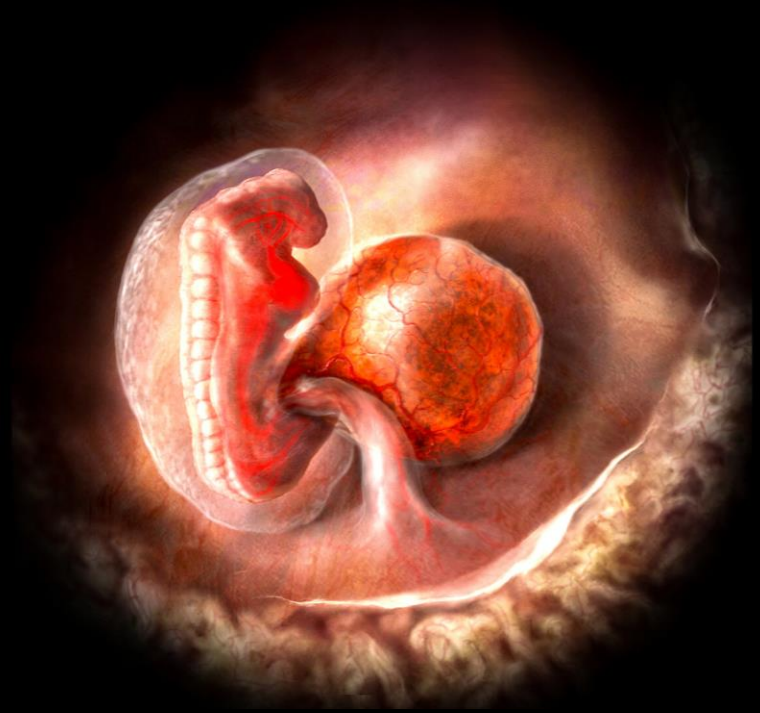
MUNI MED

Embryology III

PERIMPLANTATION DEVELOPMENT

autumn 2024

Preparing uterine tissue for implantation



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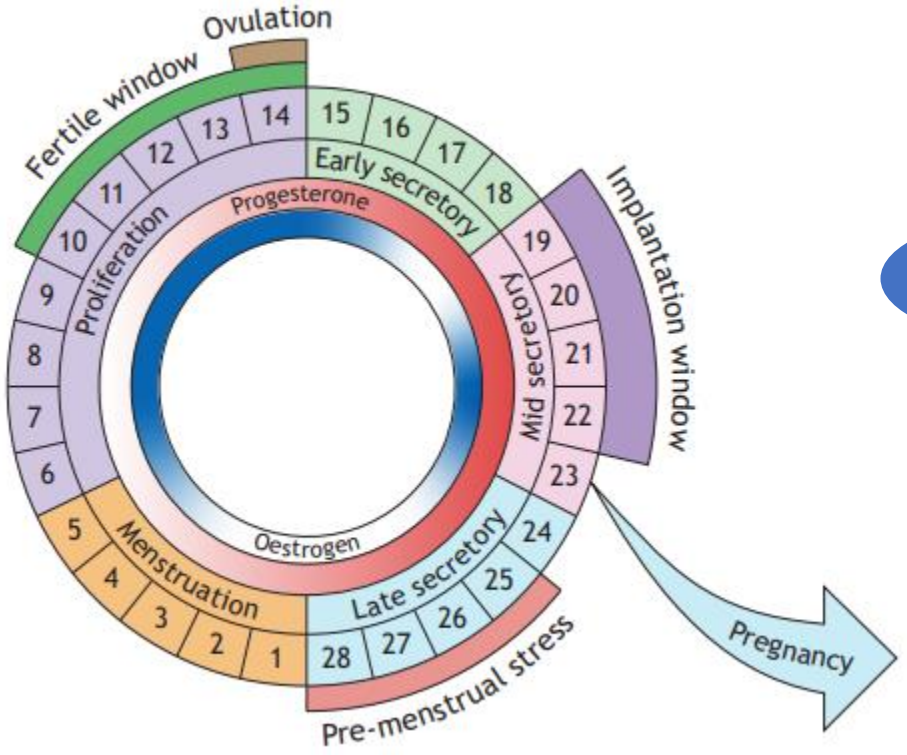
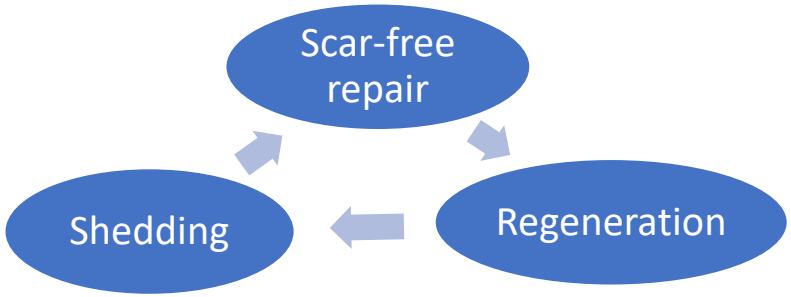
Uterine tissue remodeling

❖ Menstrual cycle

= cyclical endometrial tissue turnover and rejuvenation





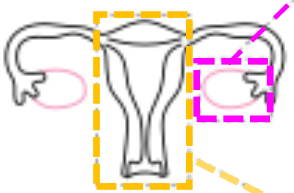
~28 days
~ 400 cycles per reproductive life
- day 1 = 1st day of menstruation bleeding

Mutter et al 2023

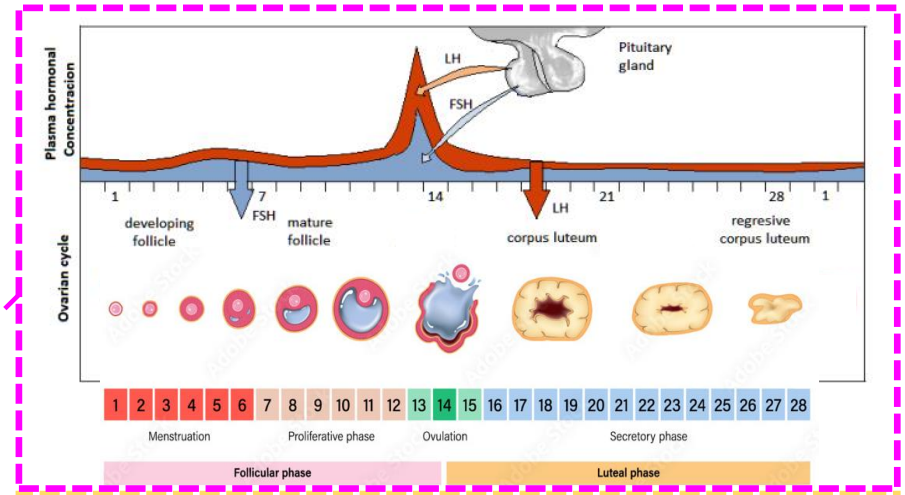
Endometrial remodelling

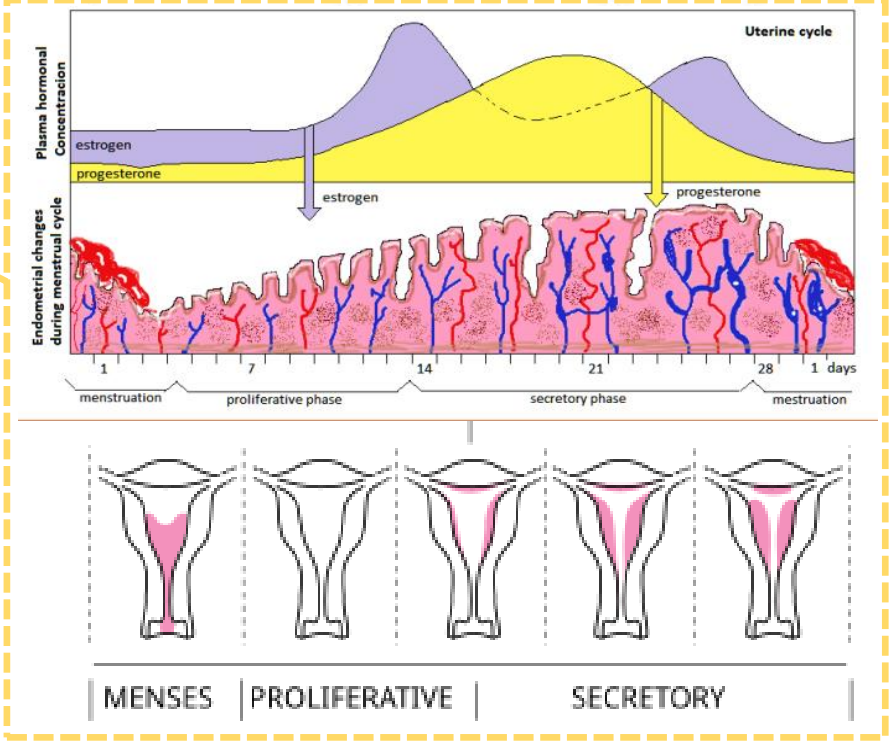
❖ Menstrual cycle

- coordinated with the **ovarian cycle** and estrogen (E2) and progesteron (P4) secretion



UTERINE CYCLE





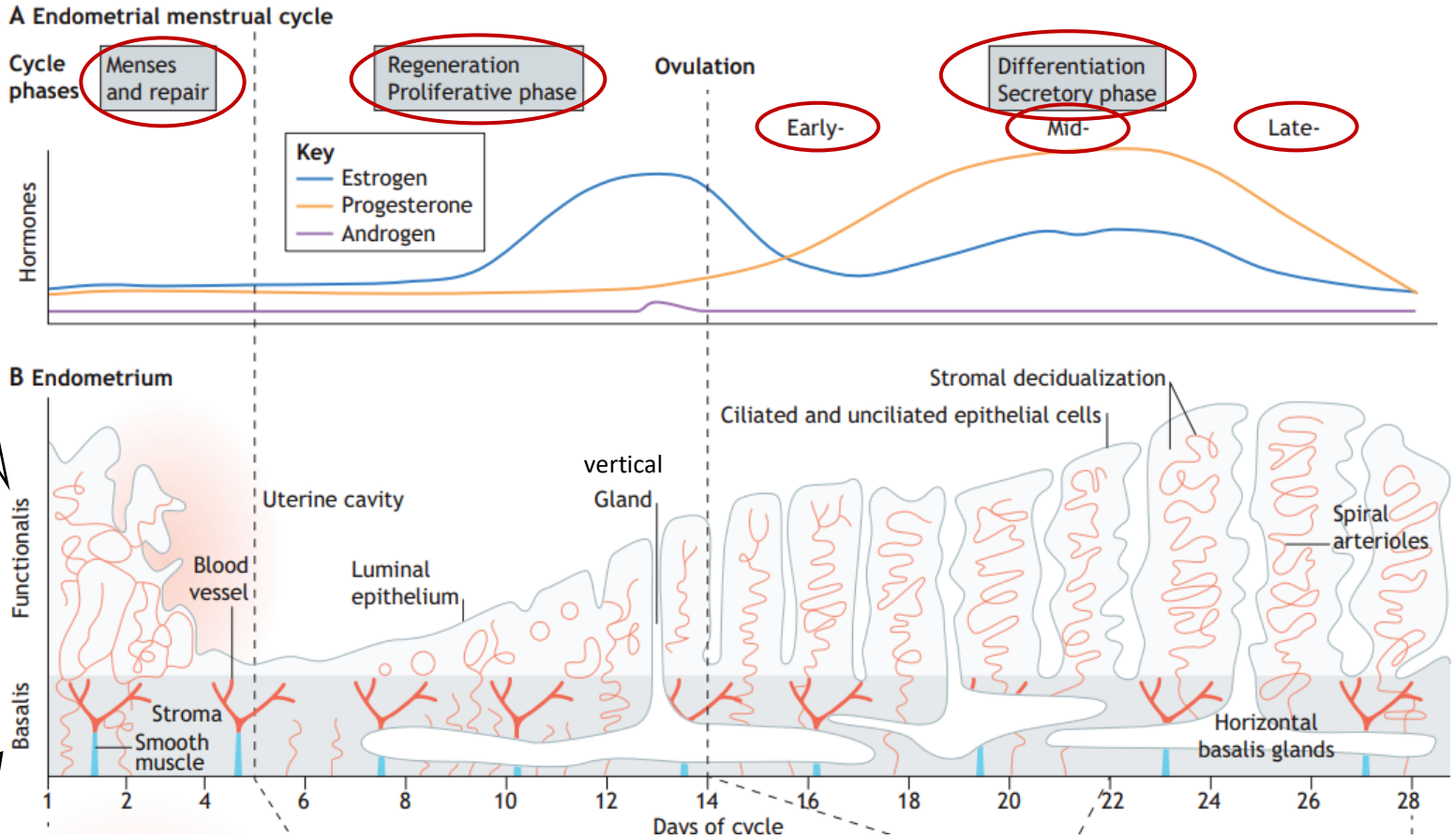
Cyclic endometrial changes

Functionalis

- hormone sentitive
- comprises luminal epithelium and vertical glands
- sheds during menstruation

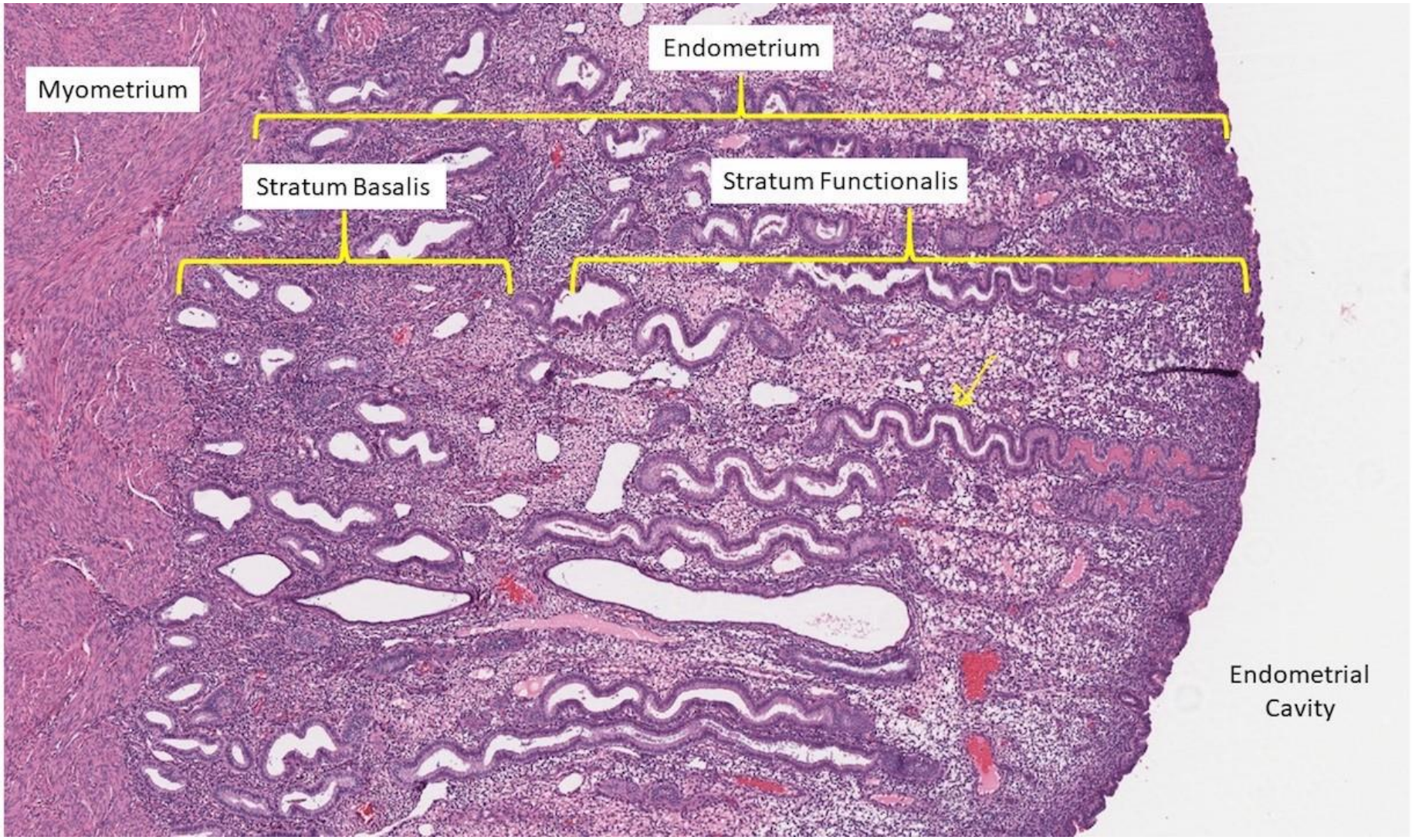
Basalis

- unresponsive to hormones
- comprises horzontal glands network, stromal cells, vasculature and stem cells
- intact during cycle



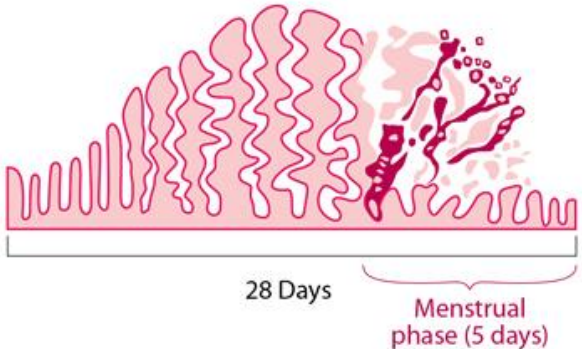
Salamonsen et al 2021

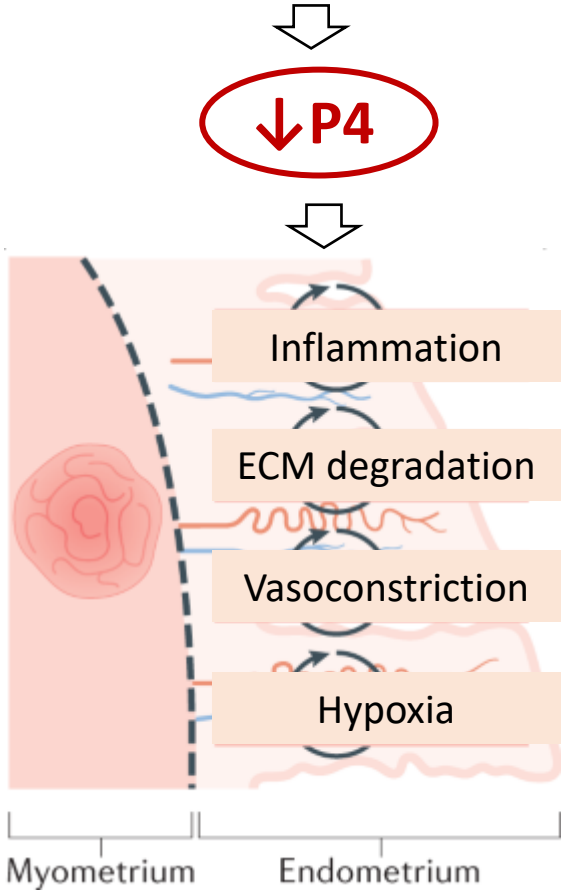
Cyclic endometrial changes

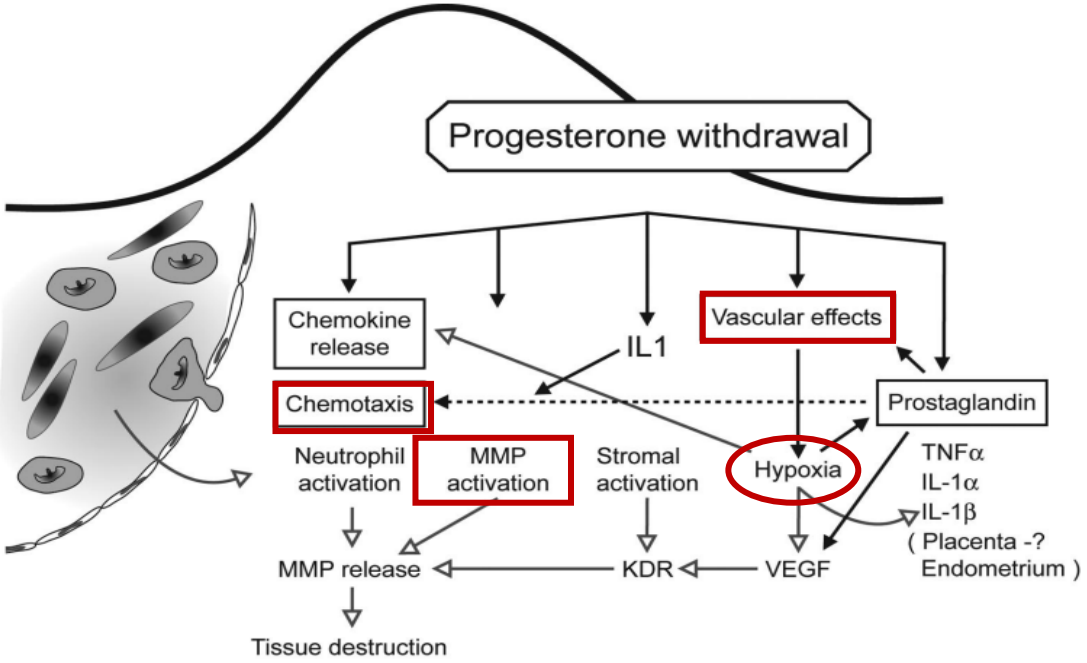


Menstrual phase

- tissue shedding, bleeding, and rapid **scar-free** repair (~48 hours) of zona functionalis of endometrium
- highly regulated inflammatory response to P4 withdrawal
- absence of anti-luteolytic signal hCG







Menstrual phase

❖ INFLAMMATORY RESPONSE

→ secretion of

- cytokines
- chemokines
- prostaglandin synthesizing enzymes

local
vasoconstriction

⇩

hypoxia

massive influx of
immune cells

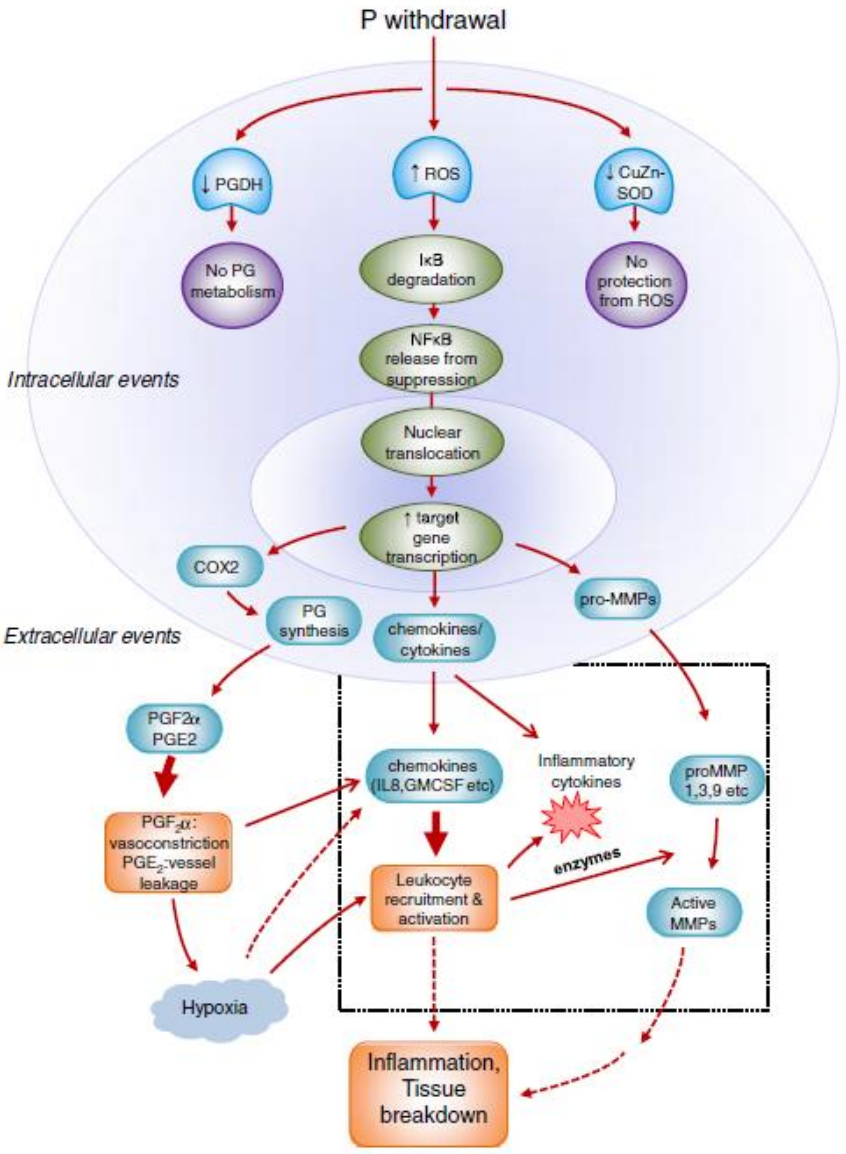
⇩

activation of MMPs

e.g. cathepsin, elastase

⇩

ECM degradation
and tissue breakdown



Evans and Salamonsen et al 2012

Menstrual phase

❖ HYPOXIA

- caused by local vasonstriction

- hypoxia-induced stabilization of HIF-1a physiologically drives endometrial repair after shedding

Maybin et al 2021

ARTICLE

DOI: 10.1038/s41467-017-02375-6 OPEN

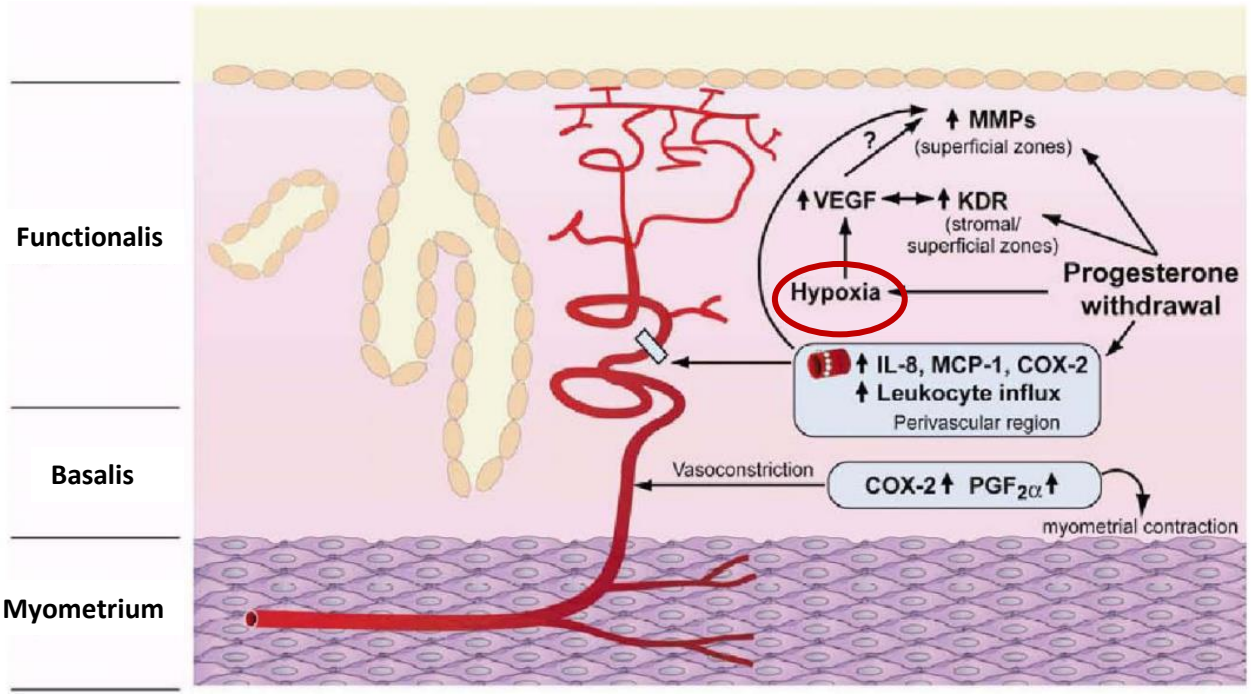
Hypoxia and hypoxia inducible factor-1α are required for normal endometrial repair during menstruation

Jacqueline A. Maybin[1], Alison A. Murray[1], Philippa T.K. Saunders[2], Nikhil Hirani[2], Peter Carmeliet[3] & Hilary O.D. Critchley[1]

Hilary Critchley

- women with heavy menstrual bleeding have decreased HIF-1α during menstruation

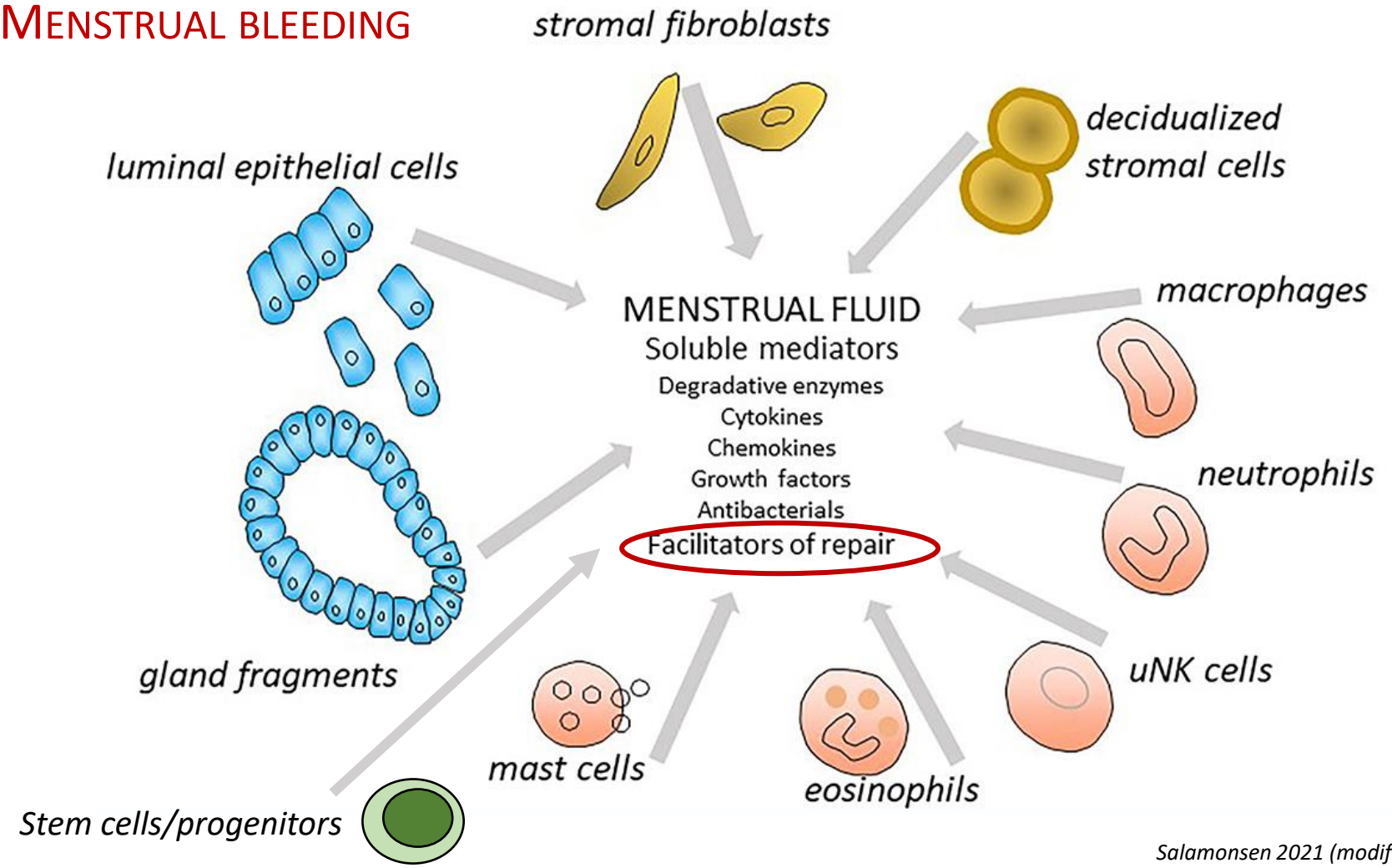
- genetic and pharmacological reduction of endometrial HIF-1α in mice causes prolonged menstrual bleeding



Critchley et al 2006

Menstrual phase

❖ MENSTRUAL BLEEDING



Salamonsen 2021 (modified)

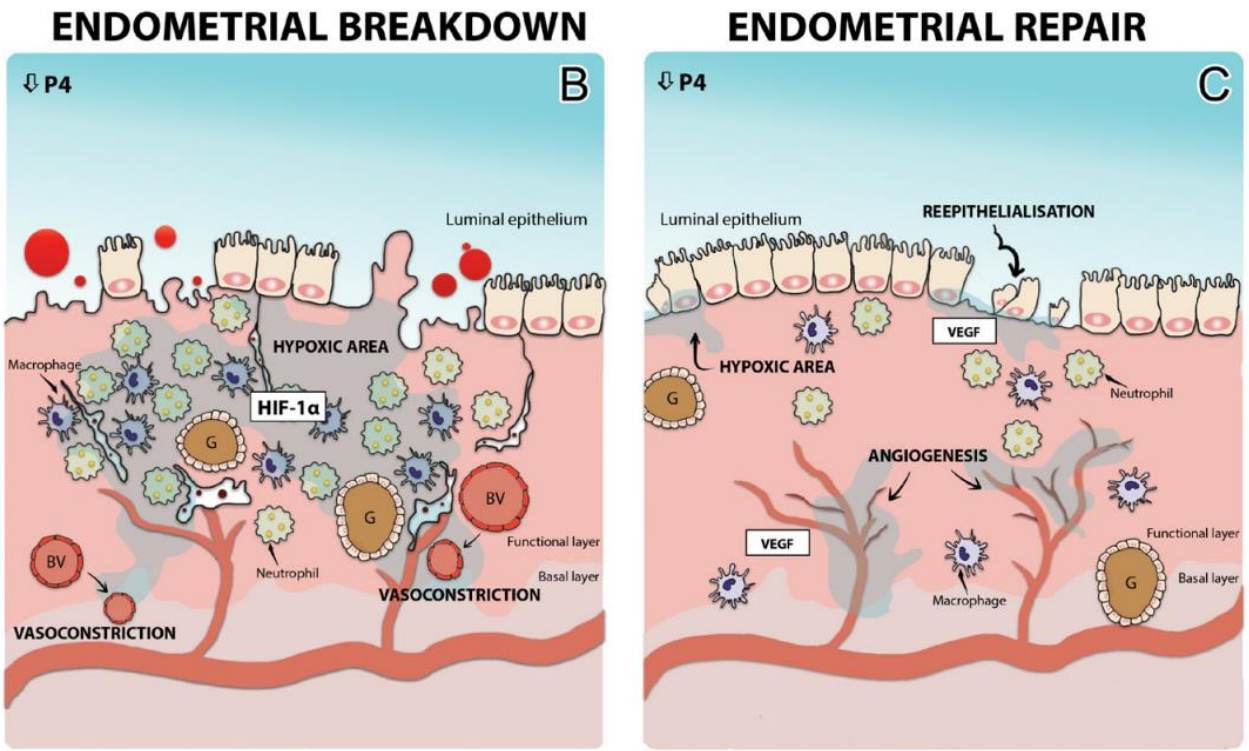
- menstrual fluid environment + preservation of *basalis* → **no scarring**

Menstrual phase

❖ ENDOMETRIAL REPAIR

- the restoration of an intact epithelial layer
- re-epithelialization of denuded areas occurs simultaneously with tissue breakdown and is completed within ~48 hours of initiation of shedding
- **no scarring** due to preservation of *basalis*

1) resurfacing of luminal epithelium
2) angiogenesis in sub-epithelial stroma
3) repair of damaged transverse arteries



Martinez Aguilar et al 2020

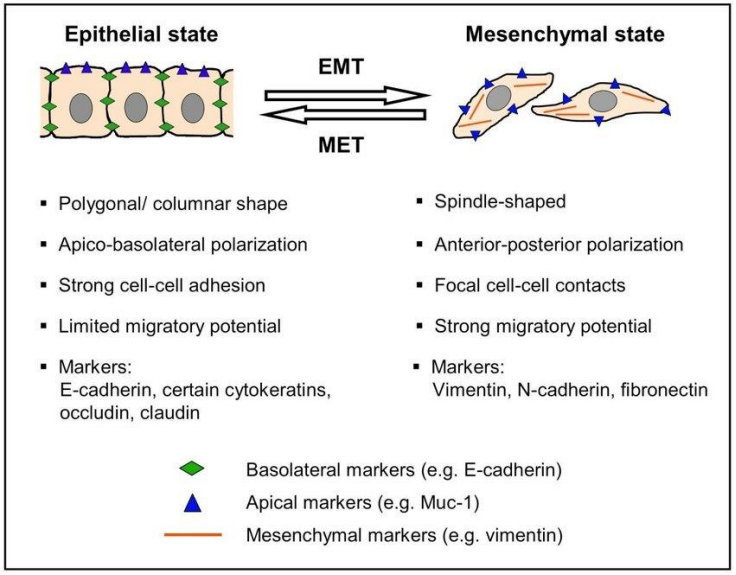
Menstrual phase

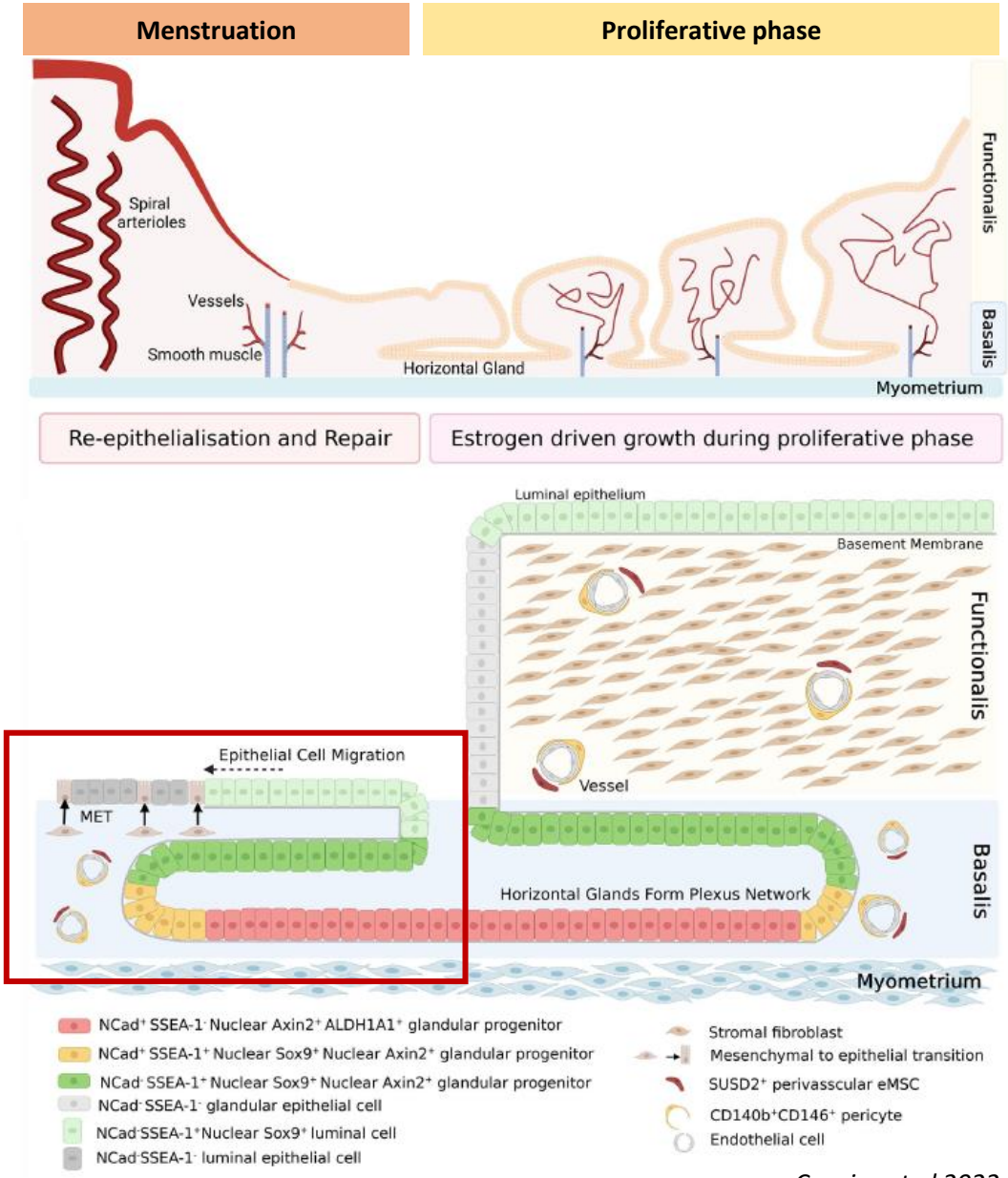
❖ ENDOMETRIAL REPAIR

Luminal epithelium resurfacing

← migration of epithelial cell progenitors from exposed horizontal endometrial glands in *basalis*

←stomal cell transformation to luminal epithelium = **MESENCHYMAL-TO-EPITHELIAL TRANSITION (MET)**

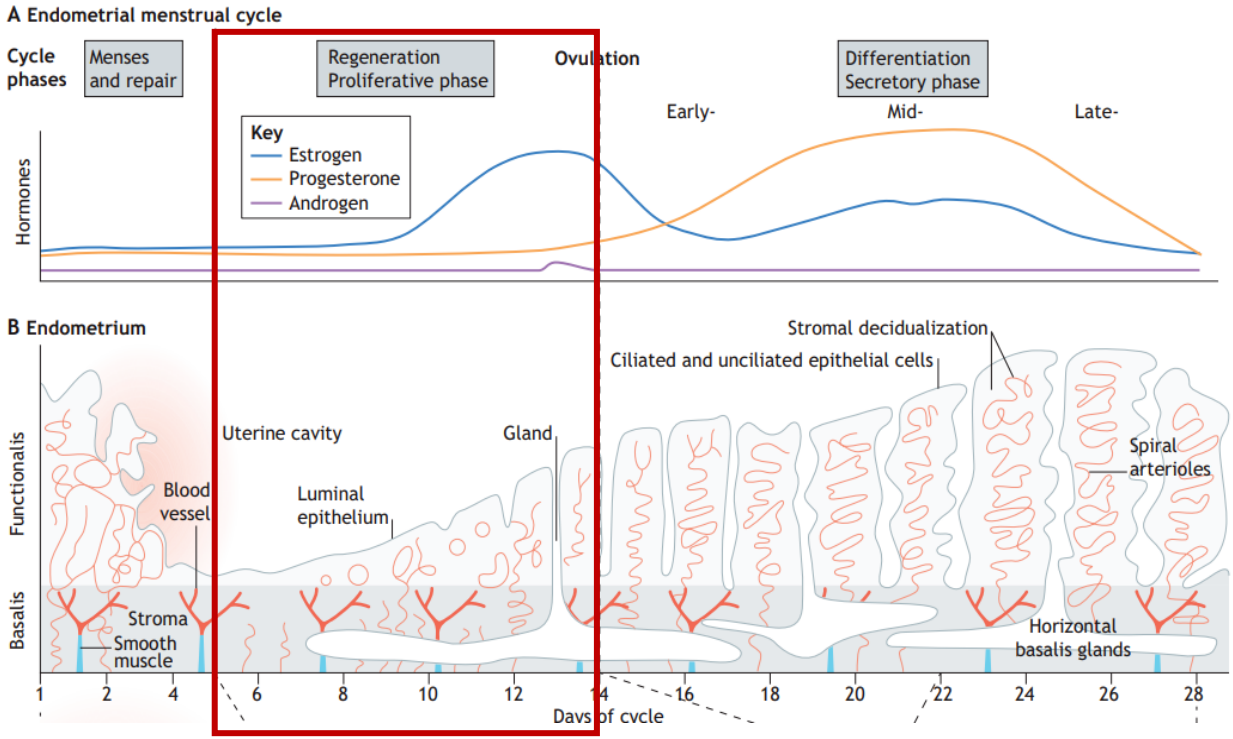




Cousins et al 2022

Proliferative phase

- post-menopausal period (starts ~ day 4, last ~ 10 days)
- increase of endometrial linning thickness (from ~ 0.5 mm to ~ 7-8 mm)
- rapid regrow and regeneration of functional layer due to massive cellular proliferation
- activation of growth factor signaling pathways, high vascular perfusion, and transient tissue edema
- positional proliferation, cell specification, and angiogenesis
- can occur only once the epithelial surface is covered



Salamonsen et al 2021

Proliferative phase

- **E2-dependent**
  - E2-receptor present in epithelial and stromal part)

  + role of androgens

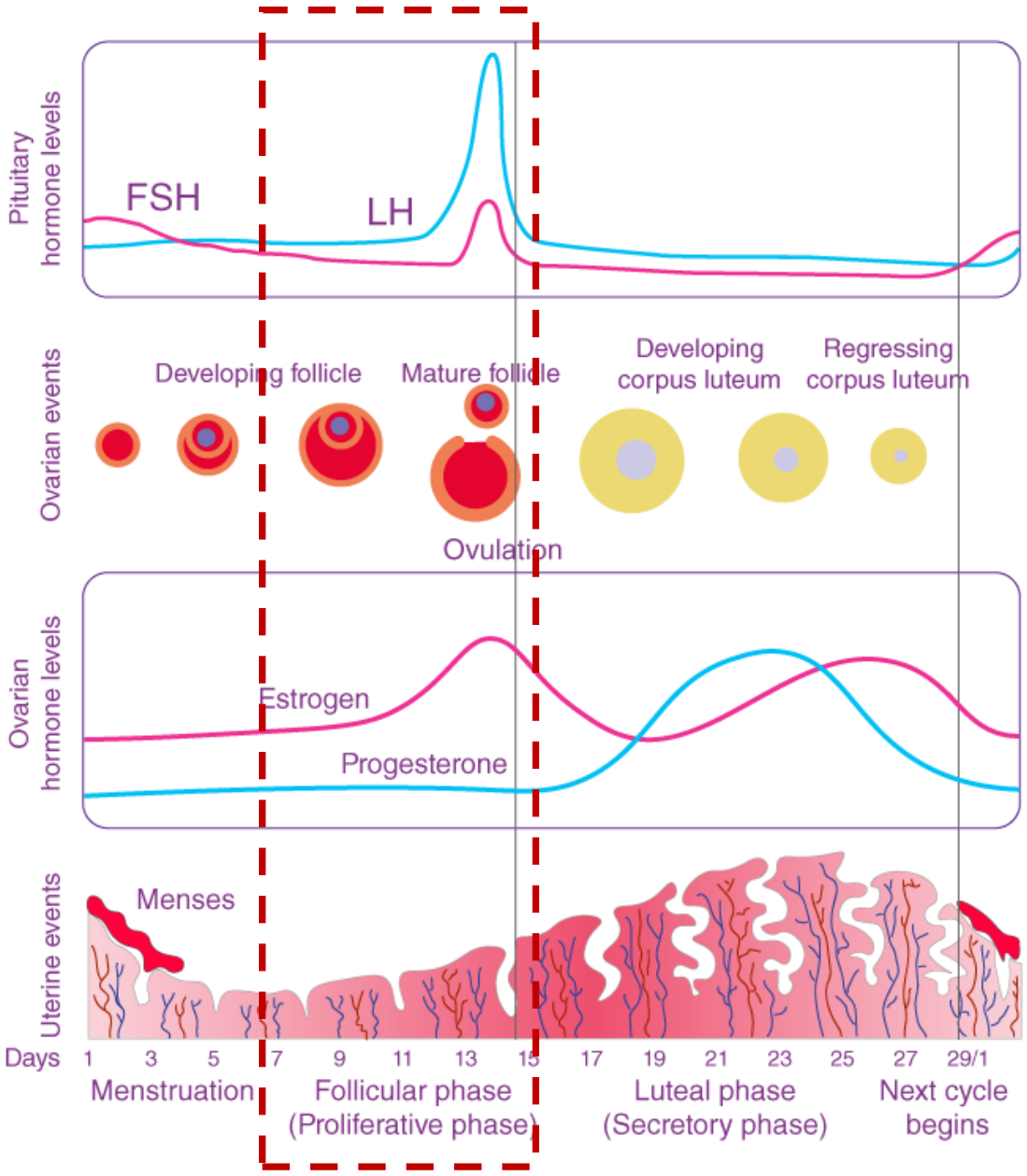
  (initiate gland reformation)

- E2 induces cell proliferation and expression of P4 receptor

  → endometrium

  thickening and priming

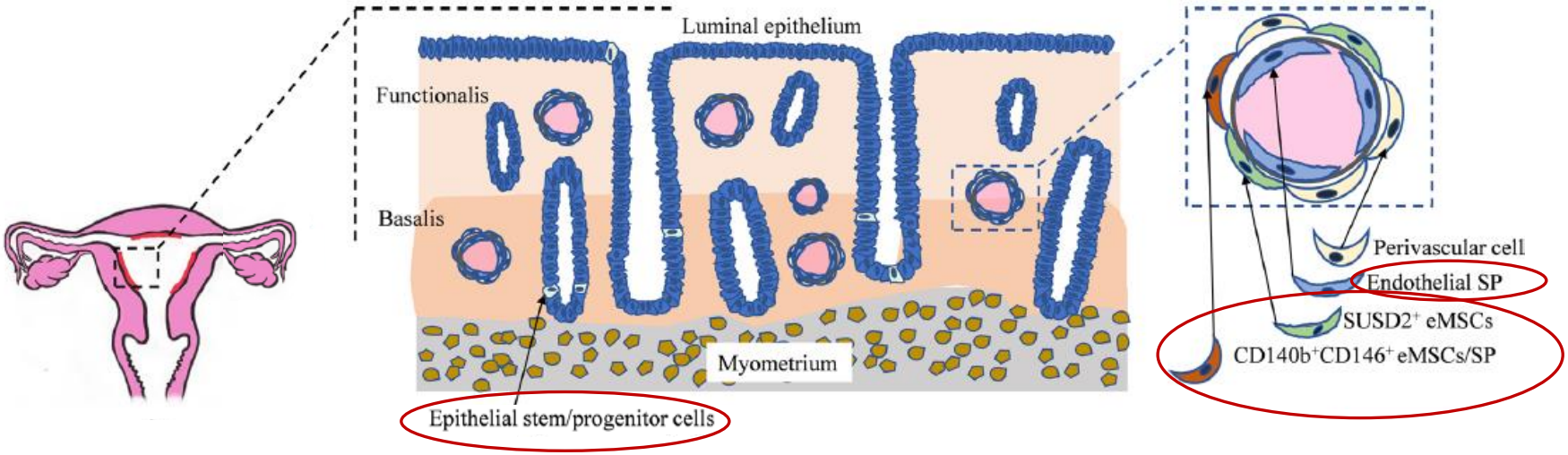
  for the structural changes that will undergo during the secretory phase



Proliferative phase

❖ UTERINE STEM CELLS

- rare clonogenic population with high proliferative potential
- capable of self-renewal and differentiation to one or more lineages of specialized tissue cells
- reside predominantly in *basalis* endometrial layer (epithelial + stromal compartment)
  - Epithelial progenitors → glandular epithelium
  - Endometrial mesenchymal stem cells (eMSCs) → stromal and endothelial cells
  - Side population (SP) cells → epithelial, stromal, and endothelial cells



Proliferative phase

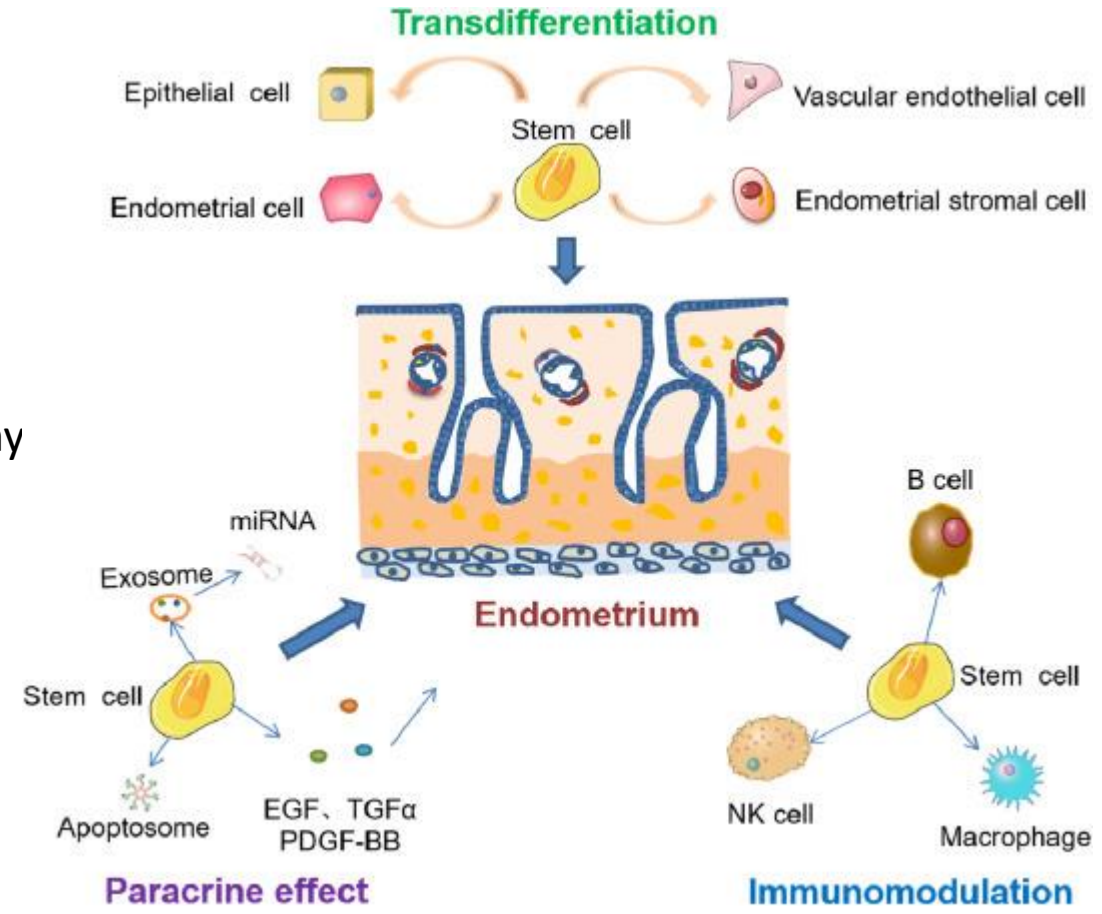
❖ UTERINE STEM CELLS

- essential for endometrial, stromal and vascular regeneration following menstruation and parturition

- distinct populations giving rise to different endometrial cell types, multizonal differentiation hierarchy

- repopulate *functionalis* and generate proangiogenic and paracrine factors promoting angiogenesis and immunosuppression

- dysregulated function leads to cancer

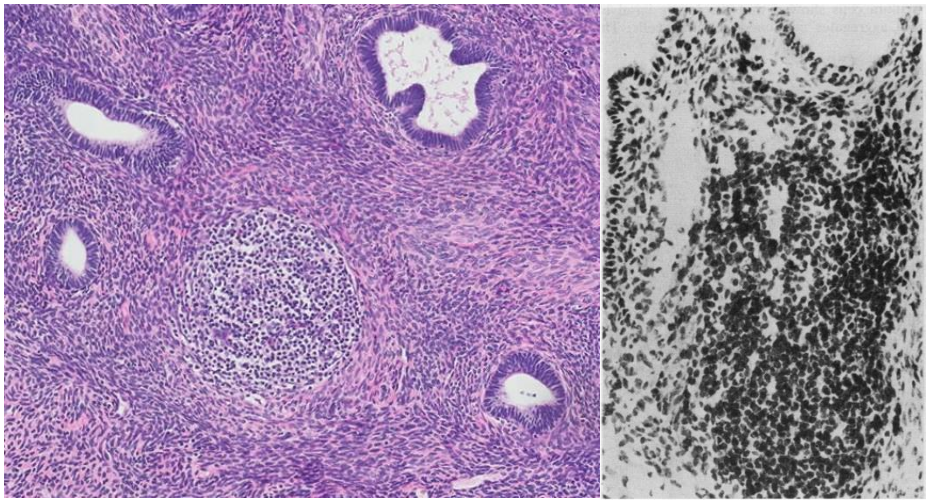


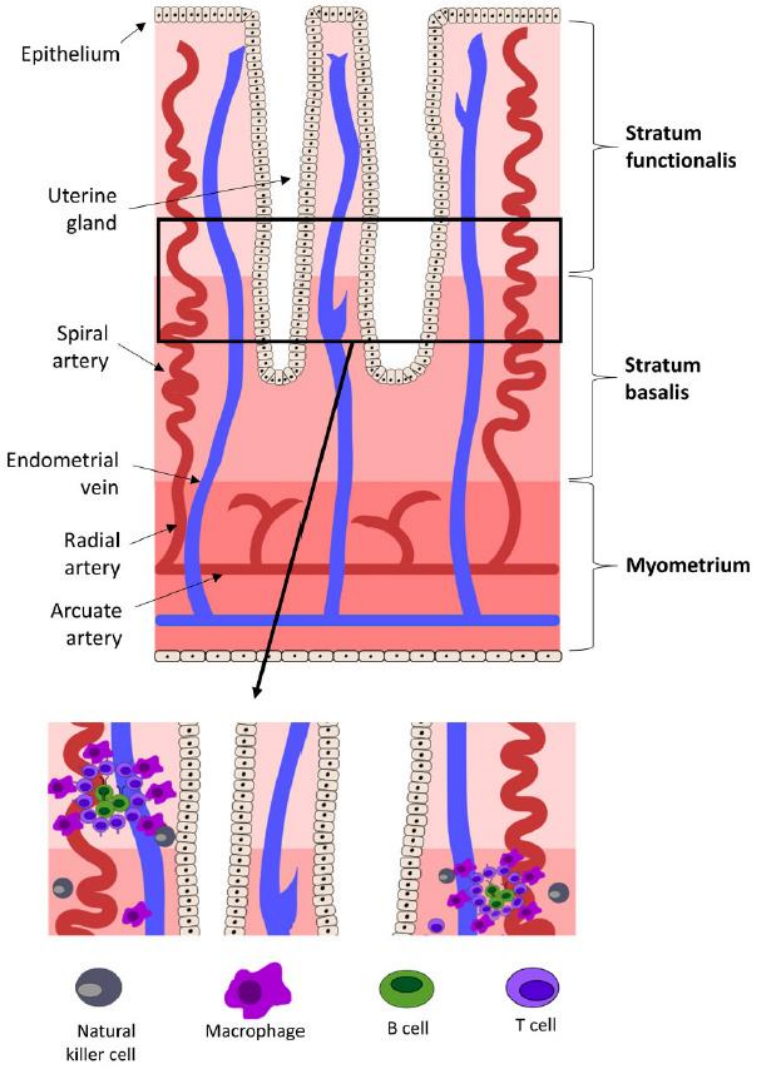
Chen et al. 2022

Proliferative phase

❖ LYMPHOID AGGREGATES

- reside in the basal endometrial layer
- clumps of several hundreds of immune cells
- core of B-cells surrounded by a circle of T-cells and a halo of macrophages
- established in each cycle by the recruitment of circulating immune cells
- regulate spatial responsiveness of endometrial tissue to ovarian hormones





Shen et al 2021

Proliferative phase

- cellular specification and tissue patterning in endometrial tissue

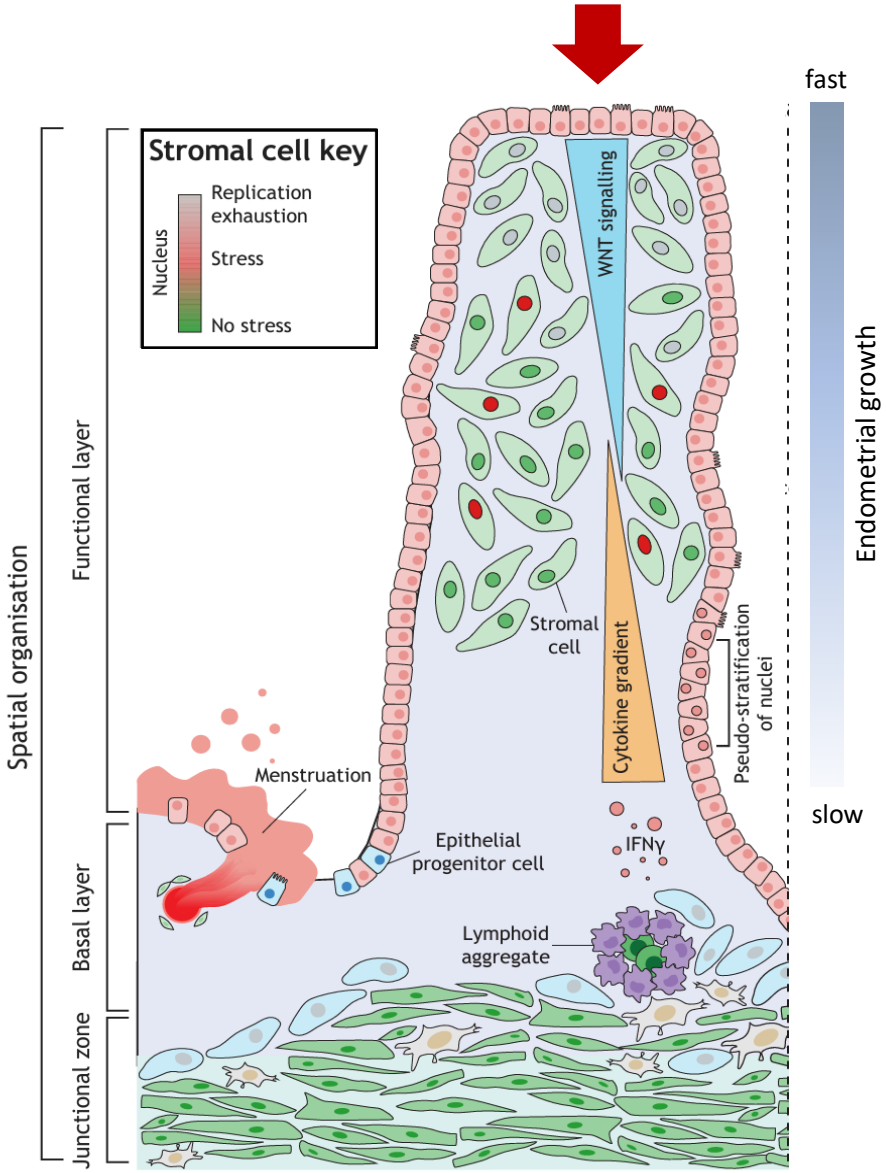
Cytokine and morphogen gradient:

- **INFγ** secreted by activated T-cells residing in lymphoid aggregates is a potent inhibitor of estrogen and P4 signaling and cellular proliferation
- **WNT signaling** (WNT7A) expressed predominantly in luminal epithelium promotes ciliogenesis in response to estrogen

→ cyclic tissue remodeling restricted to superficial layer

→ spatial patterning for P4 action in the secretory phase

- elevated replication stress and senescence markers in epithelial and stromal cells are associated with pathologically thin endometrium, E2 resistance and implantation failure



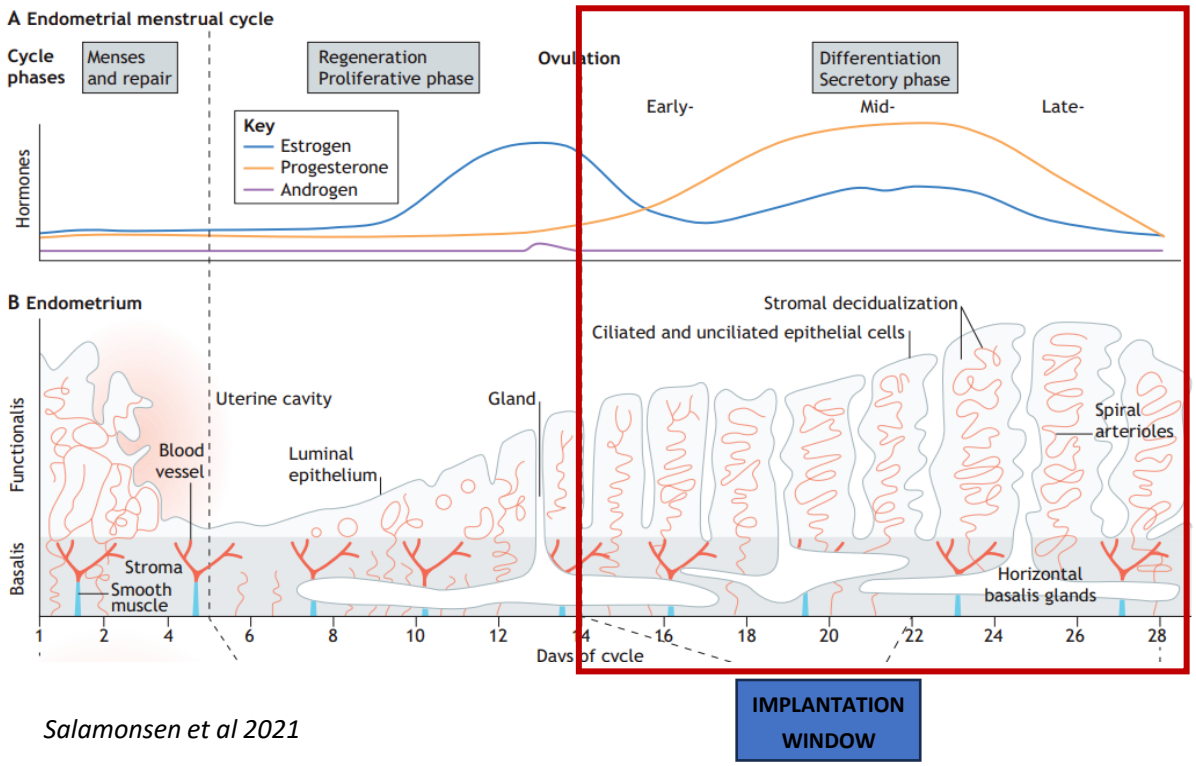
Muter et al 2023

Secretory phase

- post-ovulation stage (~ day 14-day 28)
- Ovulation → rapid drop of estrogen production (↓E2)
  → secretion of progesteron by corpus luteum (↑P4), peaks in mid secretory phase (+7-8 days)

Features:

- ↓ endometrial cell proliferation
- ↑ adhesive capacity of epithelial cells
- ↑ glandular secretion
- differentiation of stromal cells
- development of spiral arteries
- stromal edema
- stem cell recruitment
- influx of immune cells

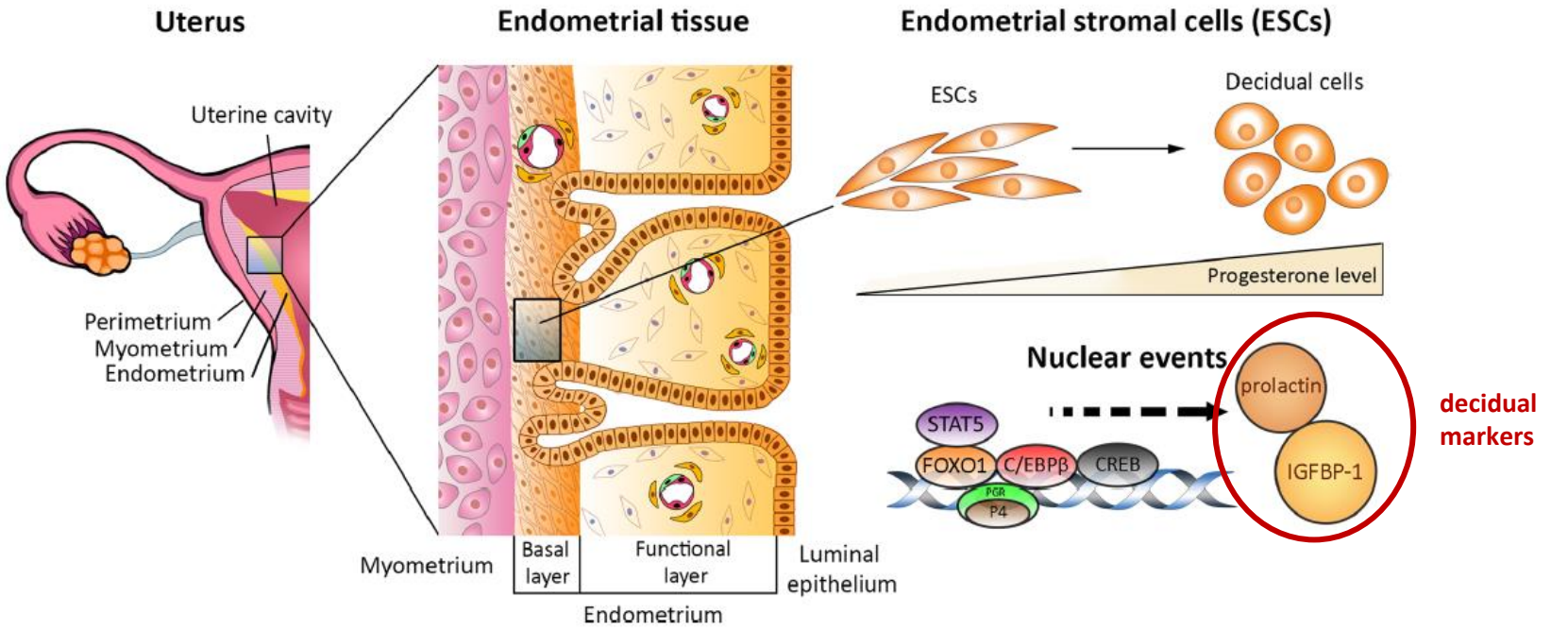


Salamonsen et al 2021

Secretory phase

❖ DECIDUALIZATION (= DECIDUAL REACTION)

- **profound mophological and functional tranformation of endometrial stromal cells**
- P4-dependent process acting on E2-primed cells
- spontaneous in humans
- regulates tropholast invasion during implantation → essential for establishing pregnancy
- *decidua (lat. „deciduus“) = maternal uterine tissue, shed off during parturition and in non-conceptous cycle*



Deryabin et al 2020

Secretory phase

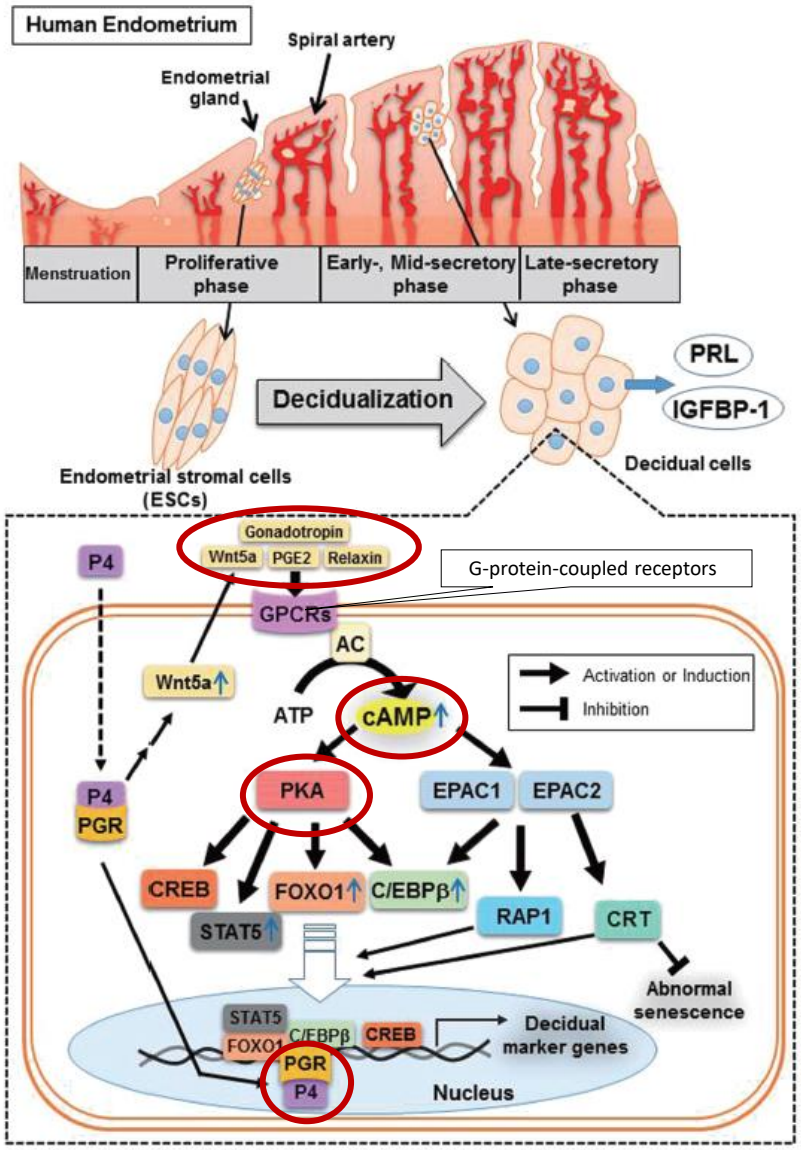
❖ DECIDUALIZATION

Trigger mechanism:

P4 activation of its nuclear receptor (PGR) is critical for maintaining decidualization process but insufficient for initiation of differentiation process

Essential role of cAMP signalling

- cAMP analogs, activators of adenylate cyclase (AC) and PDE inhibitors are potent inductors of decidualization *in vitro*
- pharmaceutical modulation of cAMP signallng pathway can affect implantation efficiency



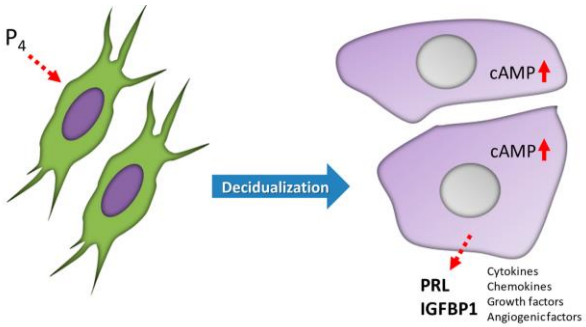
Jan Brosens

Yoshie et al 2015

Secretory phase

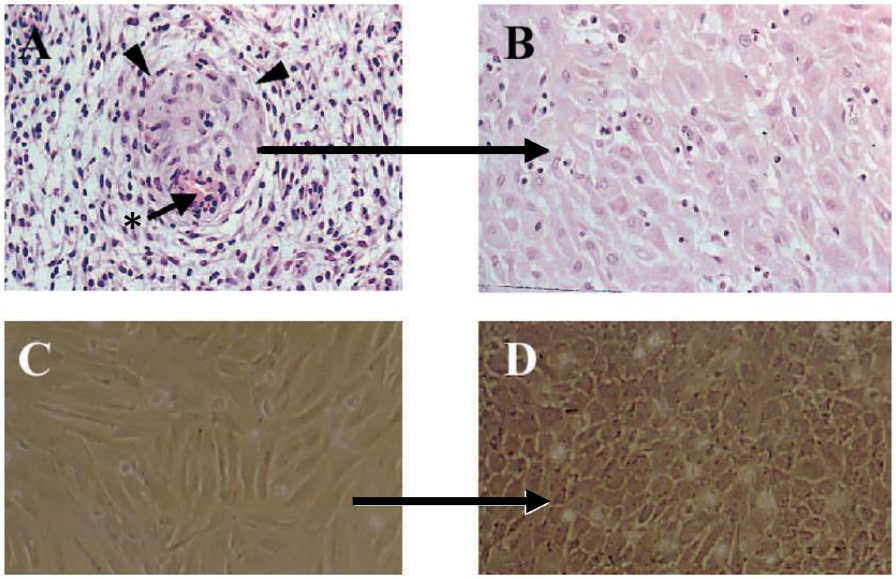
❖ DECIDUALIZATION

- morphological change:



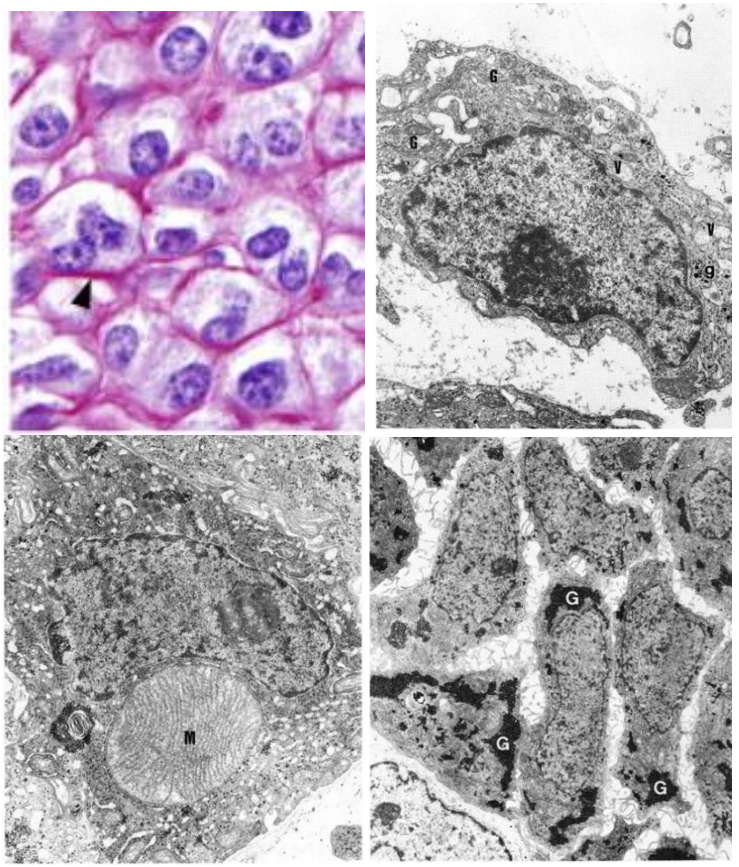
fibroblastic-phenotype
Spindle-shaped cells

large rounded cells with large nucleii and abundant cytoplasm



* deciadualization starts around spiral arteries

Gellersen and Brosens 2003



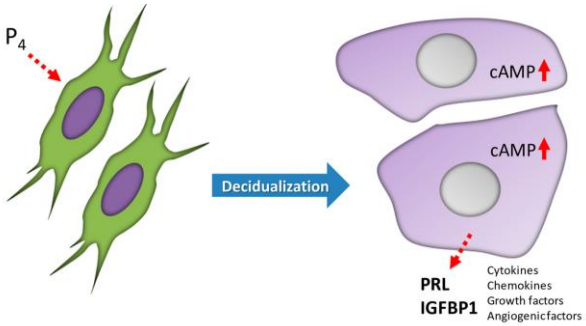
Cornillie, et al 1985

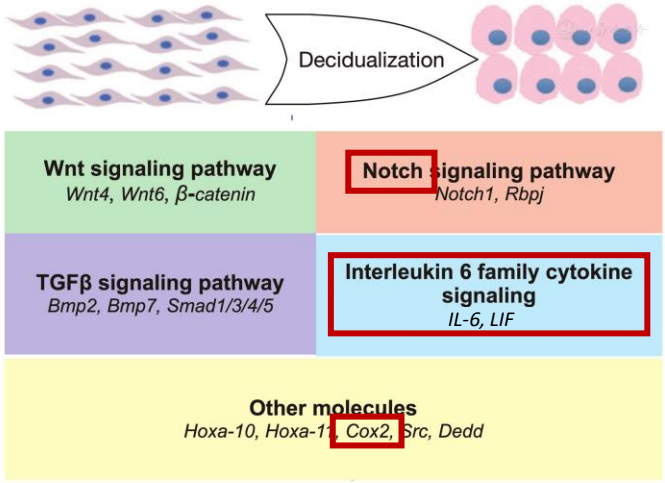
- accumulation of glycogen, lipids and glycoproteins
- the presence of giant mitochondria, prominent rER and GA, and dilated sER cisternae
- tendency to polyploidisation
- connection by gap junctions
- ↓ tissue roughness and stiffness

Secretory phase

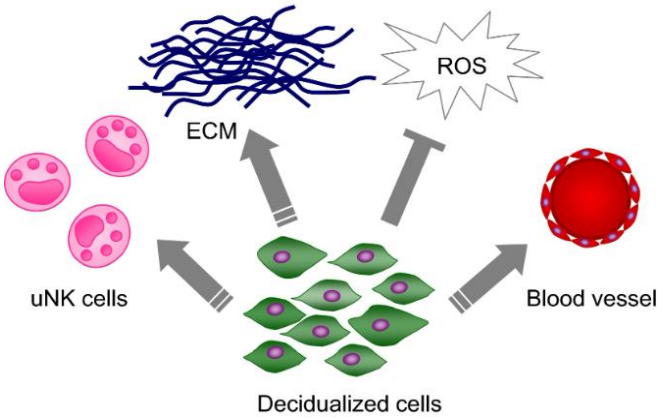
❖ DECIDUALIZATION

- functional change:





- decidualized cells express a variety of cytokines, chemokines, growth factors, and angiogenic factors
- altered expression of steroid hormone receptors
- metabolic changes
  (e.g. P4-induced downregulation of DIO2 critical for ↓T4-to-T3 conversion, silencing of stress- activated signaling and increasing ROS scavenging activity)
- upregulation of ion channels and protein transporters
  (→ increased absorption of uterine fluid facilitating embryo-endometrial interaction during implantation, increased vascular permeability)
- secretion and remodeling of extracellular matrix
  (→ deposition of hyaluronan → water perfusion → stoma edema and reduced stiffness)
- release of proinflamatory regulators
  (particularly in decidual-like senescent cells damaged by replication stress in the proliferation phase)
- accumulation of uterine NK cells (uNK)

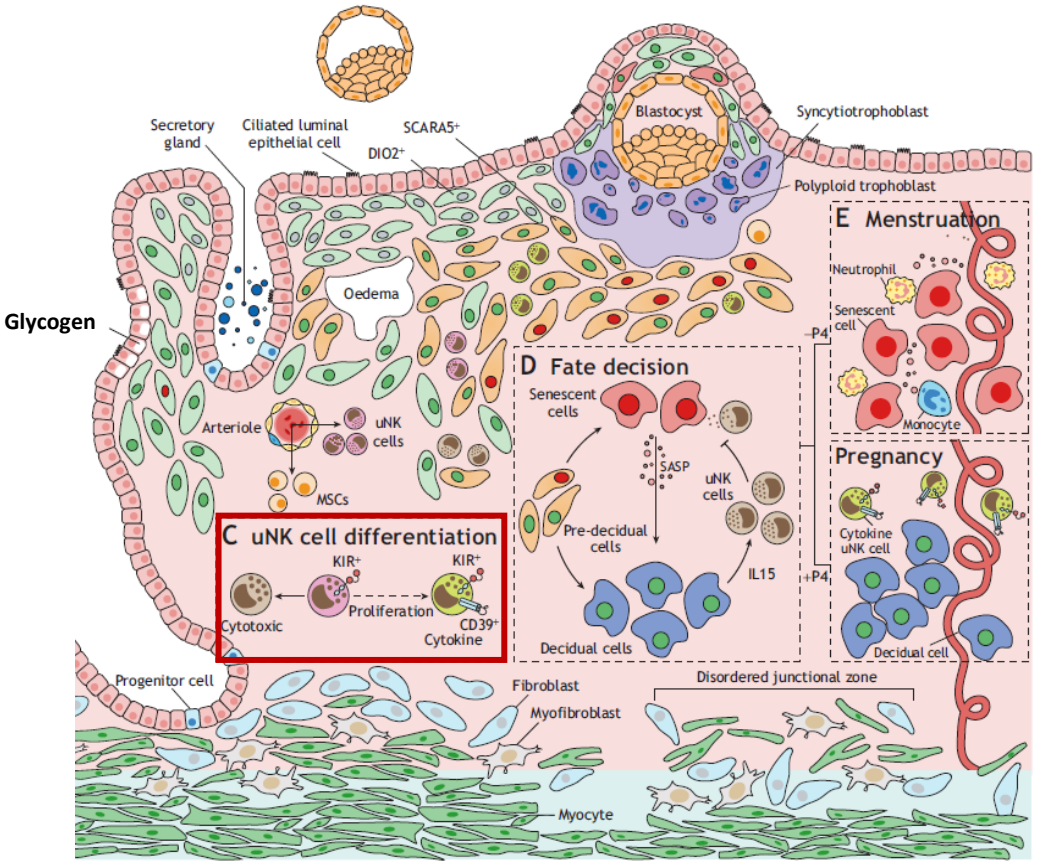


Secretory phase

❖ DECIDUALIZATION

- **Uterine Natural Killer cells (uNK)**

- subset of immune cells abundant in secretory endometrium and decidua of pregnancy
- tissue-specific characteristics
- derived from circulating NK cells
- differentiation in response to local clues
- affect uterine spiral remodeling and immunological tolerance
- alteration in uNK number/function causes infertility, miscarriage, or pregnancy complications

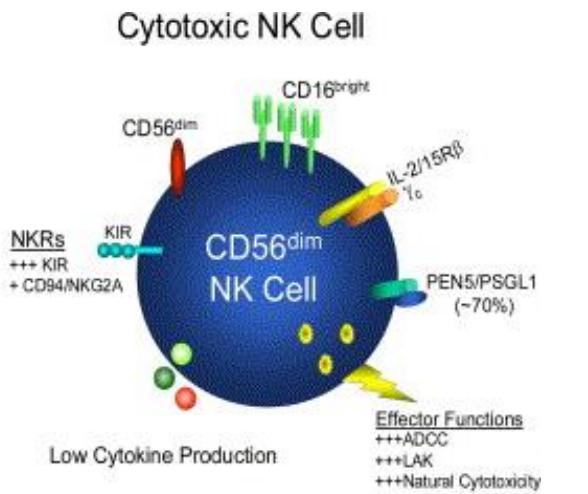


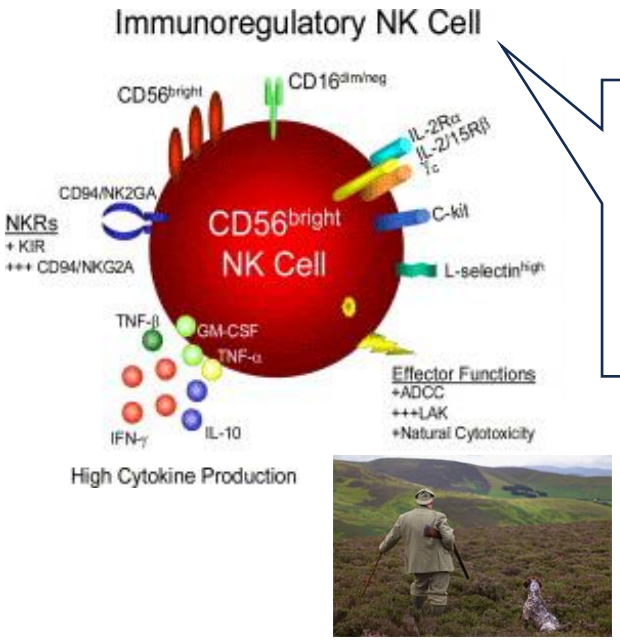
Muter et al 2023

Secretory phase

❖ DECIDUALIZATION

- **Uterine Natural Killer cells (uNK)**
  - switch from pro-inflammatory phenotype to immunomodulatory, cytokine-producing, and angiogenic phenotype





KIRs+ (killer cells immunoglunoglobulin-like receptors)
CD56^{bright} CD16^{-}
CD39^{+} CD9^{+} CD49a^{+} CD57^{-}
Ki67 nuclear proliferation marker

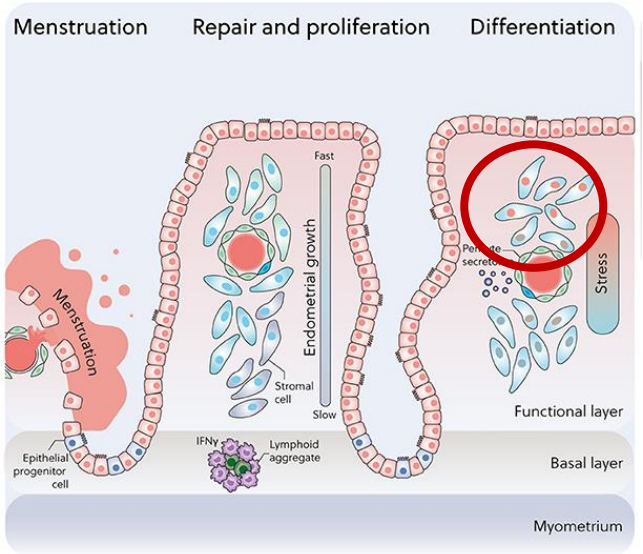
Farag and Caligiuri 2006

- maintain endometrial homeostasis by selectively eliminating senescent decidual cells
- the activity of uNKs is affected the quality of implanting embryo

Menstruation versus pregnancy

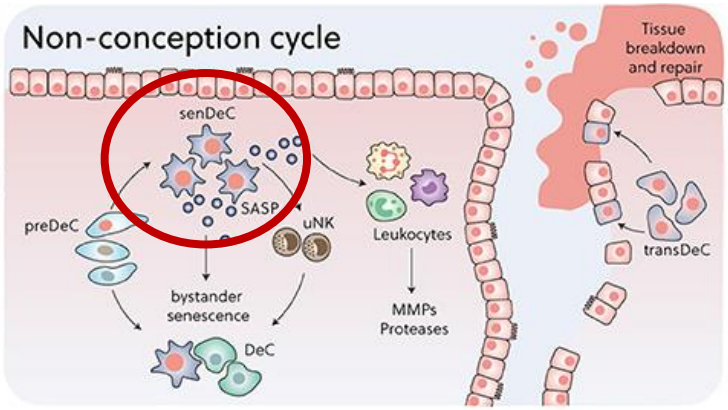
Senescent decidual cells

- damaged by replication stress during the proliferation phase
- fail to differentiate into decidual cells during the secretory phase
- insensitive to progesterone
- produce complex secretome



Muter et al 2021

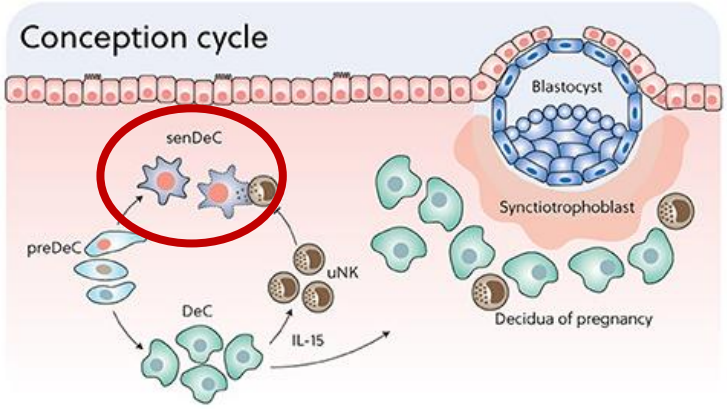
↓P4



- paracrine induction of senescence in neighbouring cells → sterile inflammation and ECM breakdown

Endometrium breaks down → menstruation

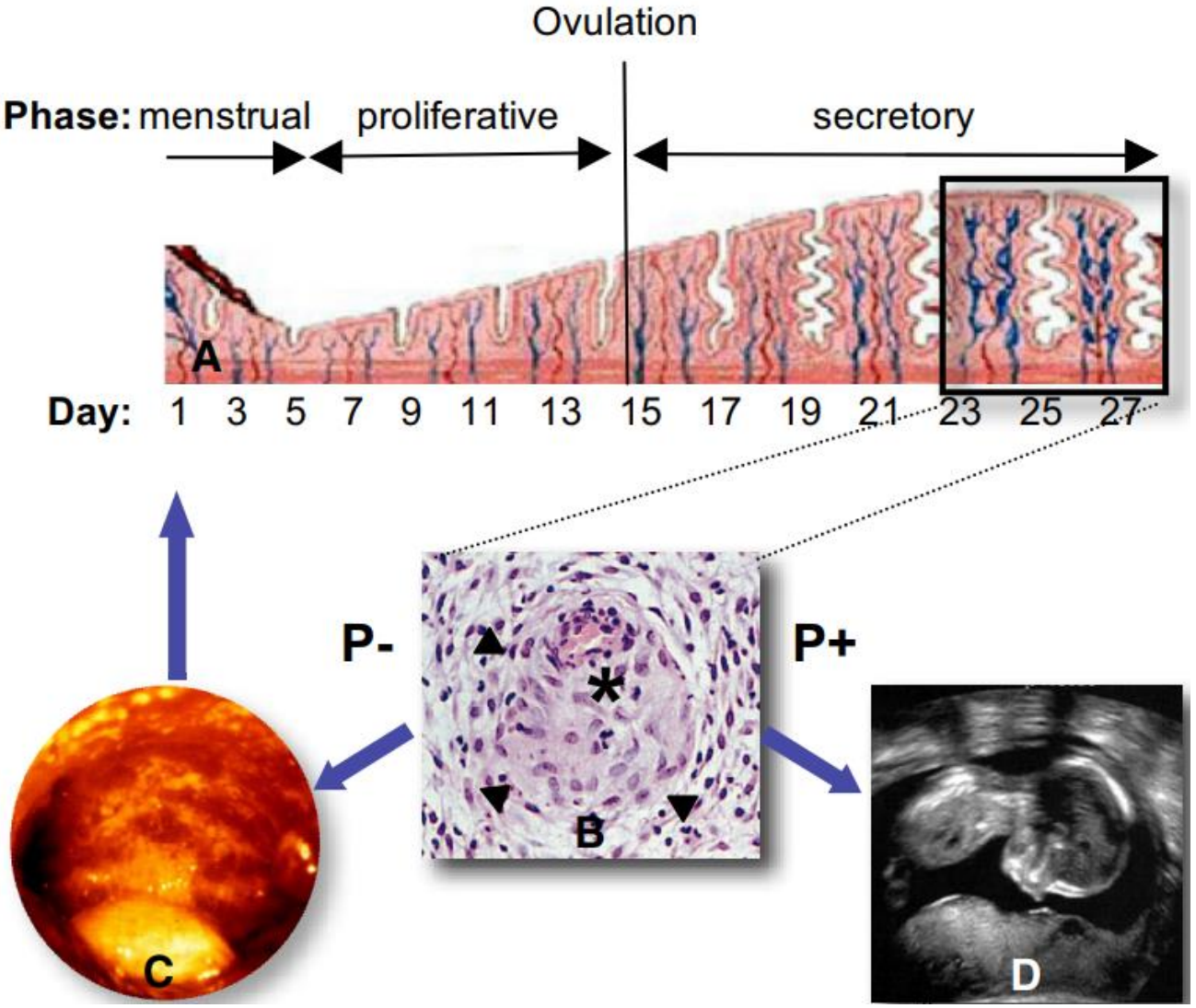
↑P4



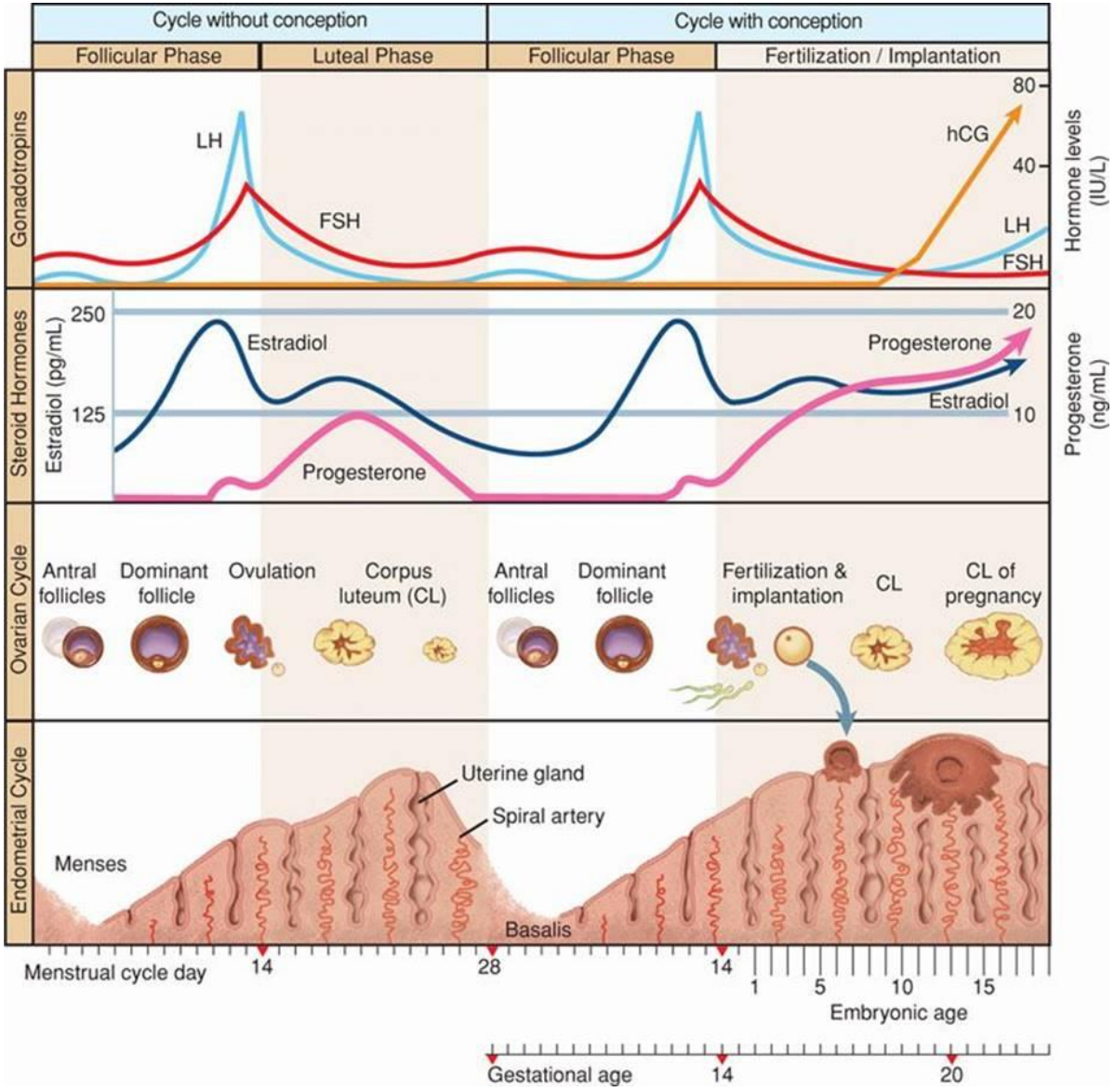
- P4 sensitive decidual cells engage uNK to eliminate senescent cells

Endometrium → decidua bed of pregnancy

Menstruation versus pregnancy



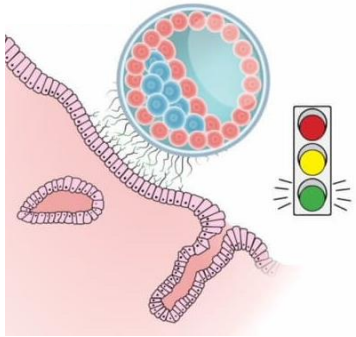
Menstruation versus pregnancy

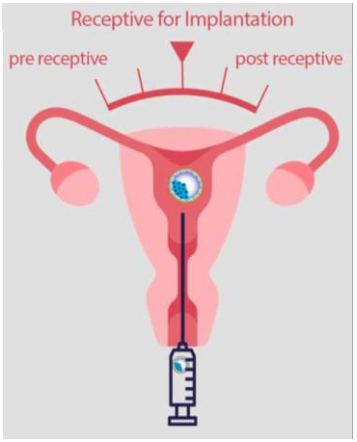


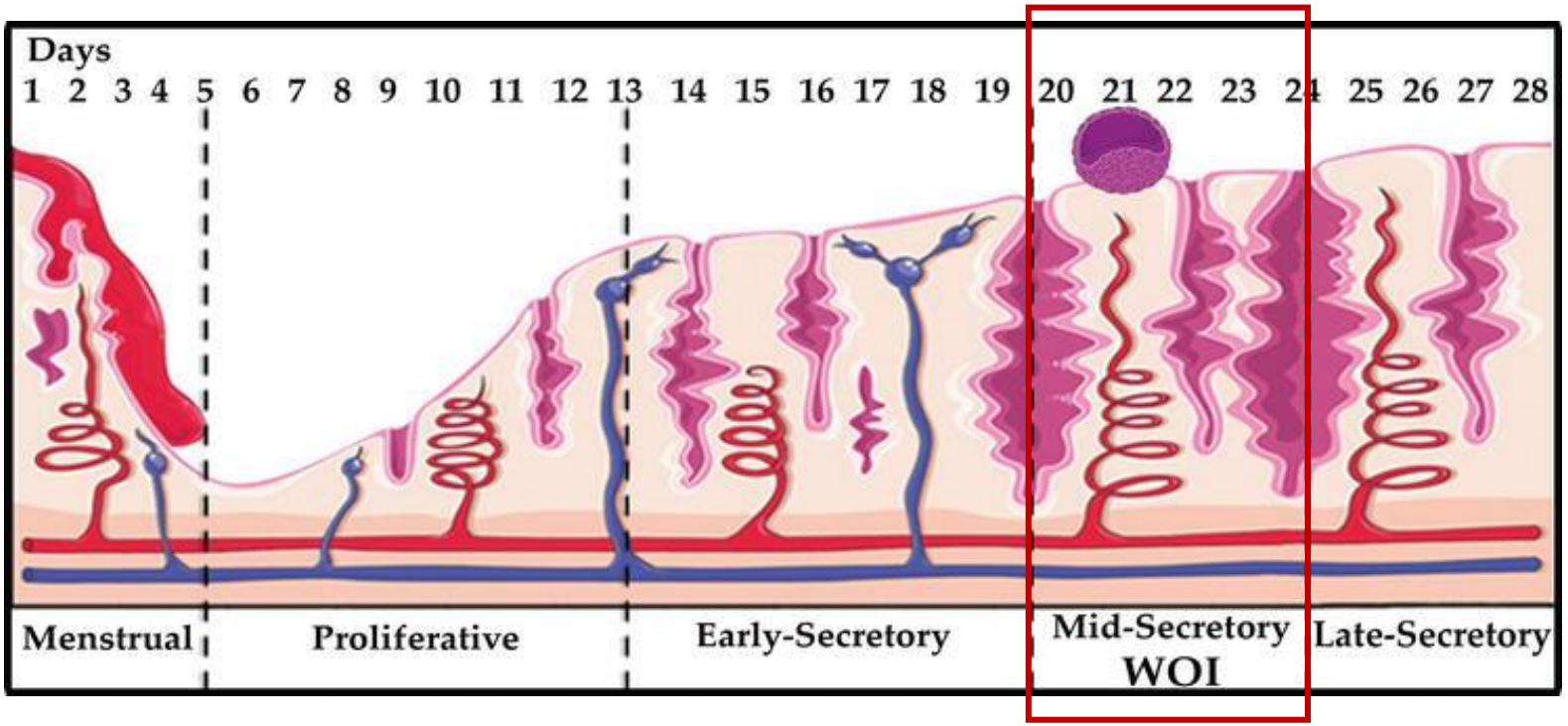
Endometrial receptivity

- uterine lining preparation for an embryo implantation
- **WINDOW OF IMPLANTATIN (WOI)**

  = limited time interval (3-6 days, ~day 20-24 of the cycle) during the mid-secretory phase, when the endometrium is ready to receive an embryo
  - optimal timing for IVF embryo transfer



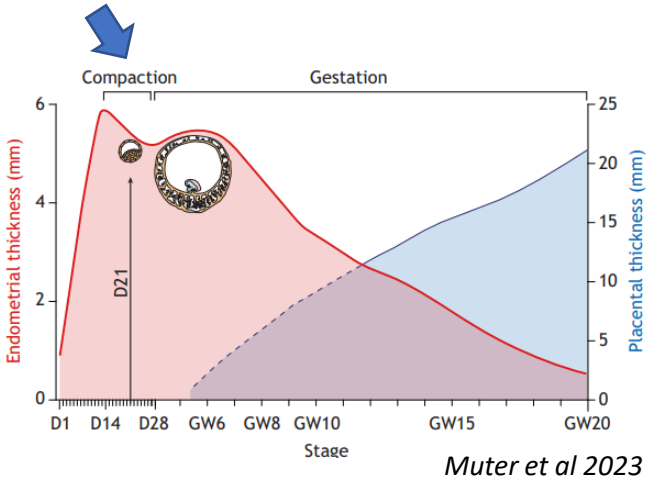




Endometrial receptivity

✓COMPACTION OF ENDOMETRIUM

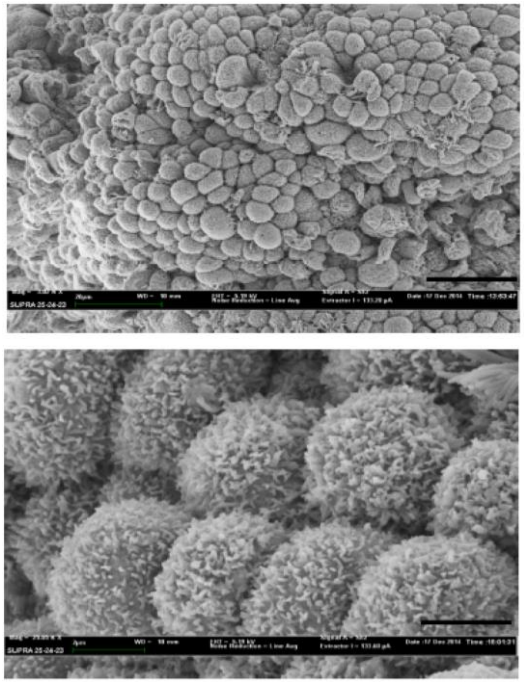
- post-ovulatory decrease in endometrium thickness
  (during luteal phase increases density of endometrium but not its volume)
- detectable by ultrasound
- indicative of P4 responsiveness and degree of decidualization



Muter et al 2023

✓PINOPODES

- surface protrusions facilitating embryo implantation
- appear on apical side of luminal epithelial cells in the mid secretory phase
  (~day 20-21, but ~5 day inter-individual timing variation!)
- their development is associated with level of P4, expression of implantation promoting factors
  (L-selectin ligand, LIF, αVβ3 integrin)

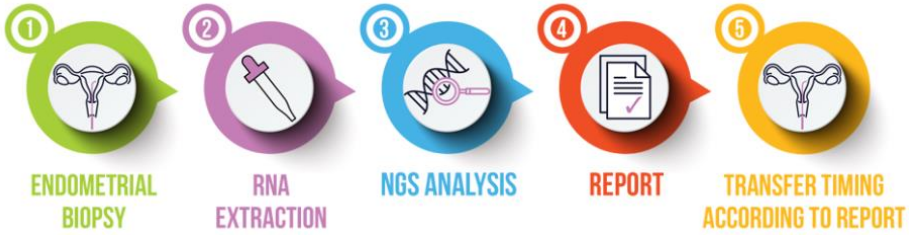


Luddi et al 2020

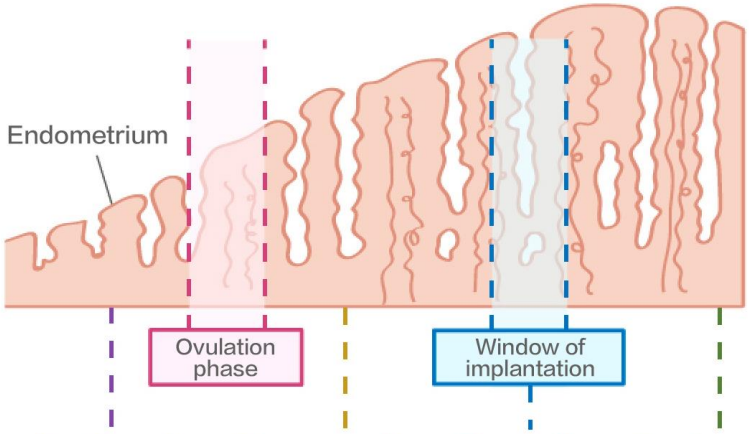
Endometrial receptivity

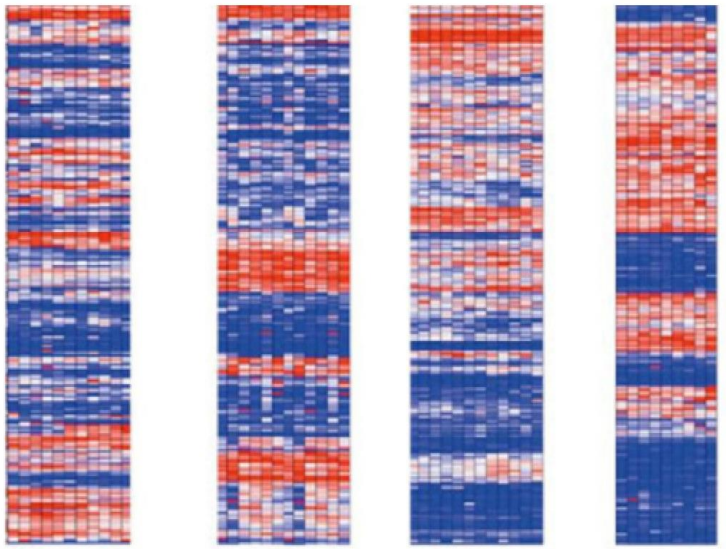
✓ Endometrial Receptivity Array (Assay/Analysis) – „ERA“

- gene expression profiling assays
- NGS of 248 genes related to endometrial receptivity



identification of patient´s WOI for personalized embryo transfer

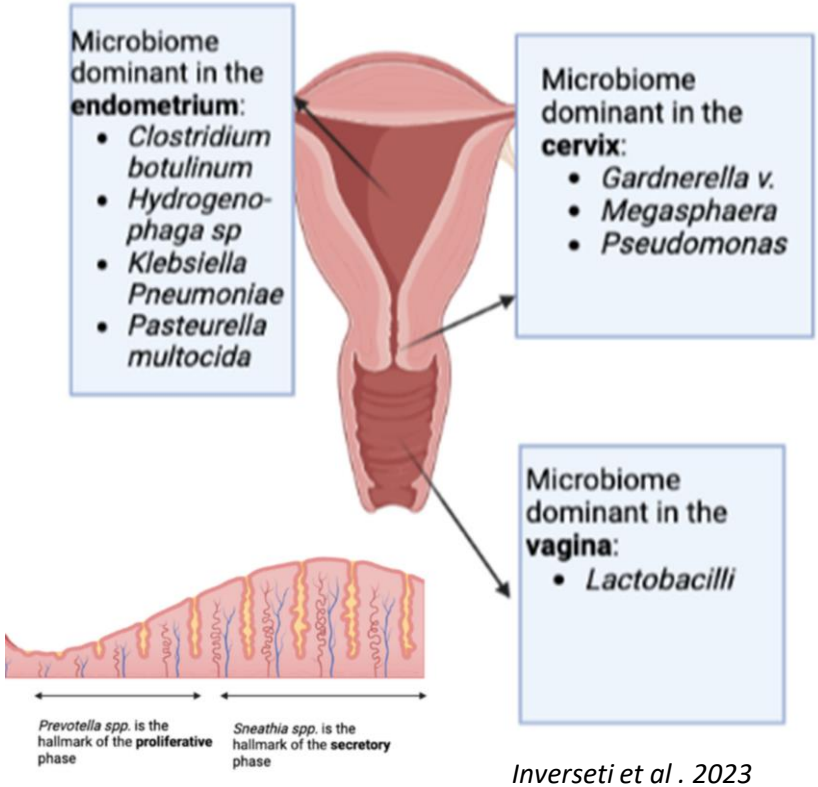




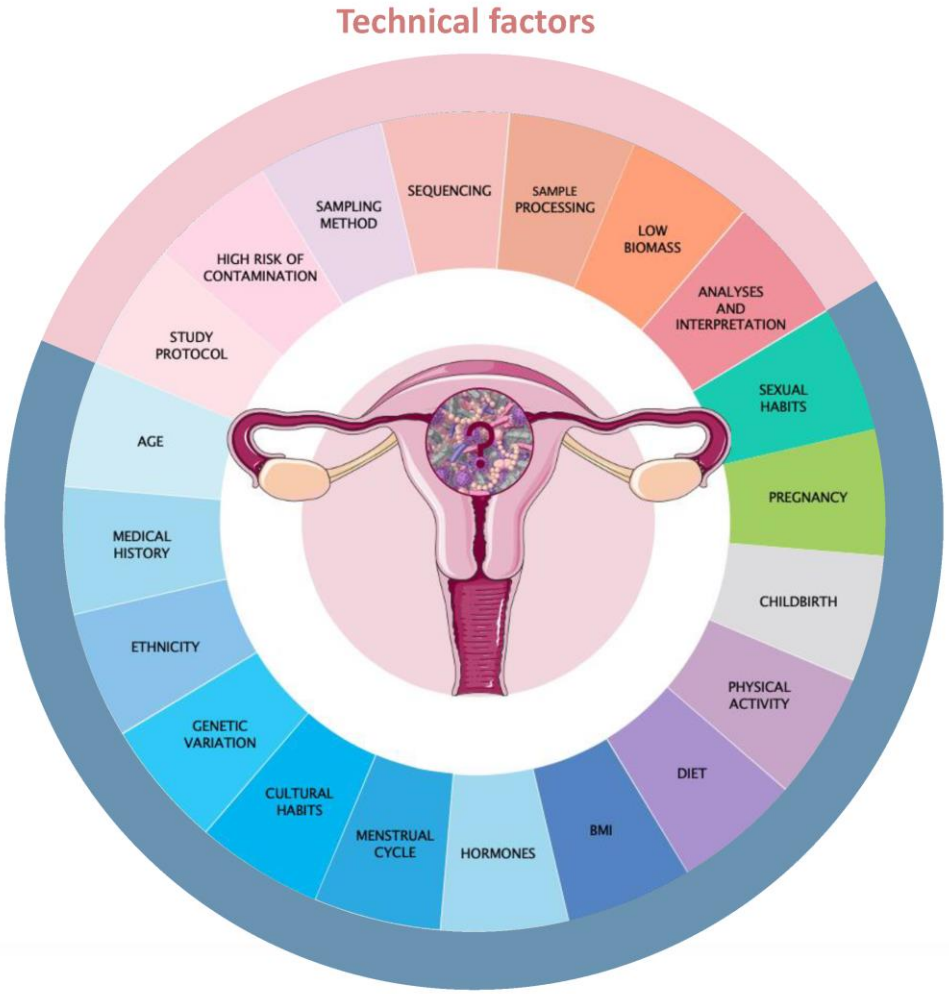
- inter-patients differences in WOI transcriptomic signature
- inconsistency between individual patient´s cycles (E2 and inflammation)
- invasiveness of biopsies (mini inavasive procedure)
- NGS results available with a delay
- cost burden

Uterine microbiome

- uterine cavity is not sterile
- inter-/intra-individual species diversity



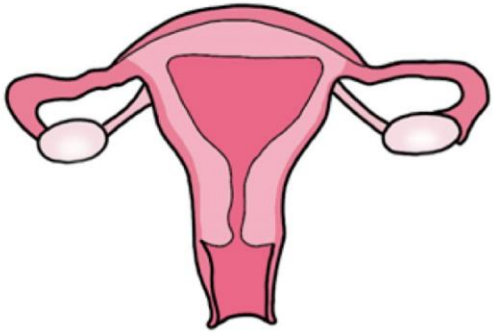
Inverseti et al . 2023

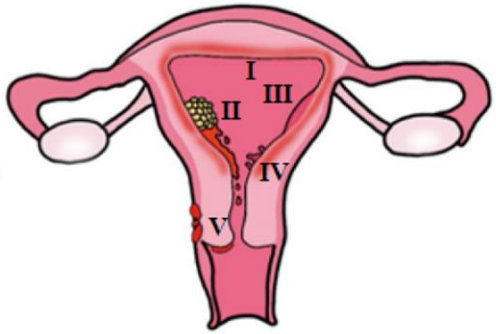


Molina et al . 2020

- dysbiosis associated with adverse reproductive outcomes
- ideal composition?
- benefits of species diversity?

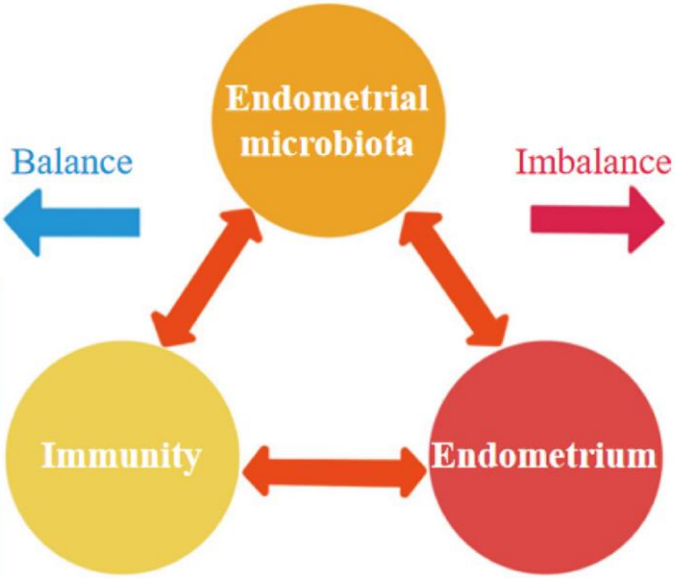
Uterine microbiome





Balance

Imbalance



Balance of uterine microecology

①Mucosal barrier function ↑

②Risk of endometrial pathological changes (eg, inflammation, polyps, hyperplasia, endometriosis and cancer) ↓

③Uterine functions (eg, endometrial immune tolerance, endocrine function) ↑

Imbalance of uterine microecology

①Mucosal barrier function ↓

②Risk of endometrial pathological changes (eg, inflammation, polyps, hyperplasia, endometriosis and cancer) ↑

③Uterine functions (eg, endometrial immune tolerance, endocrine function) ↓

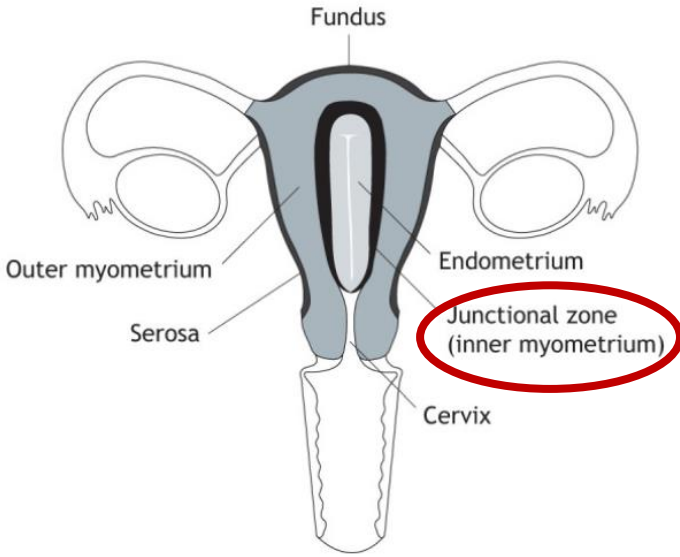
Zhu et al . 2022

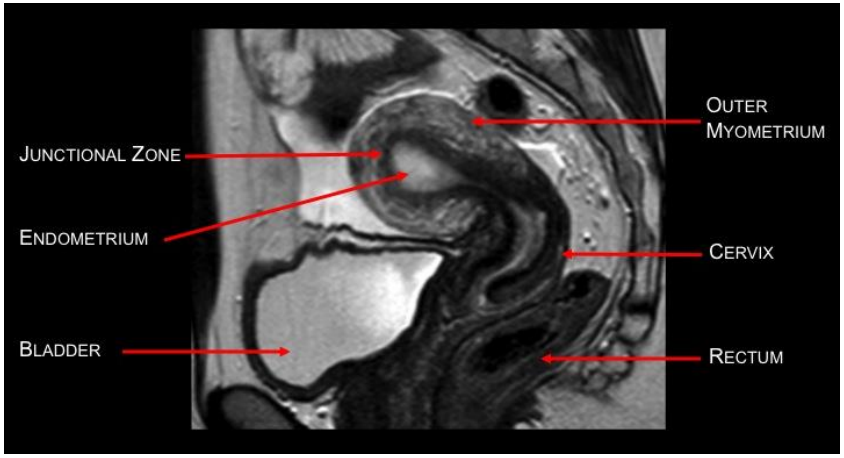
Uterine microbiome

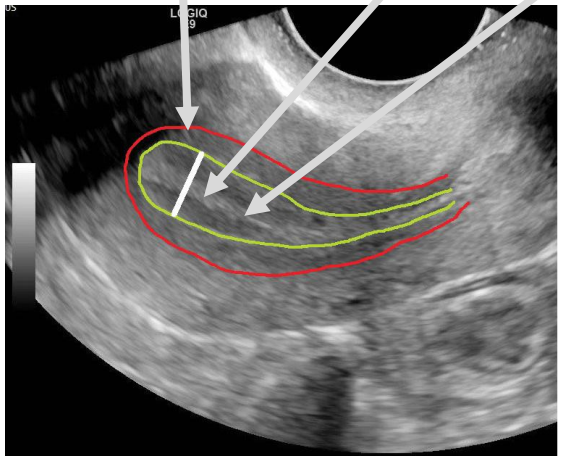
❖ Uterine microbiome diagnostic tests

Cyclic changes of junctional zone

- specialized layer of circular smooth muscle that surrounds the endometrium (inner myometrium)
- visible of high-resolution ultrasound and magnetic resonance imaging
- undegoes homone-dependent contraction and remodelling during the menstrual cycle
  - trans-differentiation stromal fibroblast to myocytes
  - cervico-fundal contractions → sperm transport during the fertile window; fundo-cervical contractions → flow of effluent during menstruation





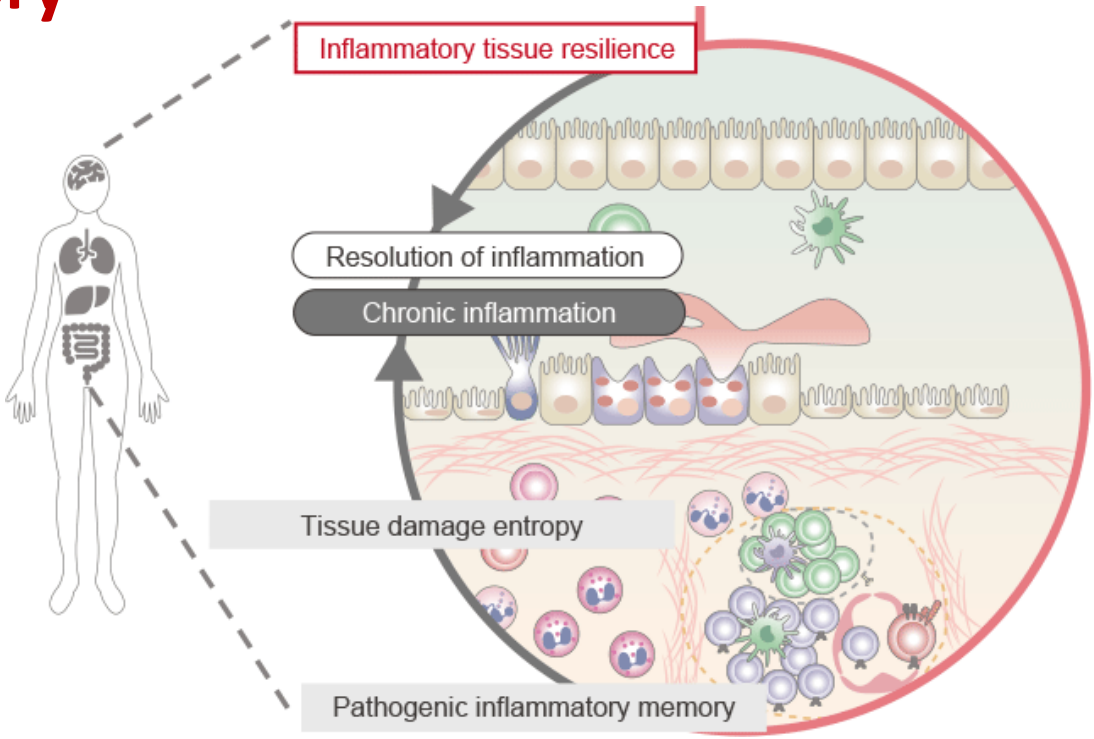


Triple line measurement

Role of uterine cyclic remodeling

❖ Inflammatory 'memory'

- *brief exposures of any organ to low levels of stress confers resistance to stress levels that otherwise cause tissue damage*



- repeated menstruation cycles might precondition the uterus for future pregnancy

CLINICAL OPINION www.AJOG.org

OBSTETRICS

A role for menstruation in preconditioning the uterus for successful pregnancy

Jan J. Brosens, MD, PhD; Malcolm G. Parker, PhD; Angus McIndoe, MD, PhD; Robert Pijnenborg, PhD; Ivo A. Brosens, MD, PhD

Brosenset al 2009

Menstrual disturbances

- Menopausal amenorrhea
- Thin endometrium
- Abnormal uterine bleeding
- Intrauterine adhesions
- Asherman syndrom
- Endometriosis

Menopausal amenorrhea

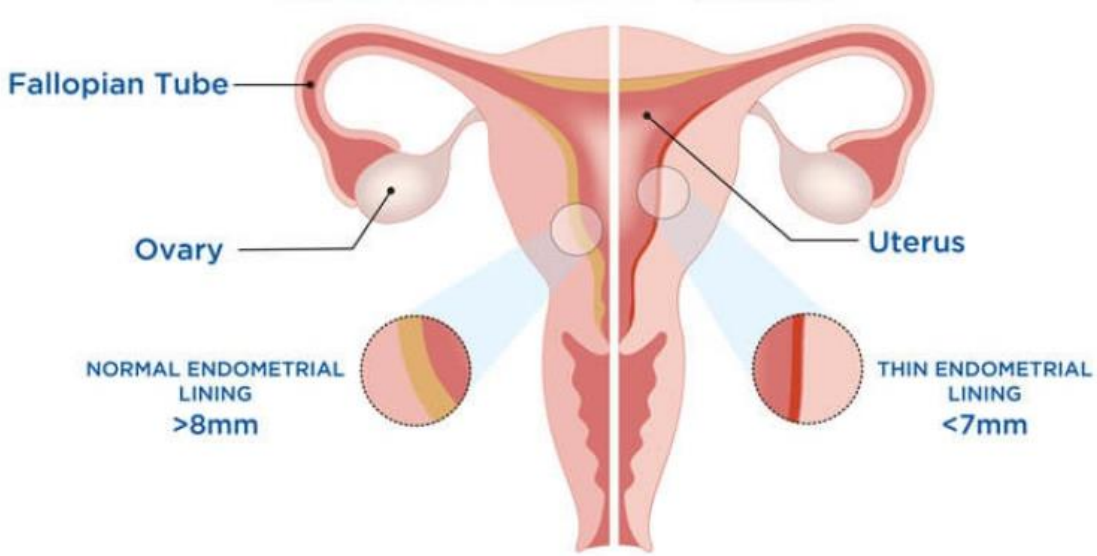
= fall in estrogen production due to ovarian reserve exhausition → **atrophic endometrium**

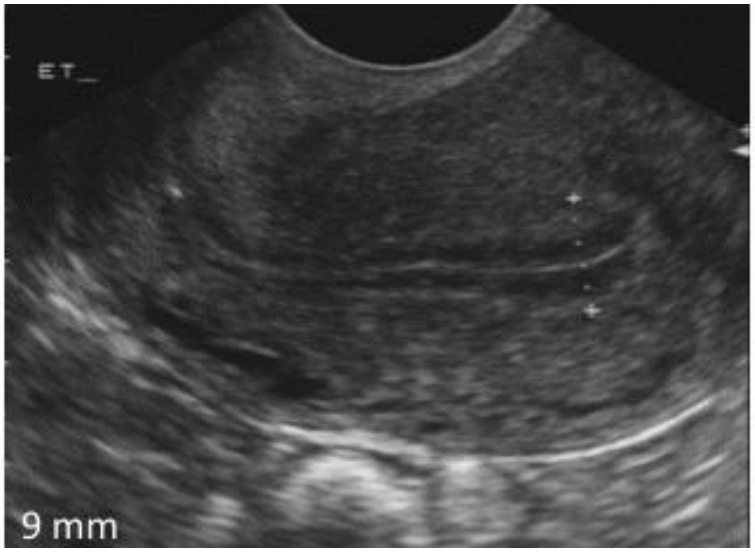
- thin (<4 mm) endometrium consisting of *stratum basalis* only
- glands are sparse and have low secretory activity, could be dilated and produce cysts
- stroma is less cellular, and contains more collagen fibers
- no apparent mitotic activity (senescence)
- physiological postmenopausal amenorrhea
- stem/progenitor cells are in a dormant state
- quiescent stem/progenitor cell can be reactivated by exogenous E2 during hormonal replacement therapy, but their clonic efficiency is lower than in premenopausal endometrium

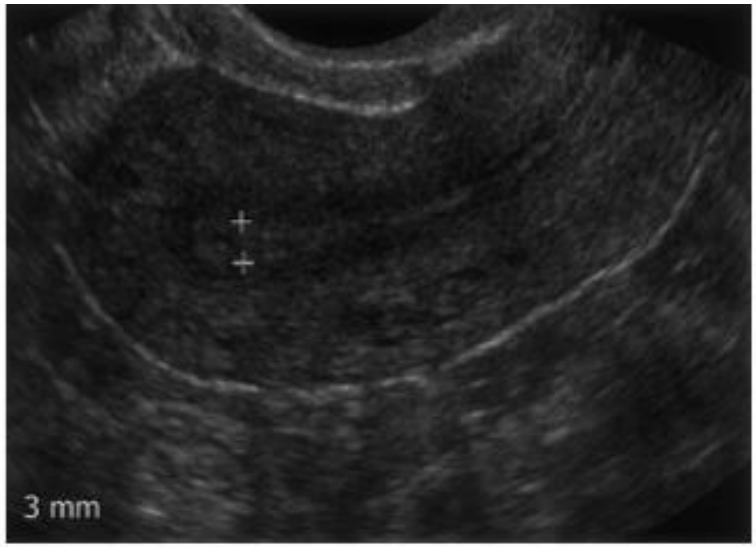
normal endometrium
(late proliferative phase)

postmenopausal endometrium

Thin endometrium







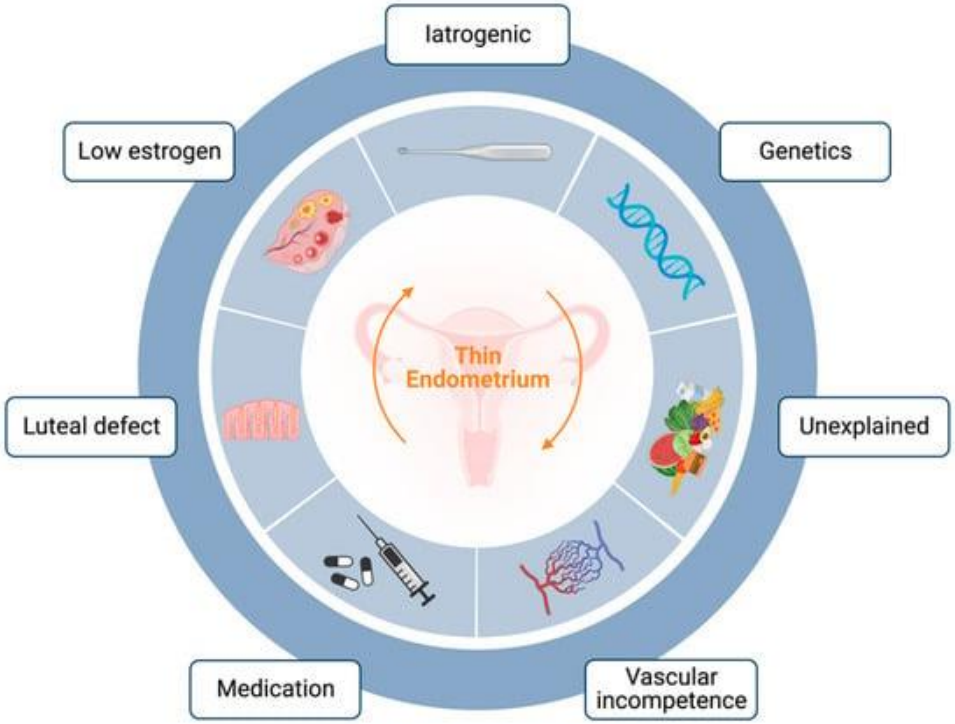
Triple line ultrasound measurement

Thin endometrium

Possible Causes
- Low estrogen levels
- Luteal defects
- Advanced age
- Fibroids
- Genetic factors
- Intrauterine Adhesions
- Poor blood flow
- Pelvic surgeries/inflammation
- Iatrogenic effect

Symptoms
- Irregular menstrual cycle
- Painful or inadequate menses
- Fertility issues

Treatment
- Hormonal therapy
- Uterine surgeries and interventions
  (e.g. endometrial scratching)
- Growth factor (PRP) therapy
- Sildenafil (off-label)



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Gynecol Endocrinol, 2014; 30(7): 481–484
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EMBRYO TRANSFER IN THIN ENDOMETRIUM

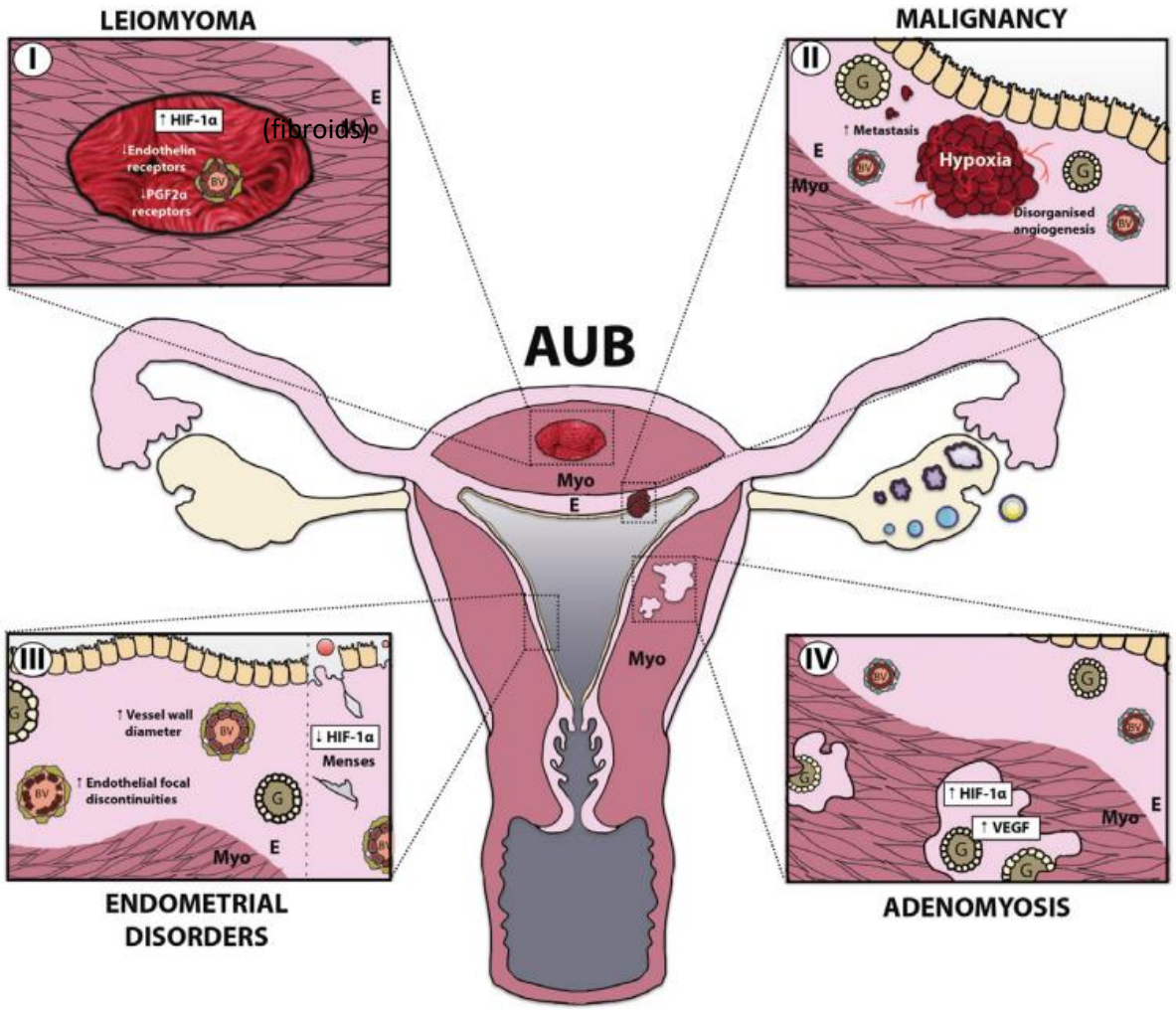
Live birth after embryo transfer in an unresponsive thin endometrium

Fábio Cruz[1] and José Bellver[1,2,3]

[1]Instituto Valenciano de Infertilidad, Valencia, Spain, [2]Fundación IVI, Instituto Universitario IVI, University of Valencia, Valencia, Spain, and [3]Department of Pediatrics, Obstetrics and Gynecology, Faculty of Medicine, University of Valencia, Valencia, Spain

Abnormal uterine bleeding (AUB)

- altered tissue oxygenation and HIF-regulation are suspected to underlie AUB conditions



Martinez Aguilar et al 2020

Intrauterine adhesions (IUA)

- damage of *basalis* layer and loss of stem/progenitor cells

→ failure of adequate repair and regeneration

Cen et al 2022

Intrauterine adhesions (IUA)

❖ STEM CELLS/PROGENITORS CELLS THERAPY

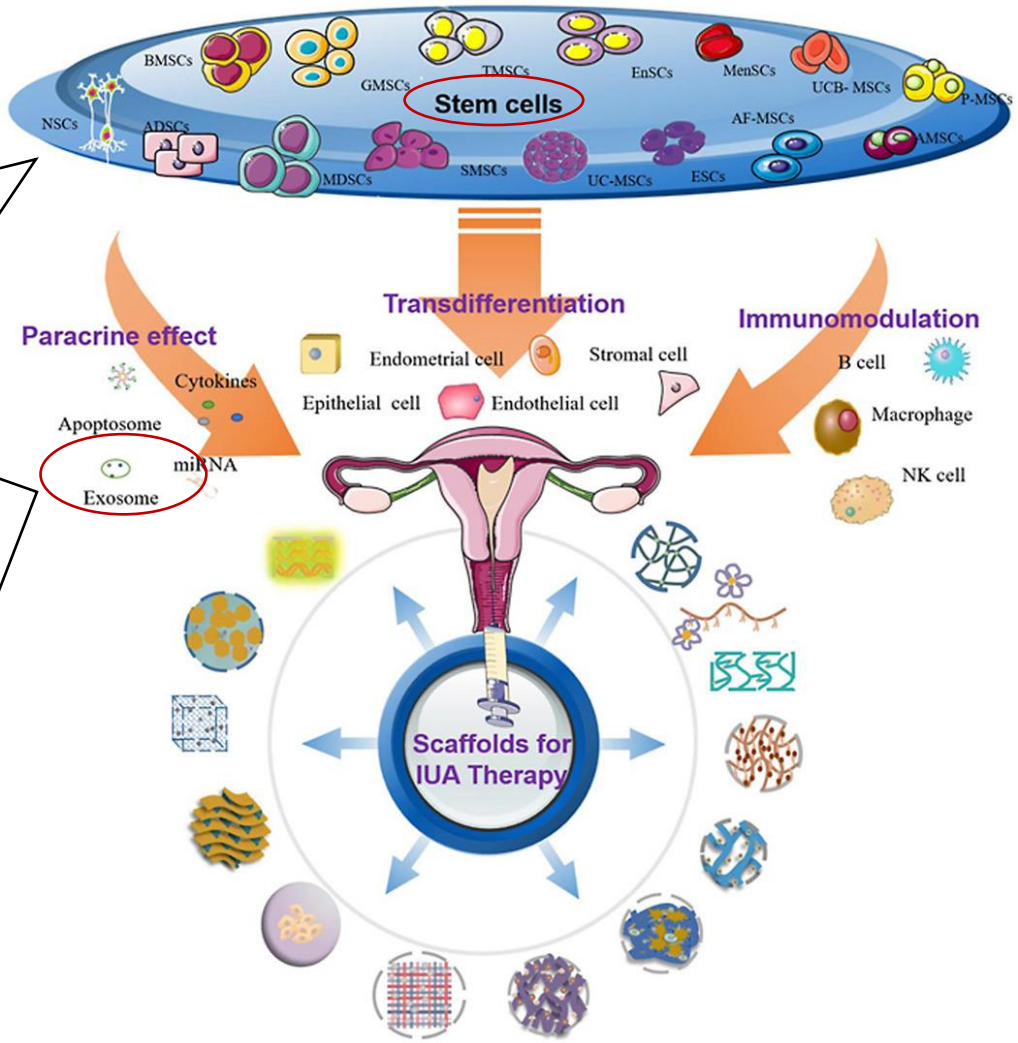
- non-invasive harvest from the menstrual fluid
  - short-lived
  - risk of off-target migration and tumor growth

MSC-secreted exosomes

- anti-inflammatory
- anti-apoptic
- proangiogenic
- immuno-modulatory

→↑ epithelisation and wound healing
→↑collagen maturity
→↓ scarring

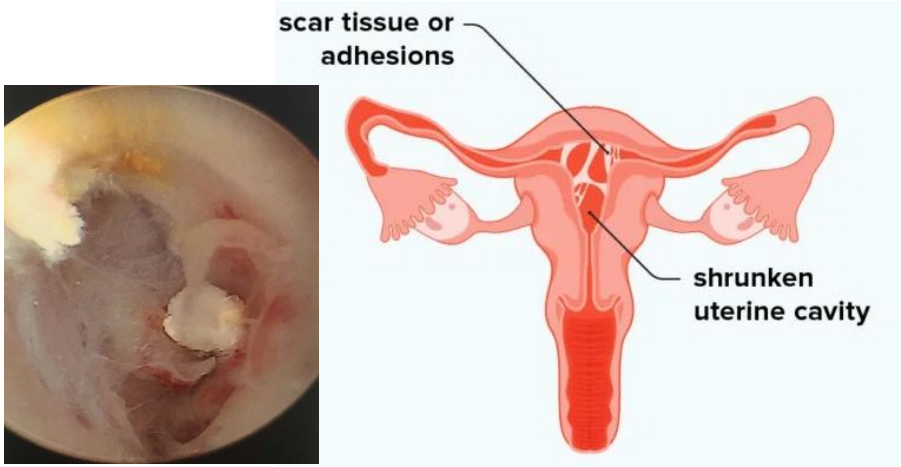
- wound healing therapeutical potential



Cen et al 2022

Asherman syndrom

- excessive intrauterine adhesions, scarring and synechiae
- causes dysmenorhea, irregular cycles, miscarriages, and placental anomalies



- etiology unknown, risk factors include uterine surgery, pregnancies, trauma, pelvic infections, genital tuberculosis. and obesity
- regenerative potential of stem celll demonstrated in clinical trials

Carlos Simon

Endometriosis

= presence of cycling endometrial tissue outside of the uterine cavity

Chronic inflammation

Dysmenorrhea and AUB
Dyspareunia
Painful defection and urination
Pelvic and back pain
Infertility

Endometriosis lesions origin

✓ retrograde menstruation

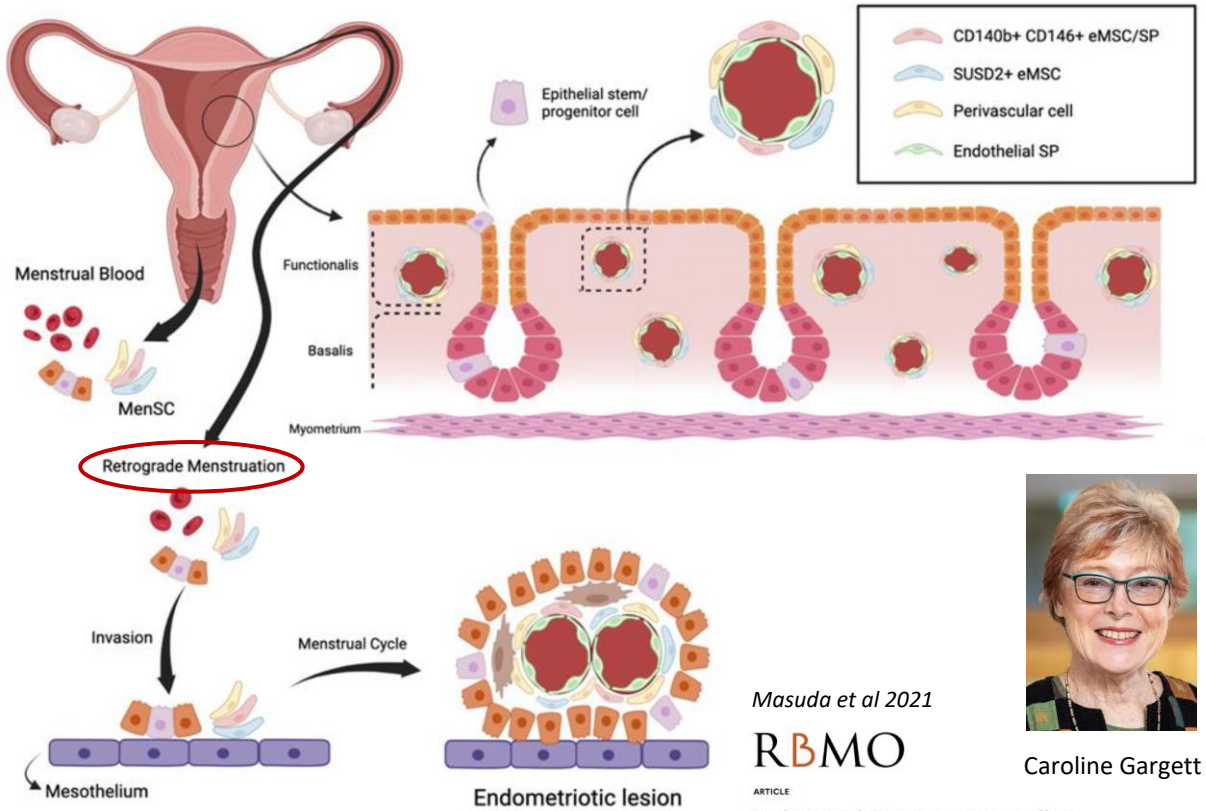
(shedding fragments of endometrium containing uterine stem cells into the Fallopian tube and pelvic cavity)

✓ ectopic adhesion and survival of uterine stem cells

(enhanced by genetic background, eMES/ePSC population composition and proliferation profile, and/or local environment)

✓ persistence and invasion of small superficial lessions

(dependent on proliferation, penetration, migration, proinflamatory and angiogenic capacity of deposited endometriotic cells)



Caroline Gargett

Masuda et al 2021

RBMO

ARTICLE

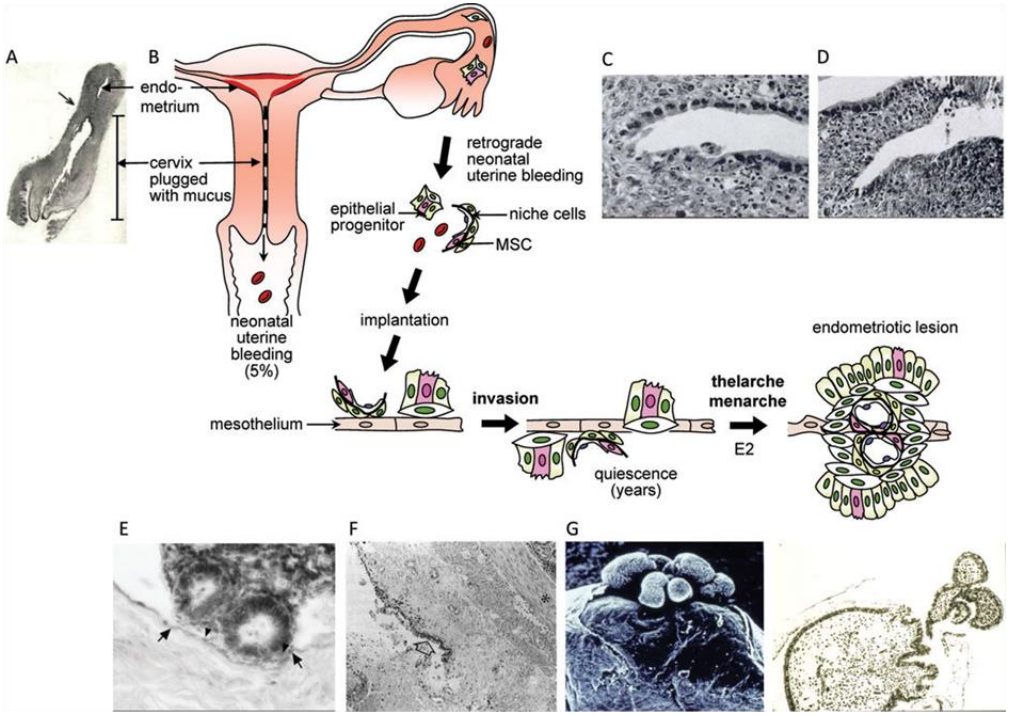
Endometrial stem/progenitor cells in menstrual blood and peritoneal fluid of women with and without endometriosis

Hirotaka Masuda[1,2,#], Kjiana E. Schwab[1,#], C.E. Filby[1,2,#], Charmaine S.C. Tan[1,2], Jim Tsaltas[2], Gareth C. Weston[2,3], Caroline E. Gargett[1,2,*]

Endometriosis

❖ Neonatal „menstrual-like“ bleeding

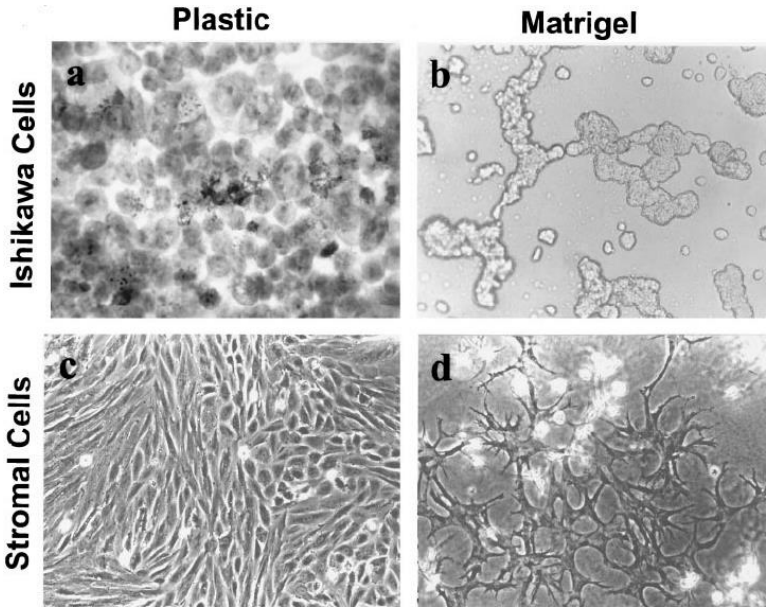
- occurs in ~5% of newborn girls (typically post-term)
- results from P4 withdrawal from neonatal circulation upon birth
- cervix blocked → premenarchial retrograde uterine bleeding
- visible bleeding from the vagina indicates intense tissue shedding with a higher risk of retrograde menstruation
- possible predisposition for early onset of endometriosis in adulthood



Gargett al 2014

Research of endometrial physiology

❖ 2D in vitro models



➢ **endometrial cancer cell lines**

- Ishikawa, ECC-1, KLE, RL95-2 And Hec50co
- genetically abnormal, single-cell type

➢ **biopsy material**

- heterogenous character
  - different cycle stage
  - individual genetic background

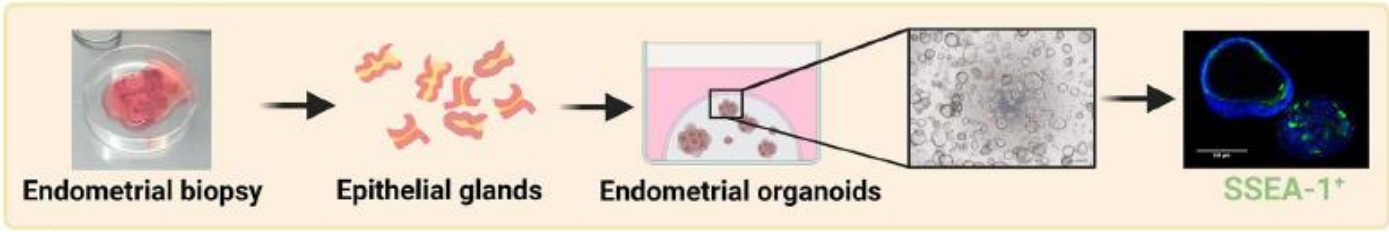
❖ 3D in vitro models

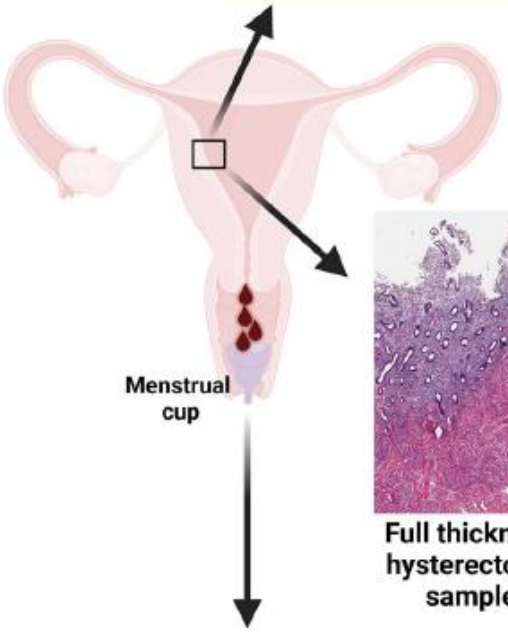
➢ **ENDOMETRIAL ORGANOIDS**

- hollow spherical structures consisting of multiple cell types, including epithelial, stromal, glandular, and vascular cells
- spontaneous self-organization in a defined serum-free medium („assembloids“)
  - ← primary endometrial cells from biopsies
  - ← endometrial cells isolated from menstrual fluid
- responsiveness to E2 and P4
- lack of complex organization of endometrial tissue

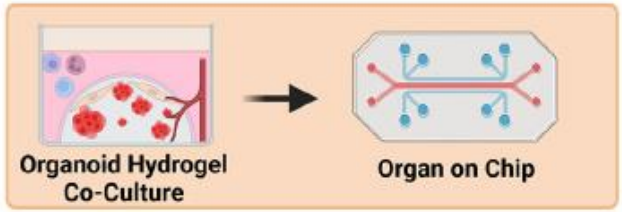
Disease modeling
Drug screening
Embryo-endometrial cross talk research

Research of endometrial physiology

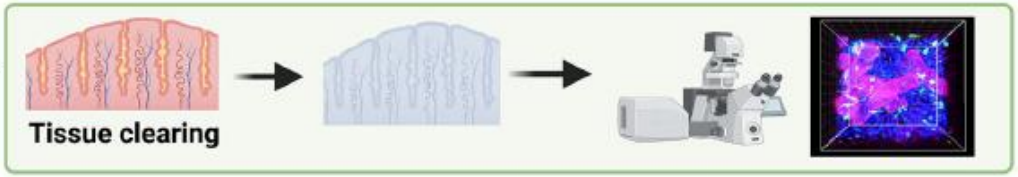


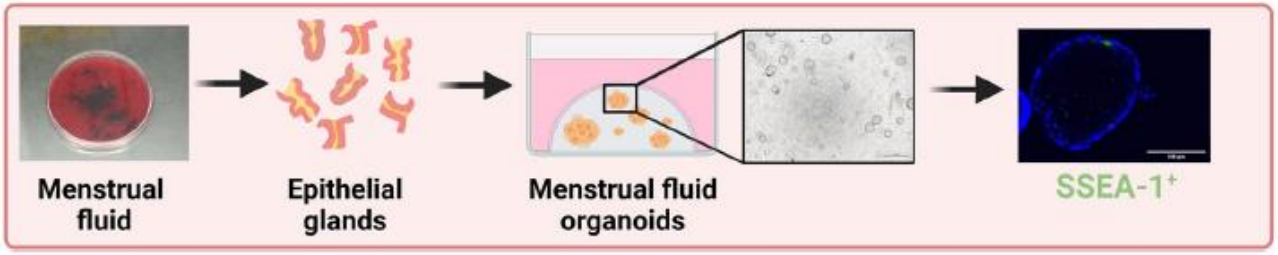


Matrix-based systems for co-culture of multile cell types



Opportunity to model complexity of uterine tissue regeneration





Cousin et al 2022

The uterus ensures that a "good" embryo lands safely