterone and Estrogen During Pregnancy and the Endometrial Cycle

COLLEEN M. O'SULLIVAN, JILLIAN L.R. UNGARIAN, KULDEEP SINGH, SHIYING LIU, JACKIE HANCE, AND DERRICK E. RANCOURT*
Southern Alberta Cancer Research Centre, Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada



Mechanism of embryo hatching

Winuthayanon et al. 2015

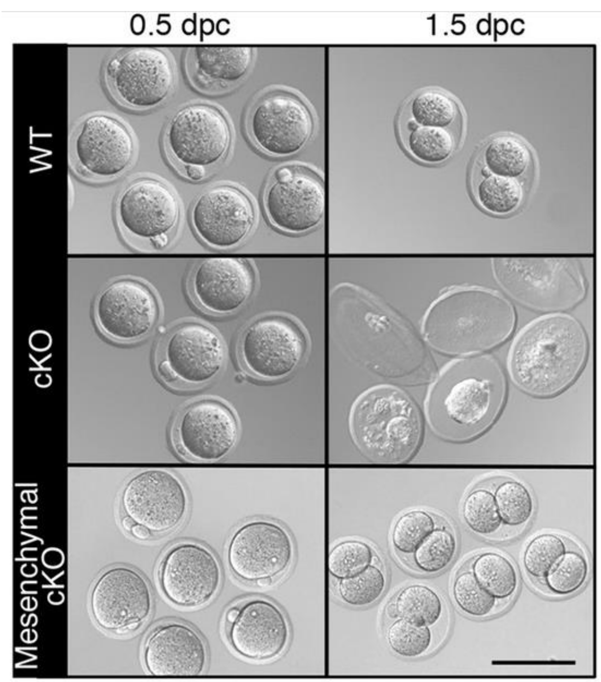

Oviductal estrogen receptor α signaling prevents protease-mediated embryo death

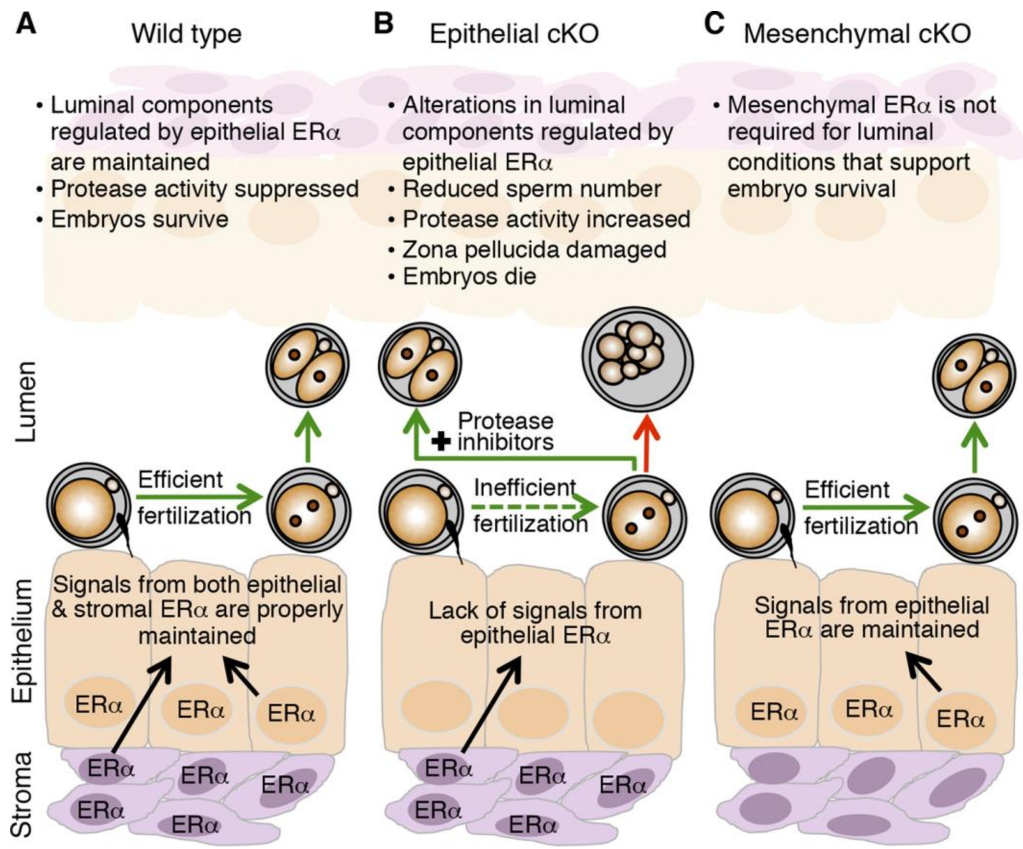
Wipawee Winuthayanon[1,2*], Miranda L Bernhardt[1], Elizabeth Padilla-Banks[1], Page H Myers[3], Matthew L Edin[4], Fred B Lih[5], Sylvia C Hewitt Kenneth S Korach[1], Carmen J Williams[1*]

❖ Maternally-derived zonolytic factors

- cKO mice lacking epithelial/mesenchymal estrogene receptor (ER)

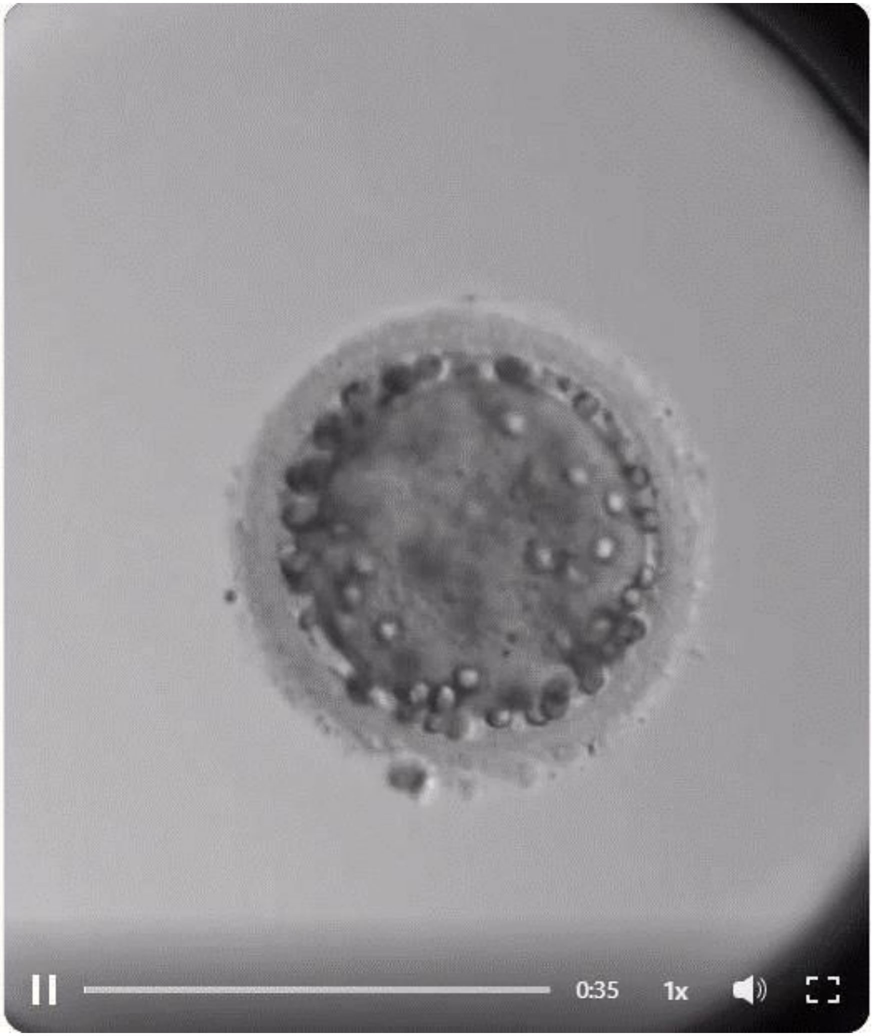
- absence of ER in uterine lining leads to elevated protease activity, premature ZP dissolution and embryo lysis by day 2





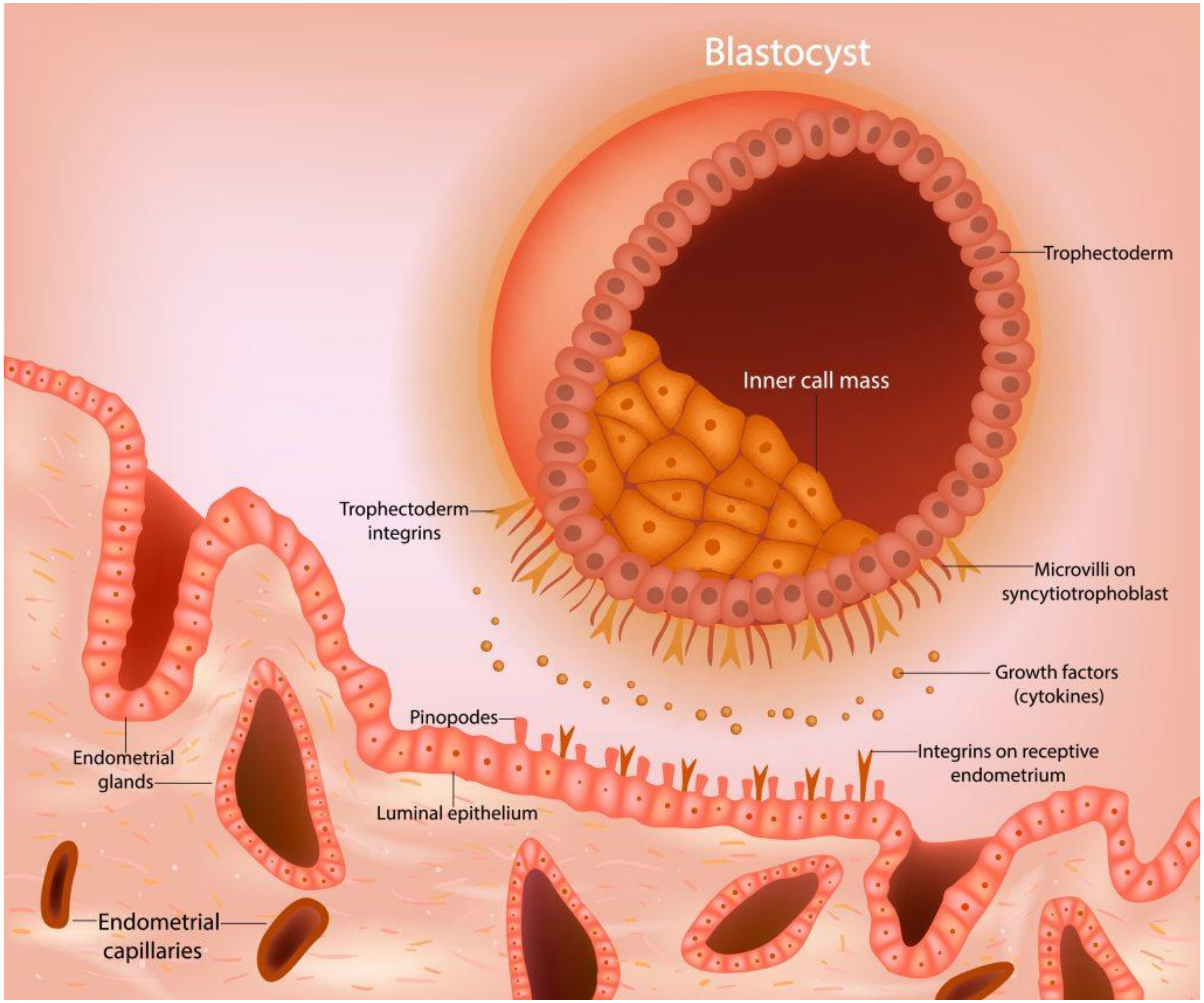
Premature ZP dissolution

- untimely dissolution of ZP rarely observed *in vitro*
- cause unknown
- risk of blastomere separation and compaction failure
- linked to poor embryo development and



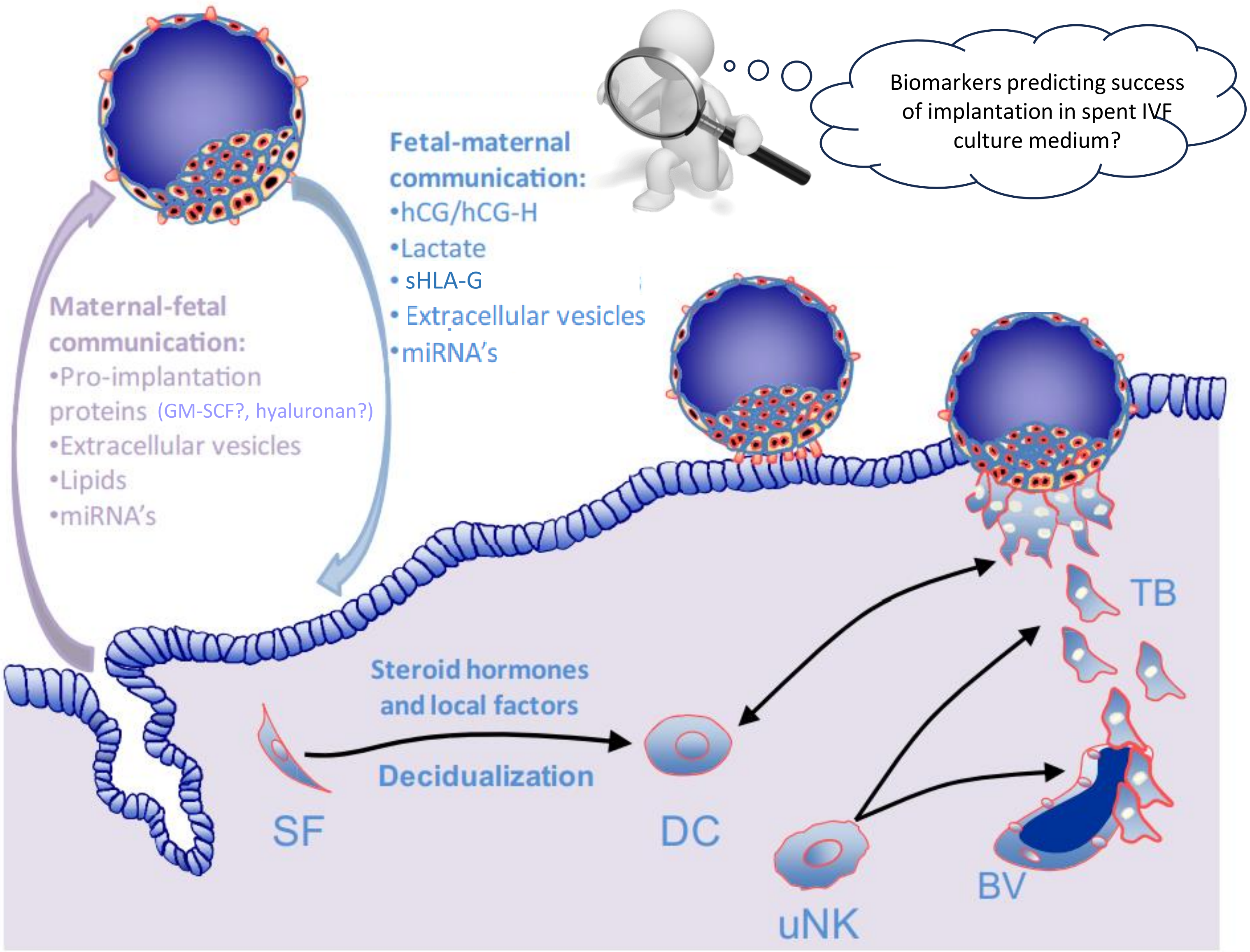
LinkedIn Emma Whitney

Embryo-endometrial signaling



Embryo-endometrial signaling

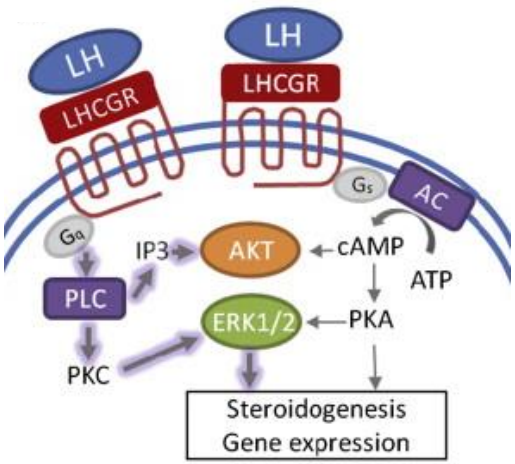
- Paracrine signaling



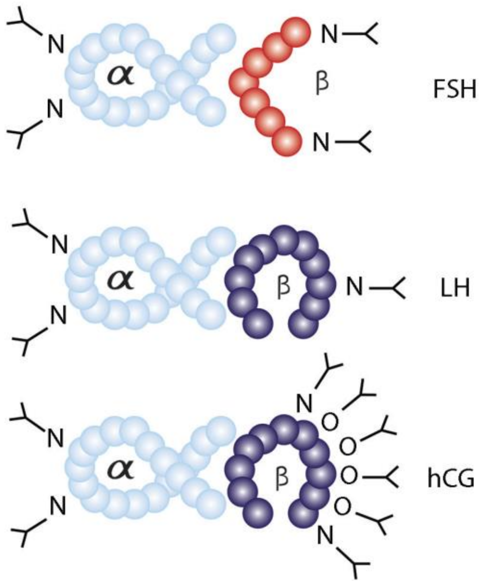
Evans 2020 (modified)

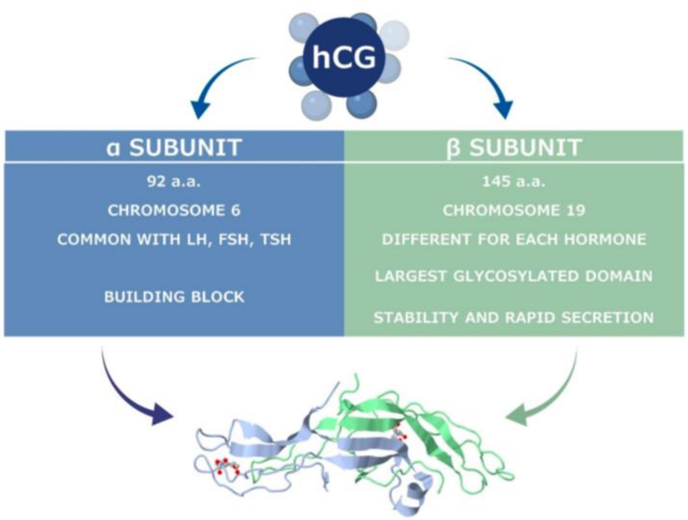
❖hCG

- 6 CBG genes encoding biologically active β-subunit in humans evolved as a result of duplication of LHB gene
- structural similarity with LH (and FSH)
  - α - unit - identical
  - β - unit - ~80% homology with LH
    - different glycosylation
    - provides specificity
- binds to common LH/hCG receptor



- hCG can mimic the bioactivity of LH

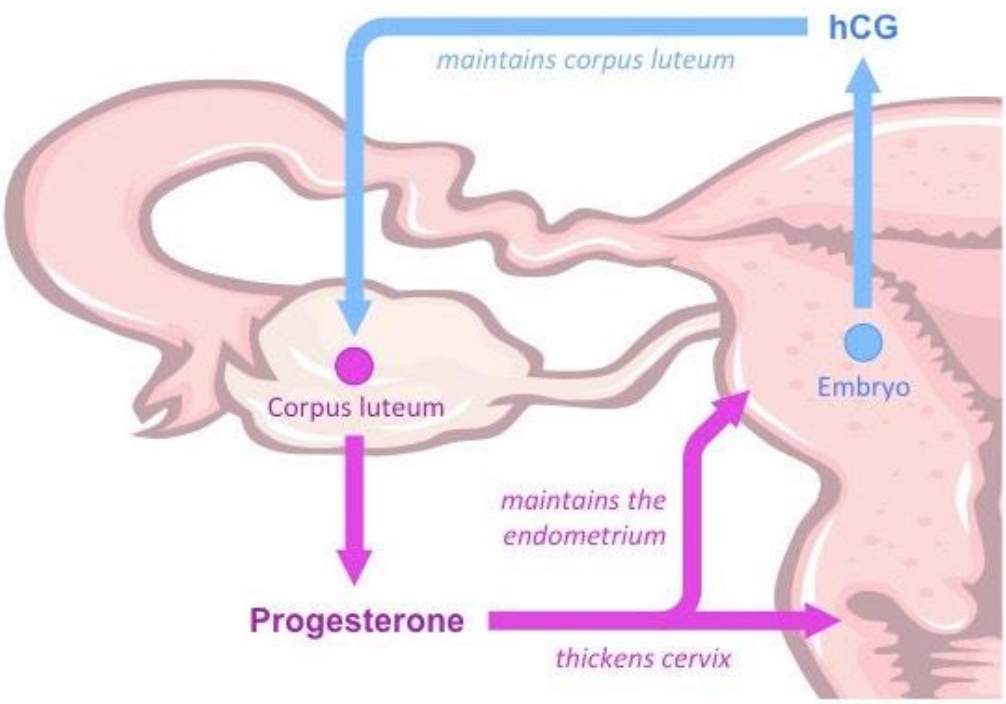




Embryo-endometrial signalling

❖hCG

Embryo-secreted hCG sustains
corpus luteum and P4 production



P4 suppress uterine inflammation
and promote establishment of pregnancy

Embryo-endometrial signalling

❖ hCG

5 isoform, differing activities

➢ Classical (regular) hCG

← preimplatation embryo and syncytiotrophoblast

➢ Hyperglycosylated hCG (hCG-H)

- lower activity but longer half-life than regular hCG

← preimplatation embryo

← extravillous cytotrophoblast during early pregnancy

← malignant hCG-producing tumors

➢ Sulphated hCG

← pituitary gland

- assumed to supplement pituitary LH functions

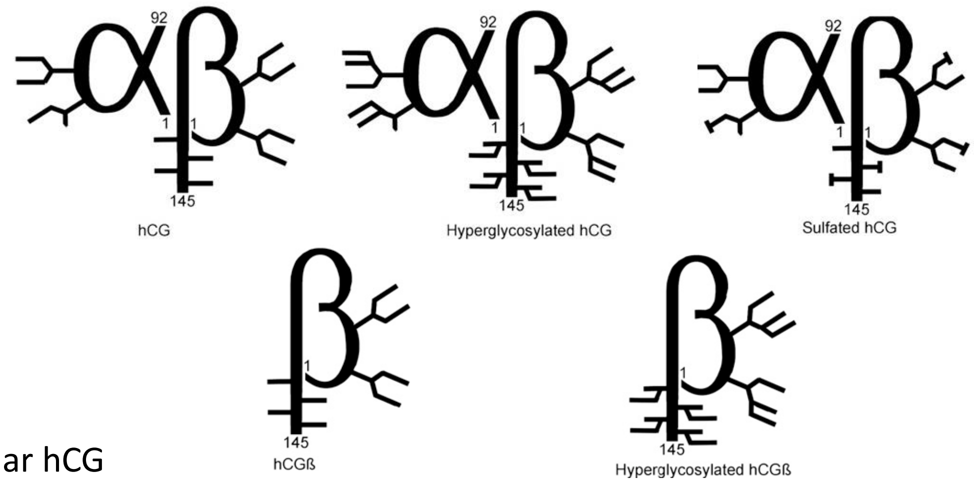
➢ Free β unit of classical hCG

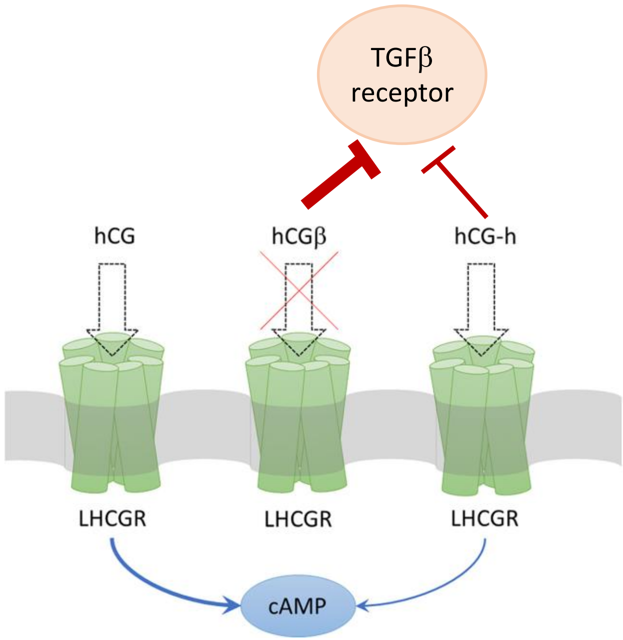
➢ Free β unit of hyperglycosylated hCG

← preimplatation embryo

← malignant hCG-producing tumors

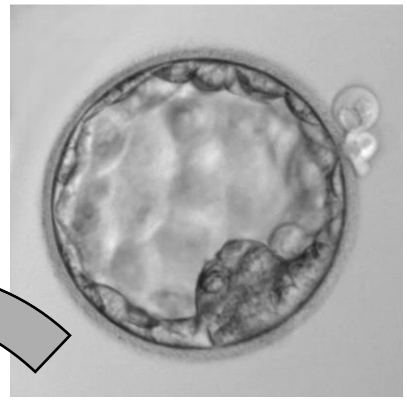
*urine of pregnant women contains also proteolytically processed („nicked“) forms of hCG (hCGn), hCG-β (hCG-βn), and core fragment of hCG-β (hCG-βcf),





Embryo-endometrial signalling

❖hCG



secretion

8 cells<blastocyst (TE) < <syncytiotrophoblast

trigger shot

50% regular hCG **40% HCG-H** **10% hCG-β**

≠

A) rec-hCG = pure regular hCG	B) purified **urinary hCG** - **molecular heterogenity** (2.2-12% hCG-H)

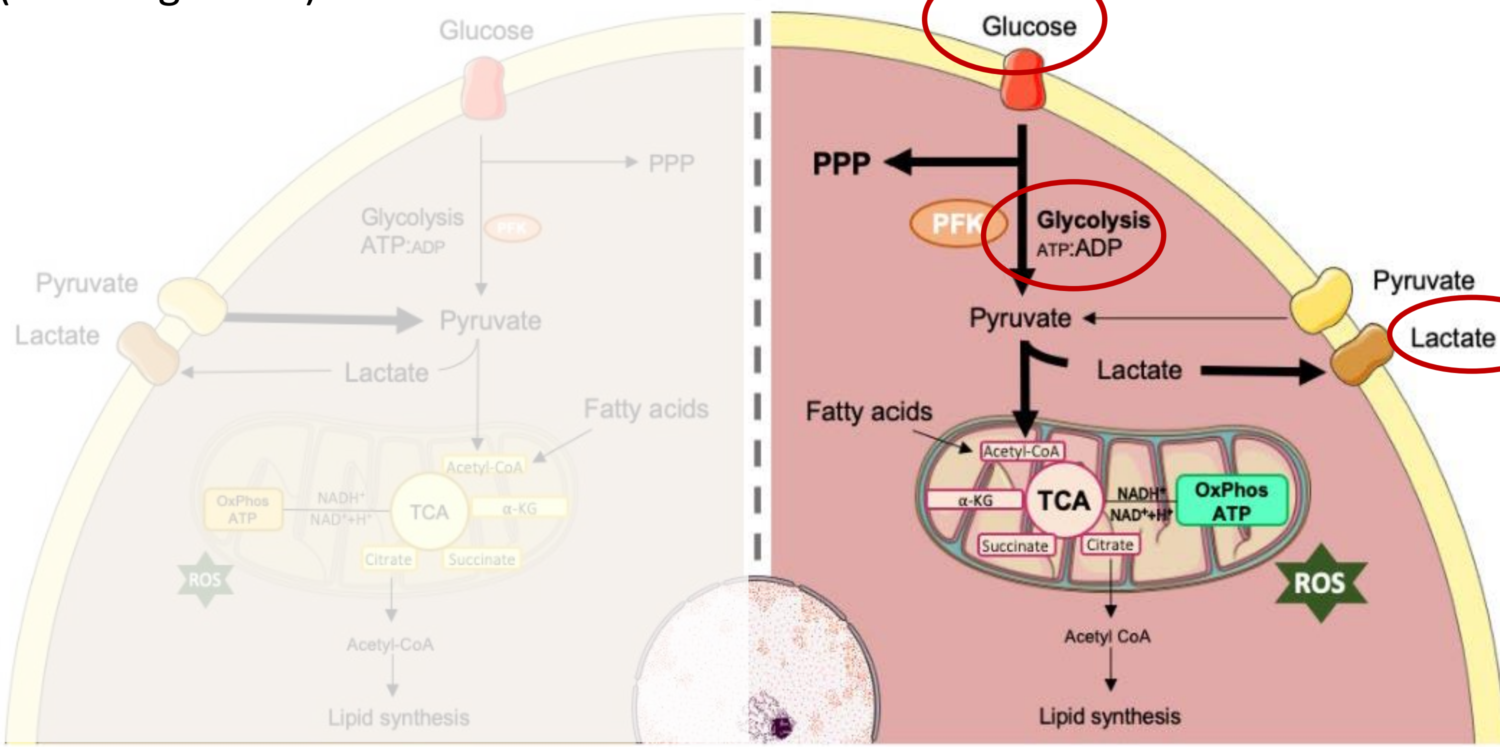
Embryo-endometrial signalling

❖ Lactate

- > 50% of Glc consumed by a blastocyst is not oxidized but converted to lactate (Warburg effect)

Instead of being only a metabolic by-product, lactate serve as a key embryo-derived signal augmenting implantation

David Gardner



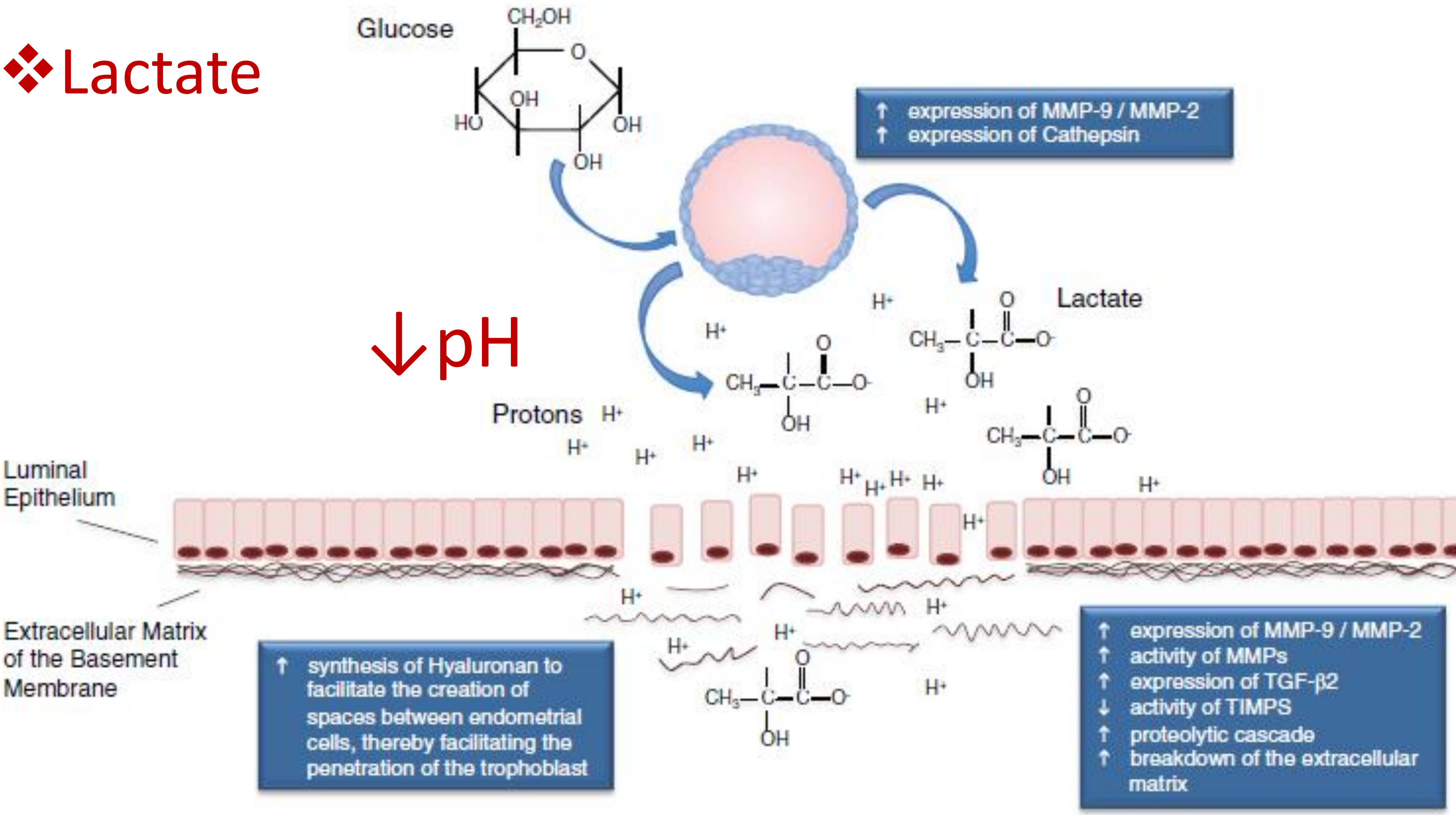
>50%

Miazzoto et al 2020

Embryo-endometrial signalling

- Lactate

↓pH

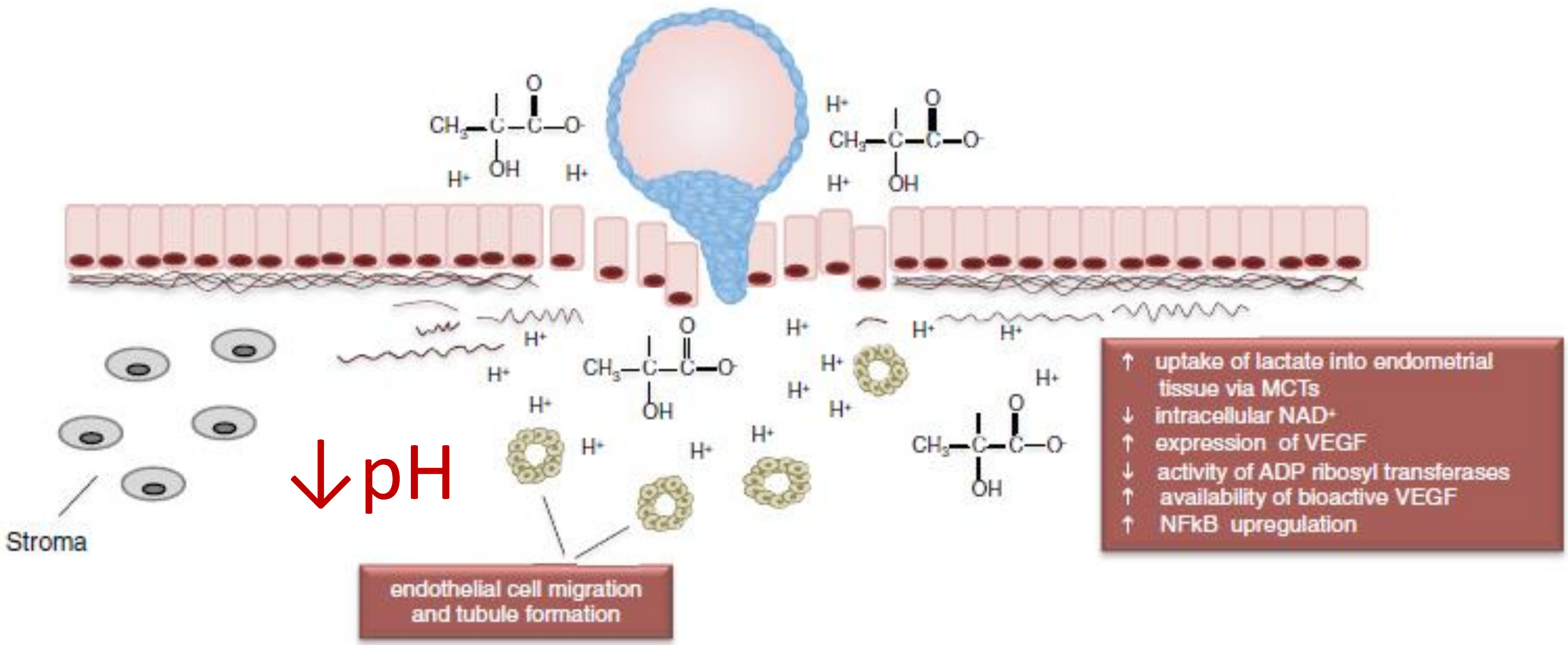


Facilitates embryo hatching, endometrial tissue disaggregation and trophoblast invasion

Gardner 2015

Embryo-endometrial signalling

❖Lactate

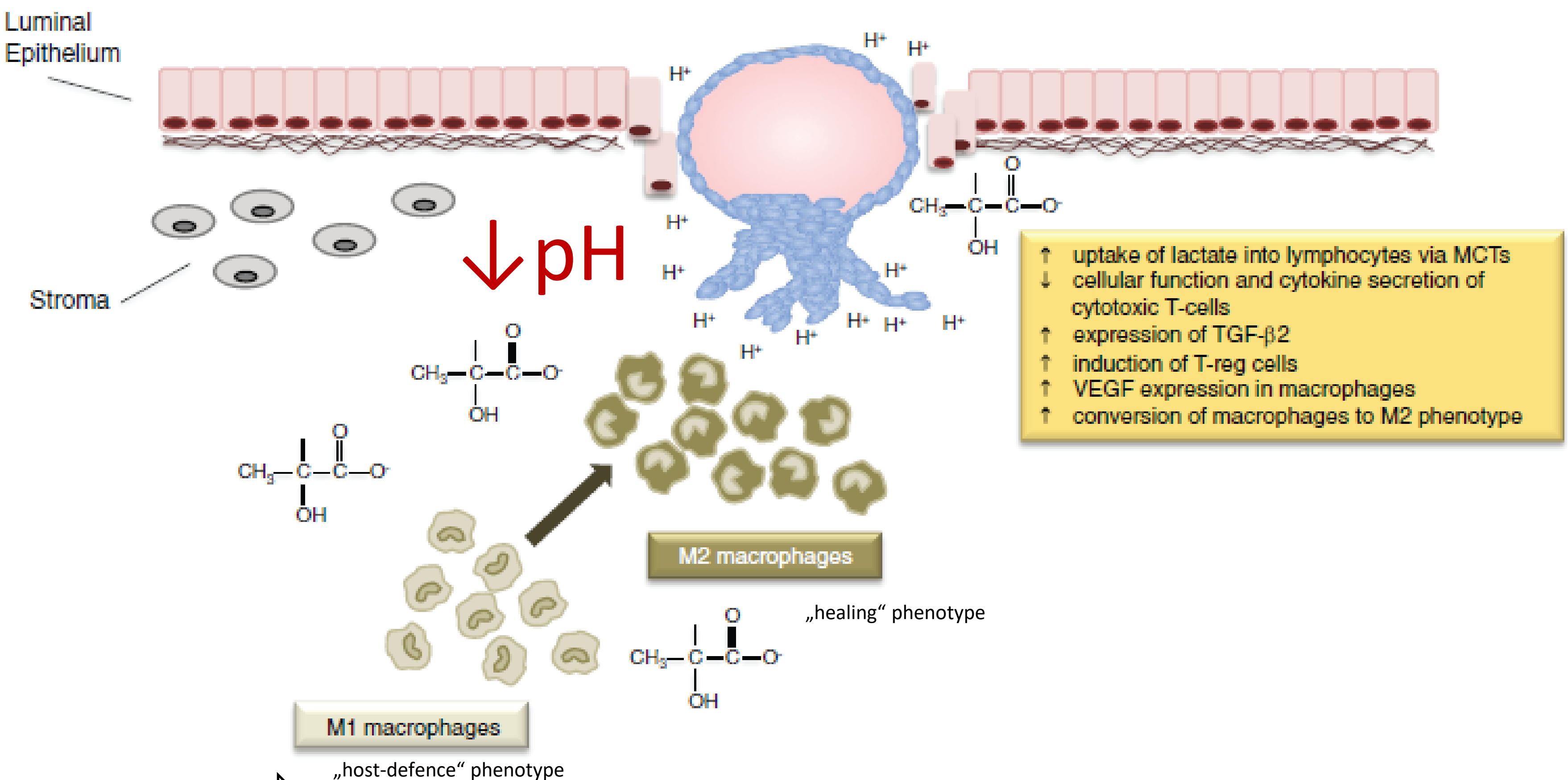


⇨ Induces angiogenesis and increases vascular permeability

Gardner 2015

Embryo-endometrial signalling

❖Lactate



⇨ Modulates the immune response of the the mother

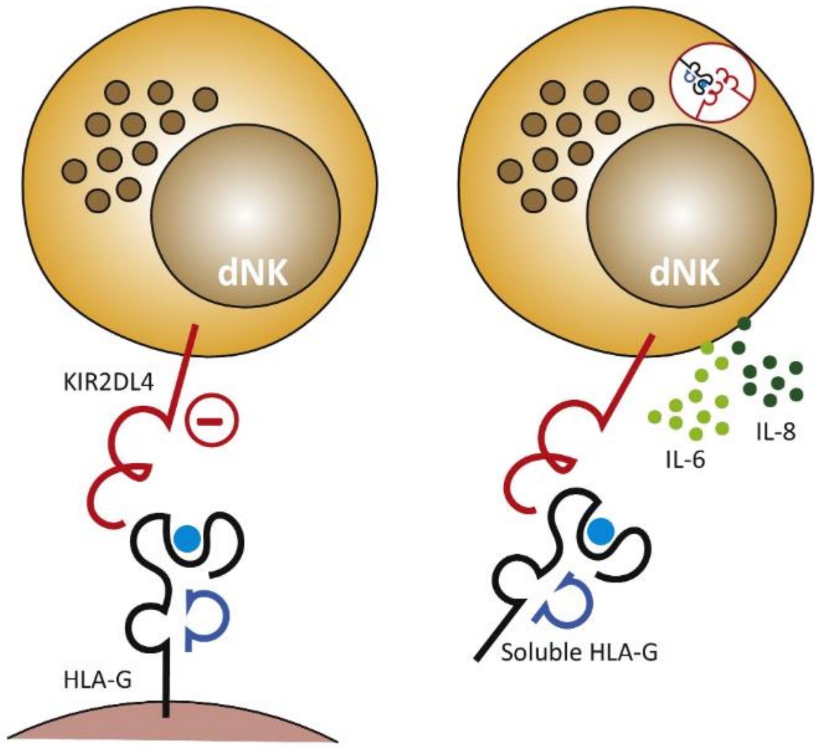
Gardner 2015

Embryo-endometrial signalling

❖ sHLA-G

- soluble form of HLA-G
- HLA-G is expressed on TE and extravillous trophoblast (EVT) cells (and tumor cells)
- HLA-G plays a key role in establishing maternal-fetal immune tolerance

- sHLA-G has systemic immunomodulation effects
- present in conditioned (spent) embryo culture medium
- documented as a positive implantation predictor of IVF embryos
- in tumors, concentration reflects invasiveness and metastasis potential

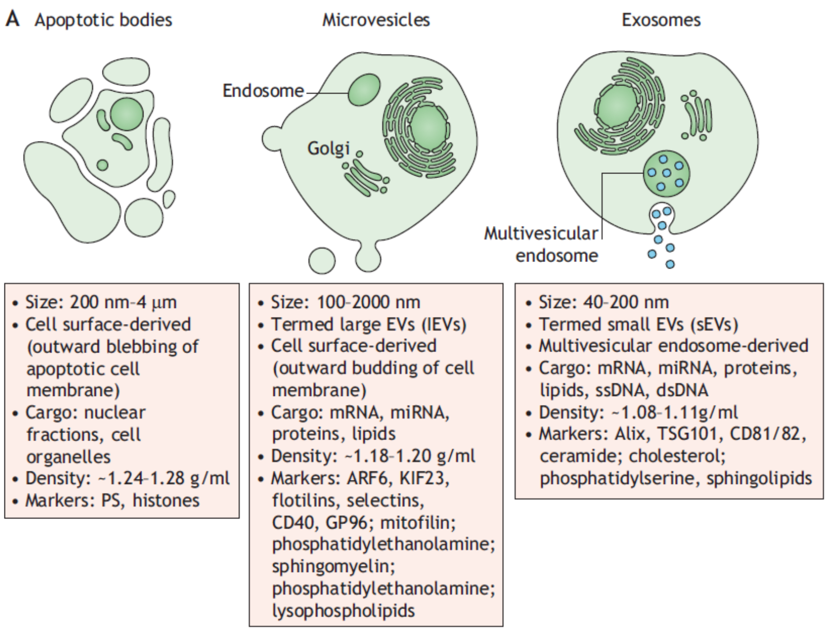


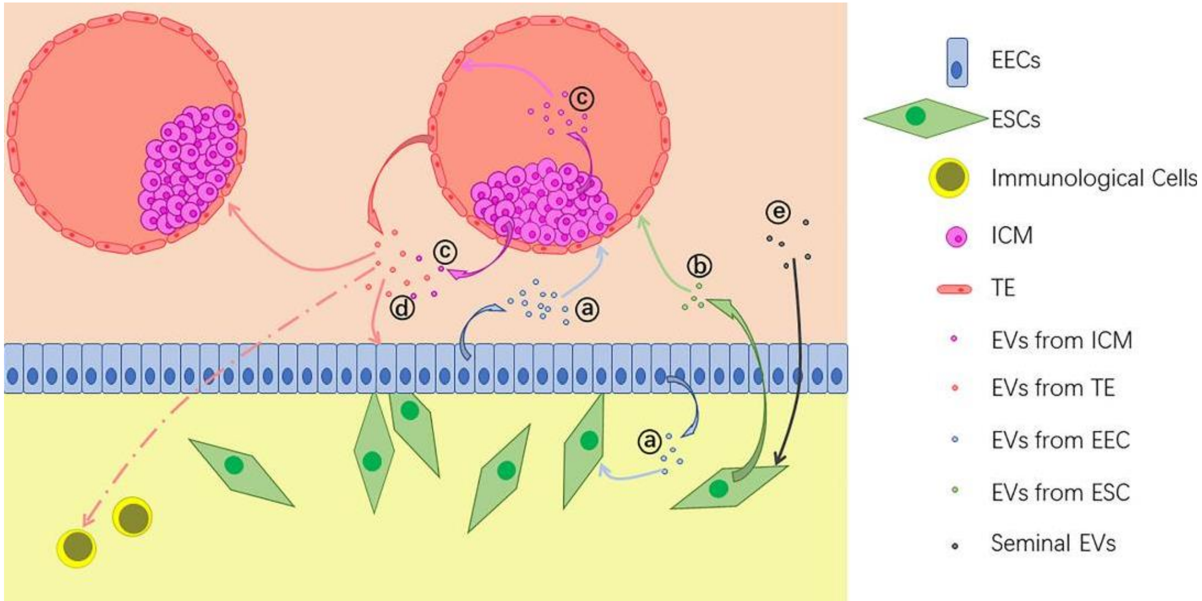
Ferreira et al 2017

Embryo-endometrial signalling

❖ Extracellular vesicles

- cell-derived membranous nano-sized vesicles
- secreted by both uterus and embryo (TE/ICM)
- different size, origin, and cargo
- known to contain proteins, lipids, and ncRNAs
- packaged content alters during the uterine cycle and embryo development





- embryotrophic effects
- TE uptake of endometrium-secreted exosomes promotes TE adhesion and invasion

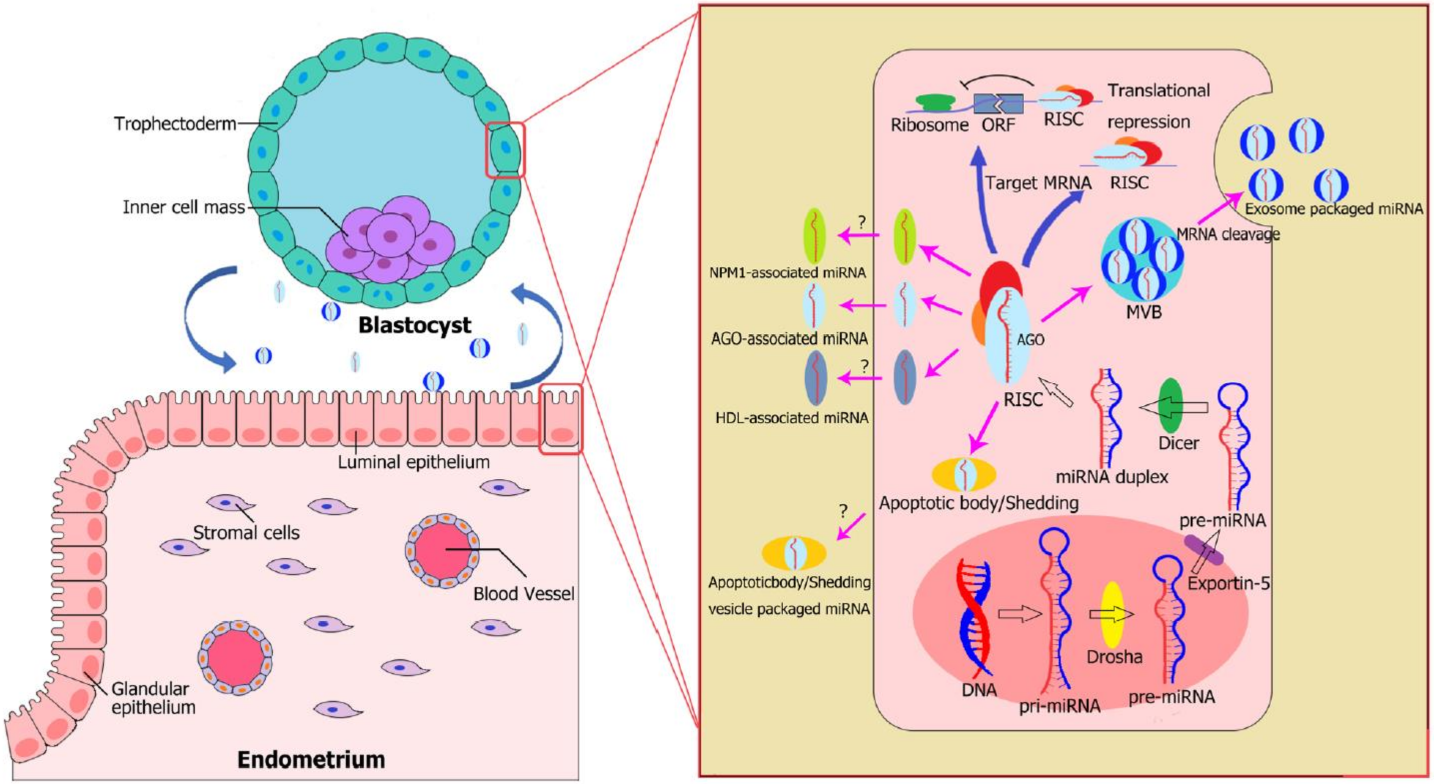
⇩

enhance embryo implantation

Embryo-endometrial signalling

❖ miRNA

- transcriptional regulator of gene expression
- involved in intercellular communication, including embryo-maternal crosstalk
- both embryo- and endometrium-derived
- different types and multiple packing forms



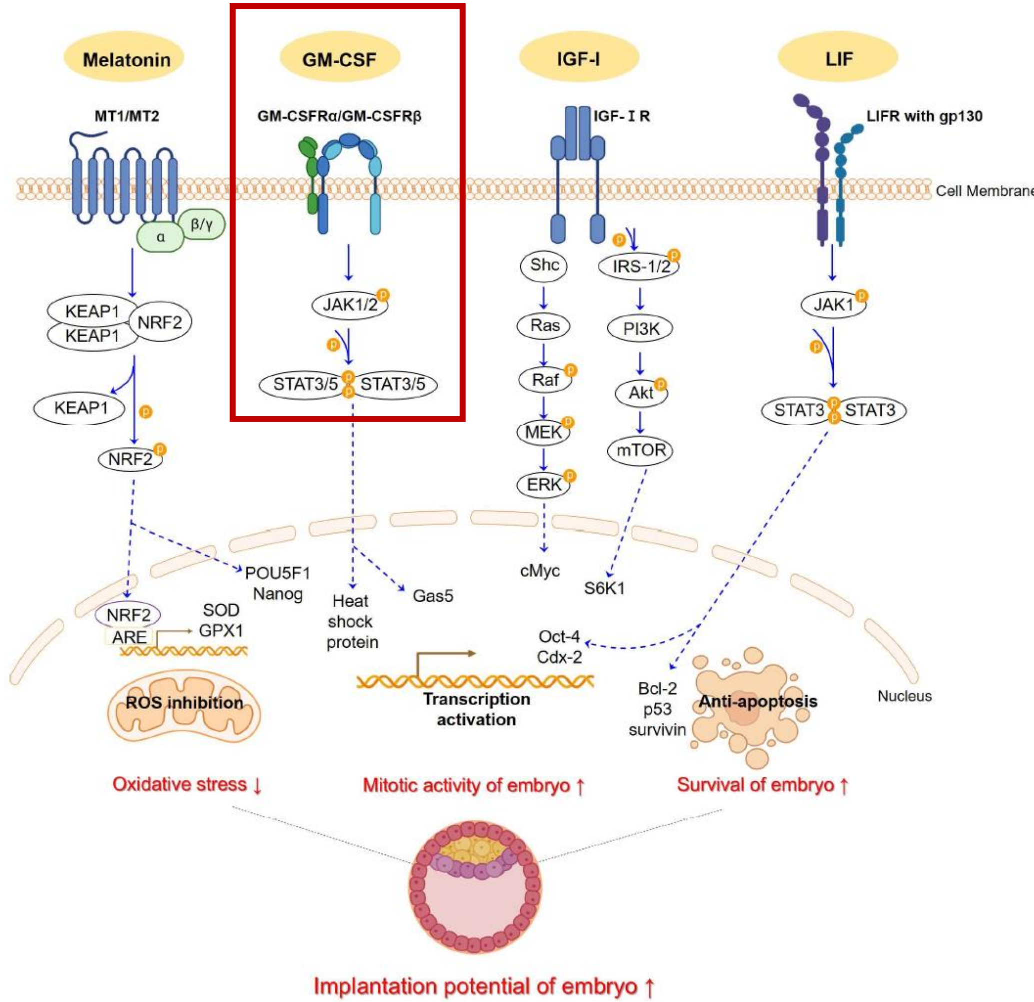
Liang et al 2017

Embryo-endometrial signalling

❖ GM-CSF

- cytokine synthesized in the female reproductive tract, including endometrium
- not expressed in the embryo
- uncertainty over its positive effect on embryo development and implantation

- commercially available embryo culture medium supplemented with GM-CSF



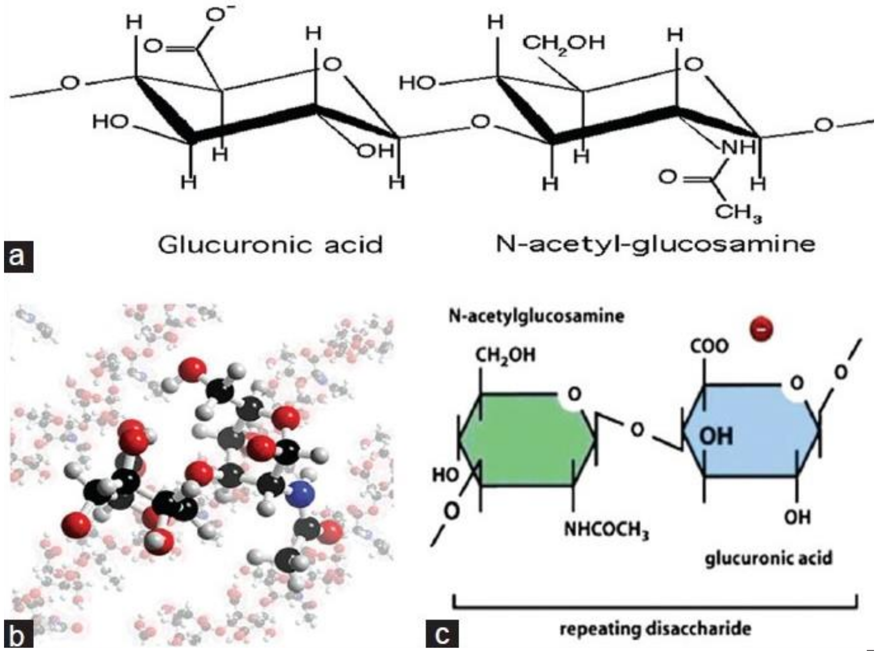
Choi et al 2024

Embryo-endometrial signalling

❖ Hyaluronan (=hyaluronic acid, HA)

- high-molecular-mass polysacharide found in ECM

- HA secretion by invasive trophoblast and synthesis in endometrial tissue can promote trophoblast penetration during embryo implantation
  BUT
  it does not act as a „glue“ promoting embryo adhesion and attachment to the endometrium



- HA supplementation of embryo culture/transfer media

- uncertainity over its positive effect on embryo development and implantation

Seeding into fertile ground.....

.....to be continued

Table 1. Some forms of clinically relevant hCG and their functions.

hCG type	Production	Function
Intact, biologically active heterodimeric, normally glycosylated hCG	Syncytiotrophoblast hydatidiform moles	Pregnancy: • Promotes progesterone production by corpus luteum cells • Promotes angiogenesis in the spiral arteries of the myometrium during pregnancy[20] • Promotes the differentiation of cytotrophoblast cells to syncytiotrophoblast • Promotes quiescence of contractions in the uterine myometrium
Hyperglycosylated hCG	Extravillous cytotrophoblast cells[2,21]	• Promotes trophoblast invasion • Promotes cytotrophoblast cell growth and placental implantation • Promotes invasion in choriocarcinoma[2,22]
Free hCG β-subunit	Pregnancy: Implanted blastocysts and trophoblasts[23] Malignancies: choriocarcinoma, nonseminomatous testicular tumors, bladder, cervical, pancreatic, lung, ovarian, endometrial cancers	Pregnancy: • Thought to play a role in implantation[23] • Maintenance of pregnancy[23] Malignancies: • Promote cell growth & malignant transformation[12] • Blocks apoptosis
Pituitary hCG[24,25]	Pituitary gonadotrophs during menstrual cycle or after menopause	• Assumed to supplement normal physiologic pituitary LH functions, i.e., follicular growth and progesterone production

hCG, human chorionic gonadotropin; LH, luteinizing hormone.