

Embryology III

PERIMPLANTATION DEVELOPMENT

autumn 2024

Preparing uterine tissue for implantation

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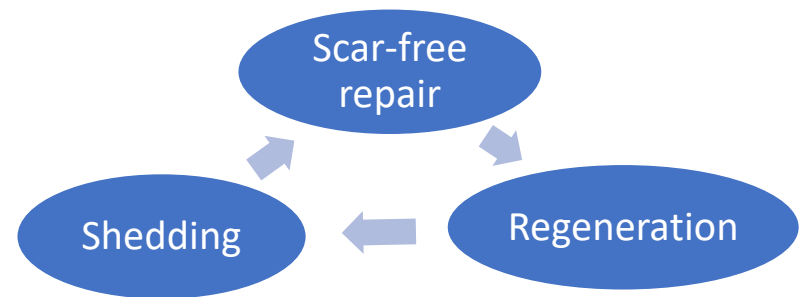
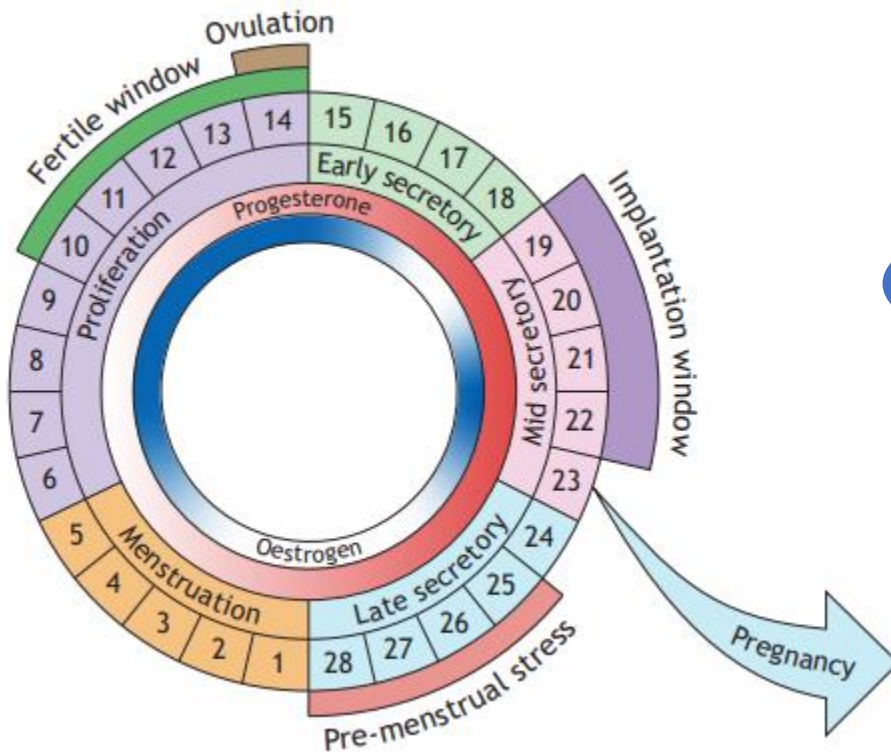
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Uterine tissue remodeling

❖ Menstrual cycle

= cyclical endometrial tissue turnover and rejuvenation



~28 days

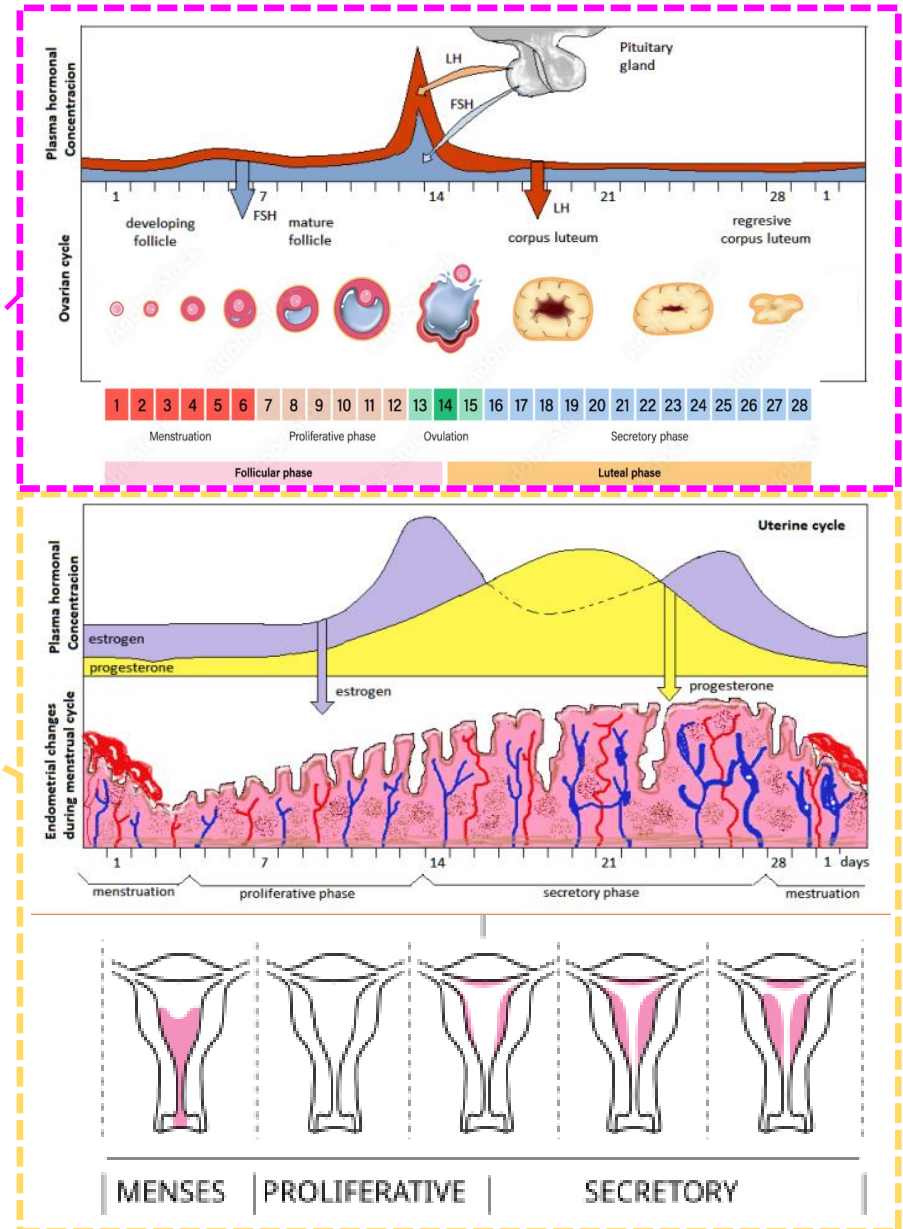
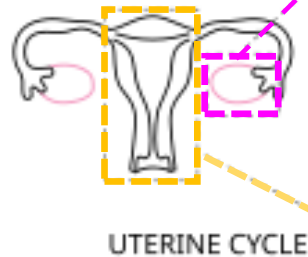
~ 400 cycles per reproductive life

- day 1 = 1st day of menstruation bleeding

Endometrial remodelling

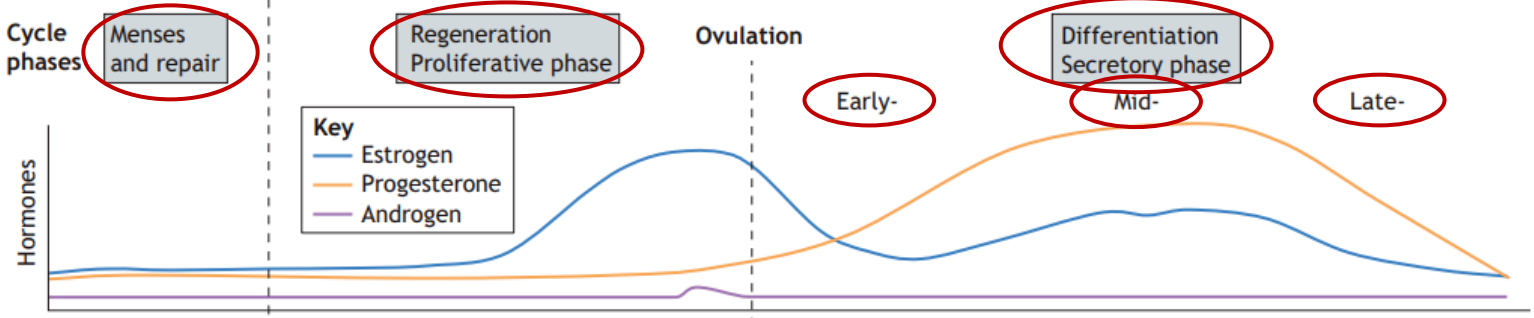
❖ Menstrual cycle

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

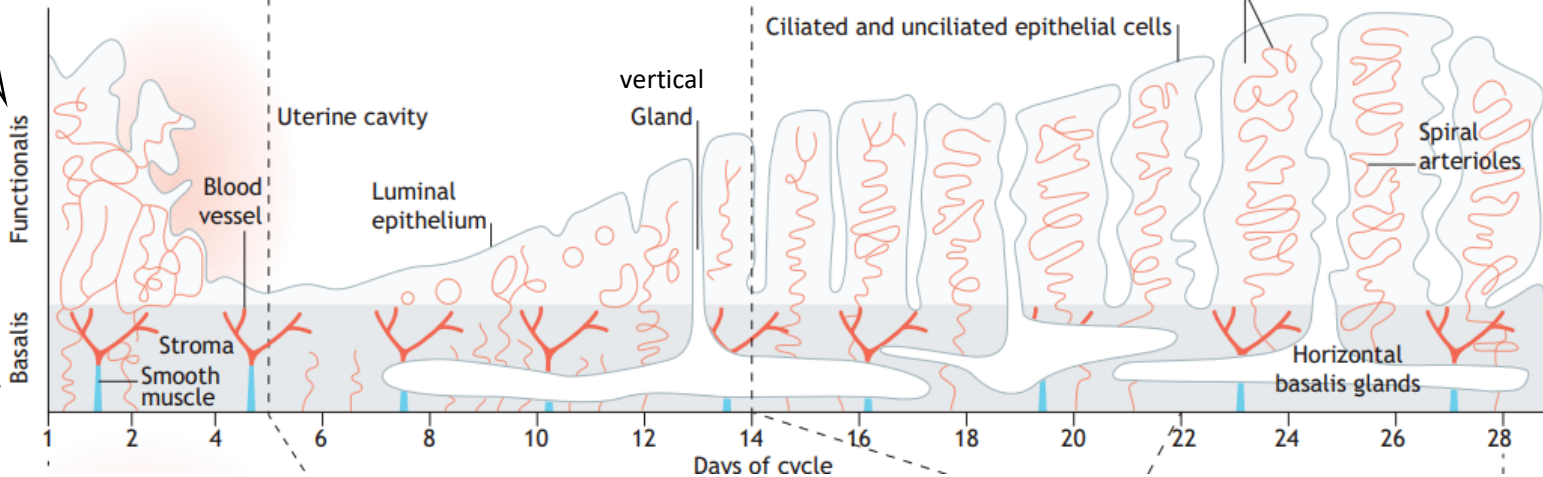


Cyclic endometrial changes

A Endometrial menstrual cycle



B Endometrium



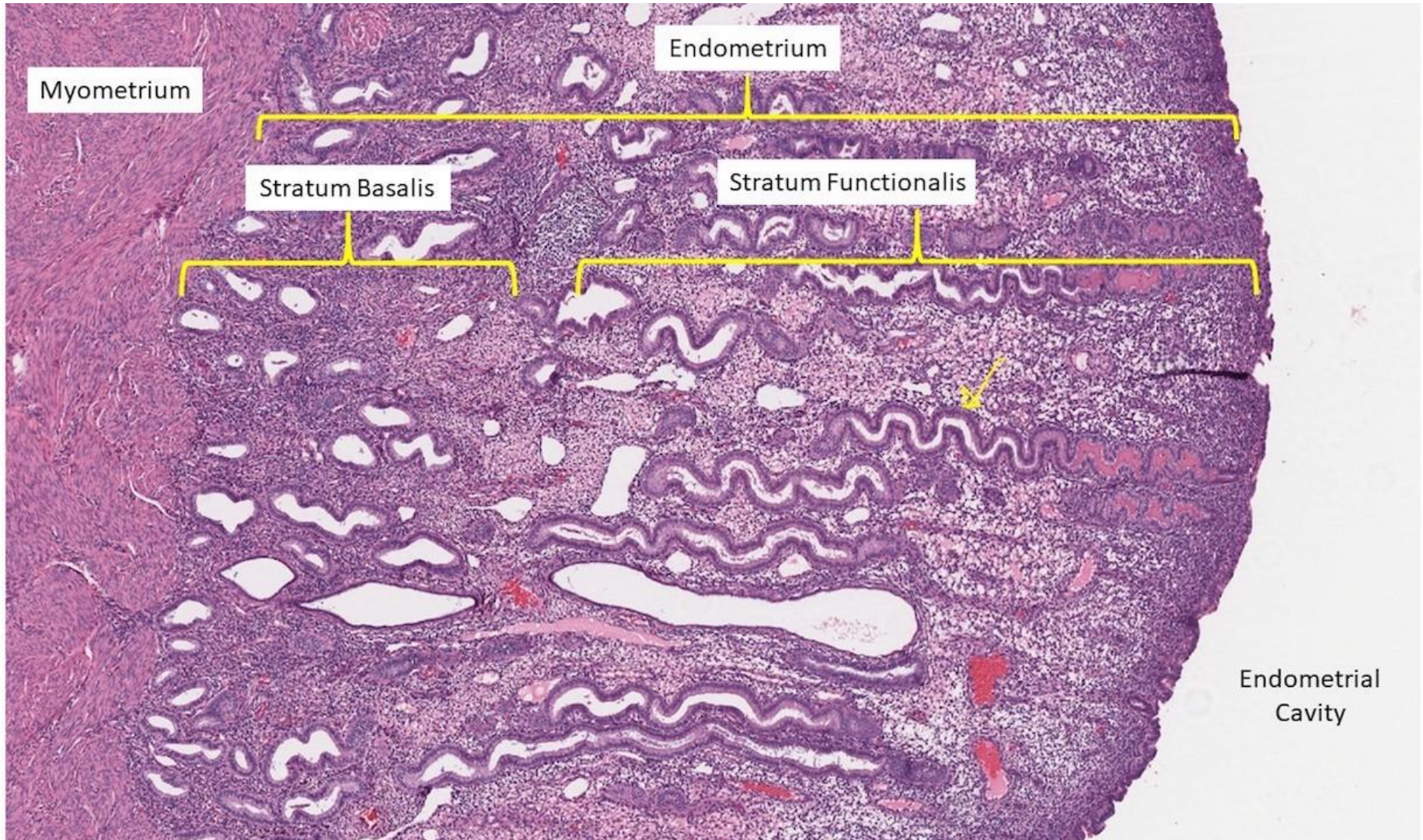
Functionalis

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

Basalis

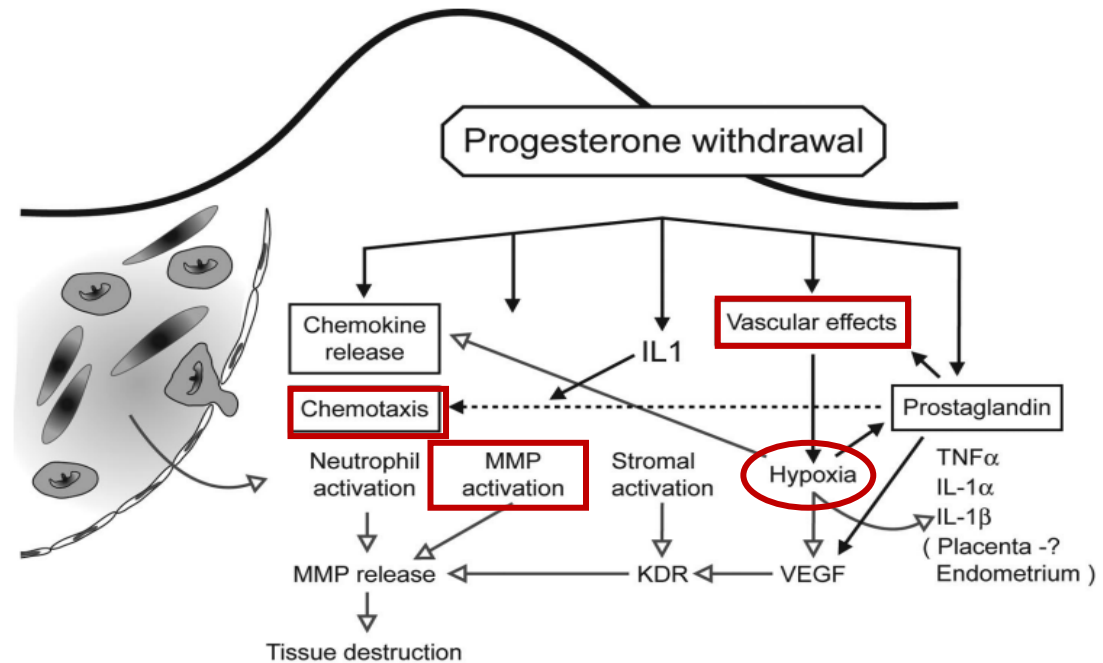
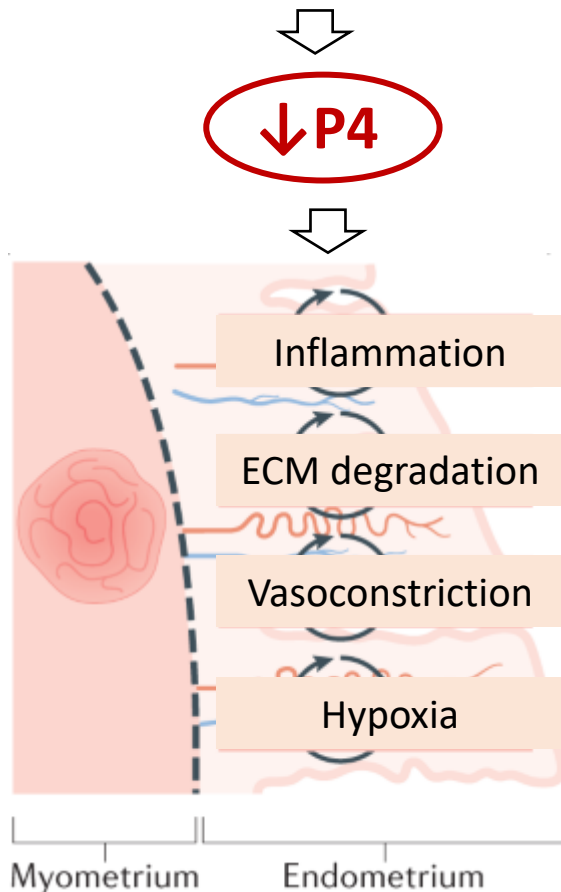
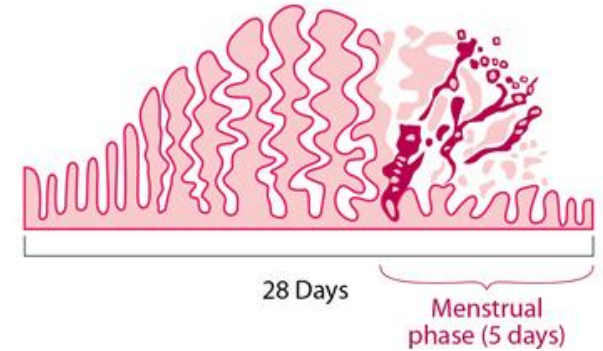
- unresponsive to hormones
- comprises horizontal glands network, stromal cells, vasculature and stem cells
- intact during cycle

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

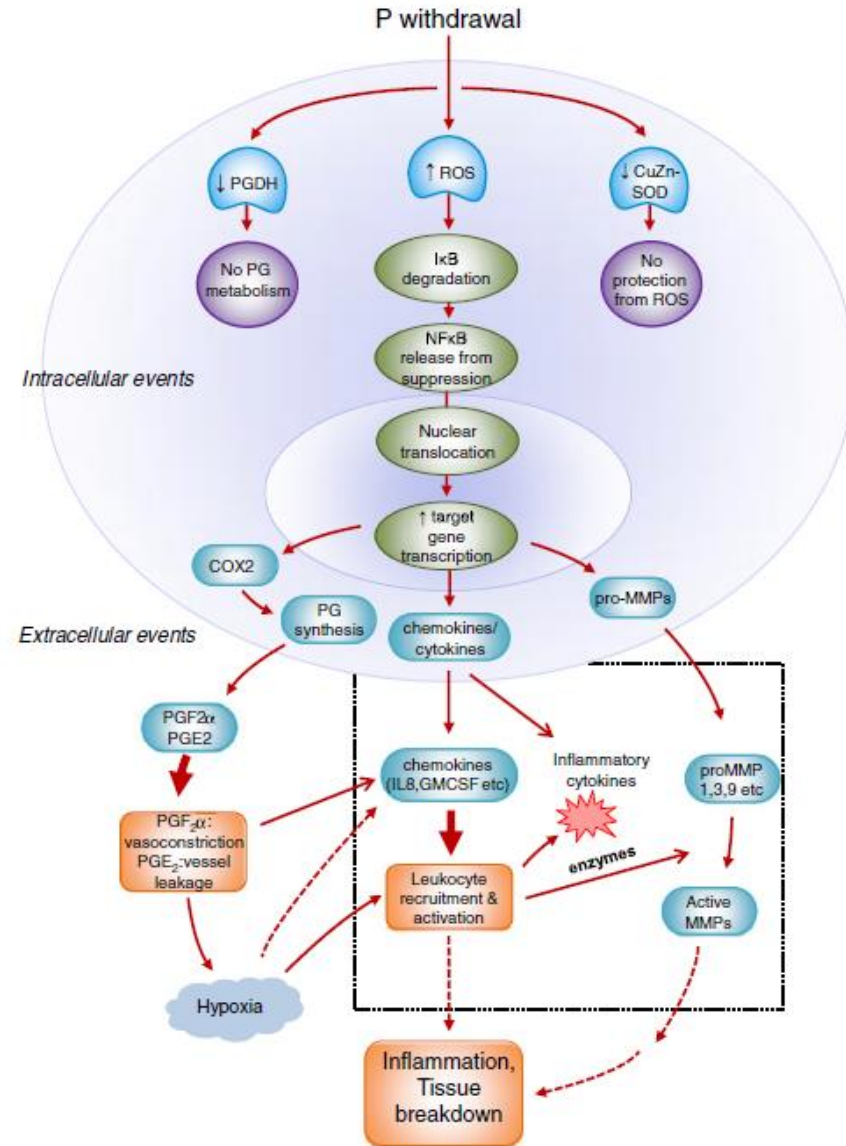
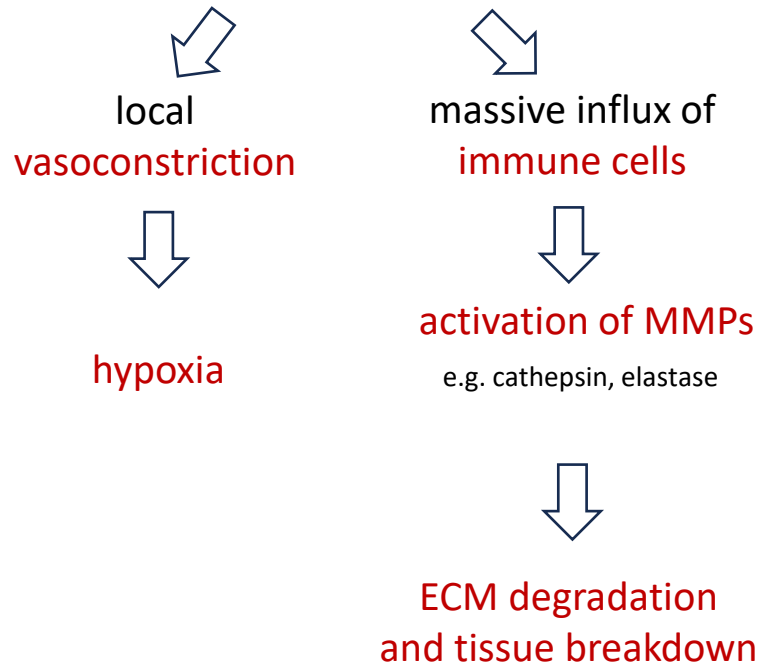


Menstrual phase

❖ INFLAMMATORY RESPONSE

→ secretion of

- cytokines
- chemokines
- prostaglandin synthesizing enzymes



Menstrual phase

Maybin et al 2021

❖ HYPOXIA

- caused by local **vasoconstriction**
- **hypoxia-induced stabilization of HIF-1 α** physiologically drives endometrial repair after shedding

- women with heavy menstrual bleeding have decreased HIF-1 α during menstruation
- genetic and pharmacological reduction of endometrial HIF-1 α in mice causes prolonged menstrual bleeding



ARTICLE

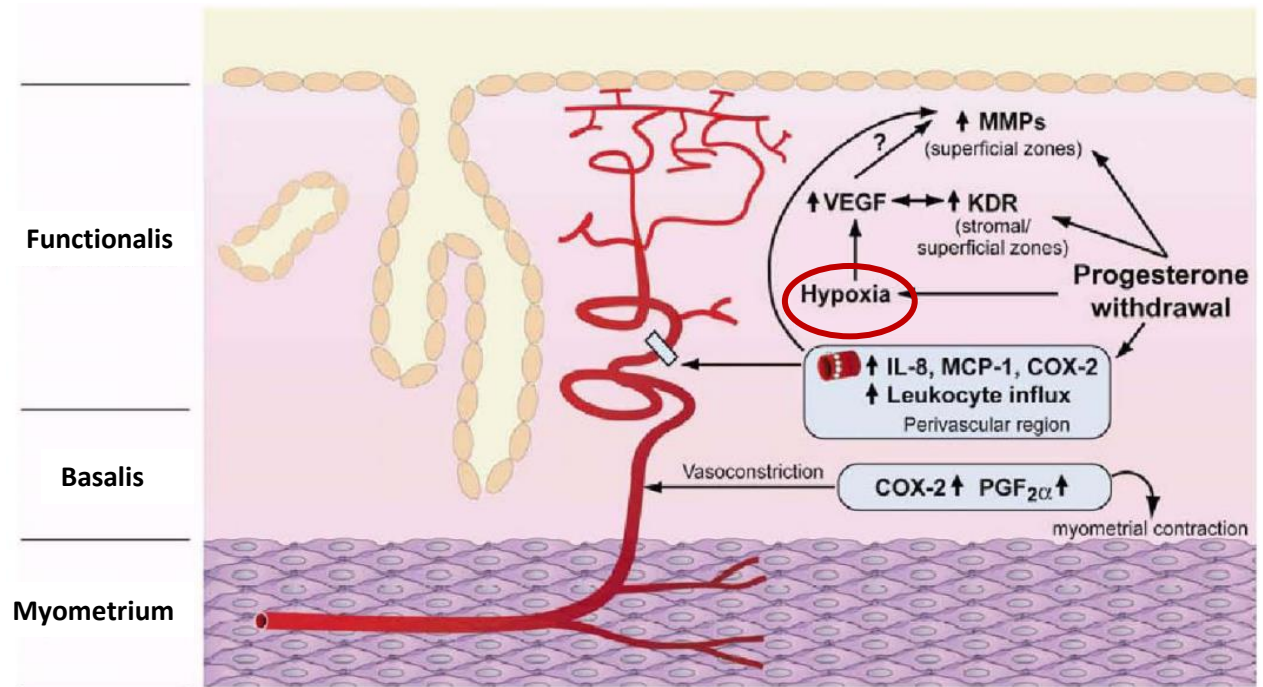
DOI: 10.1038/s41467-017-02375-6 OPEN

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

Jacqueline A. Maybin¹, Alison A. Murray¹, Philippa T.K. Saunders², Nikhil Hirani², Peter Carmeliet³ & Hilary O.D. Critchley¹

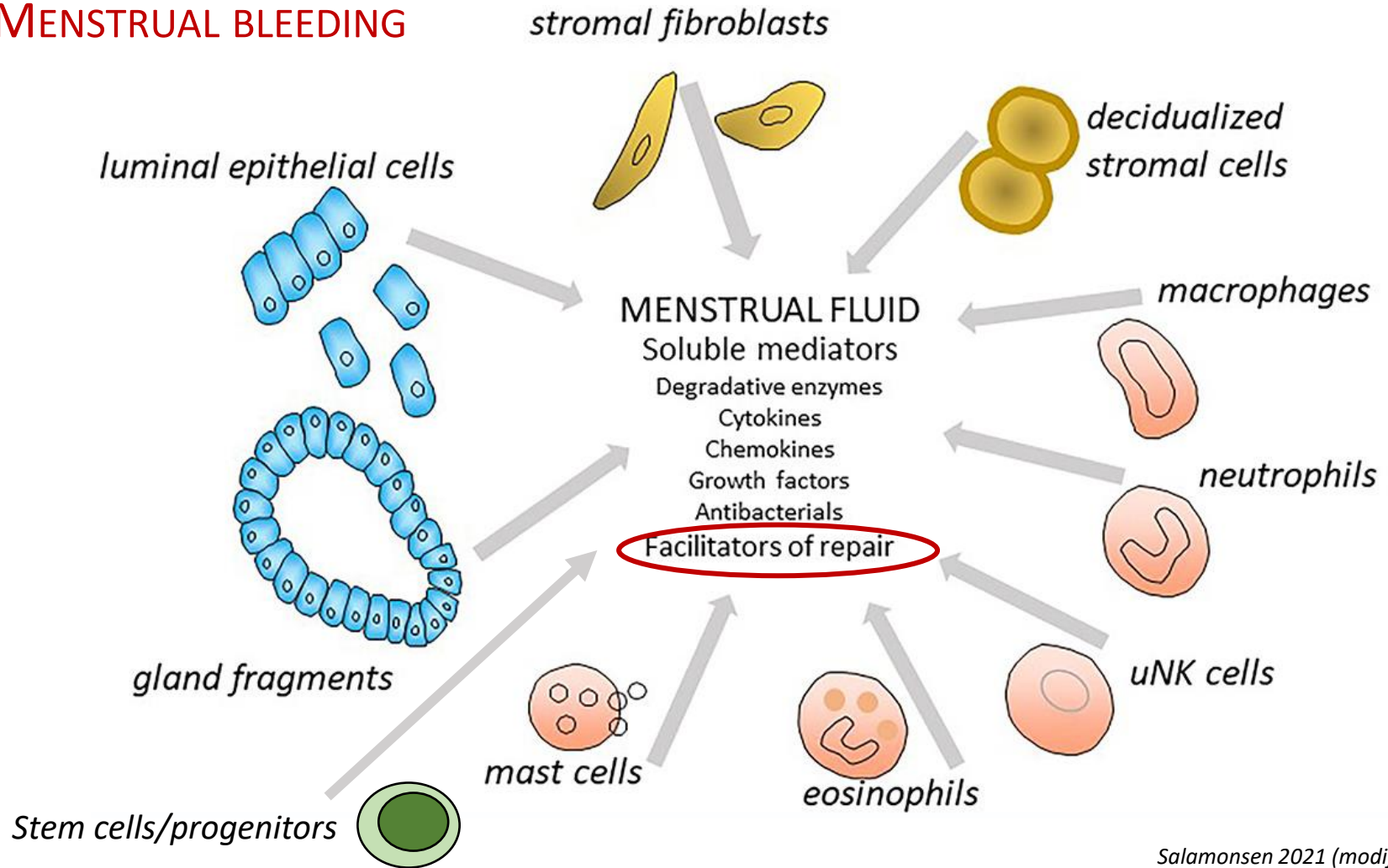


Hilary Critchley



Menstrual phase

❖ MENSTRUAL BLEEDING



Salamonsen 2021 (modified)

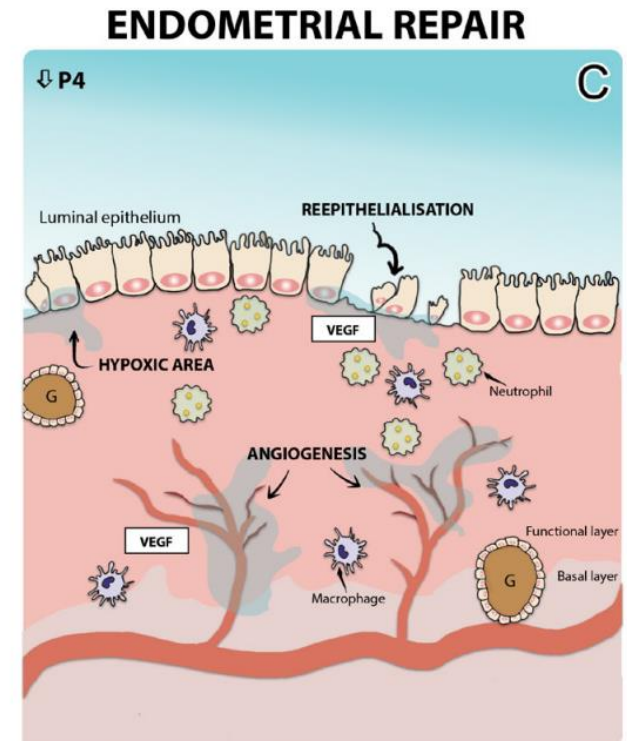
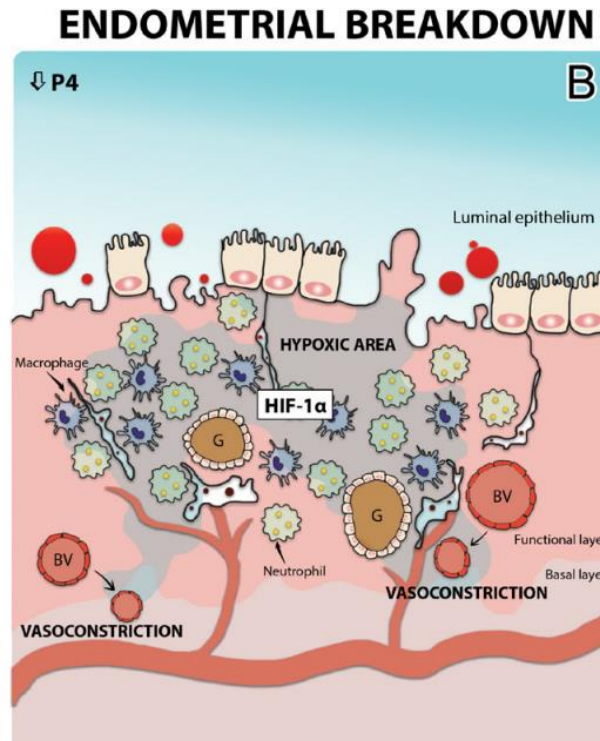
- menstrual fluid environment + preservation of *basalis* → **no scarring**

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



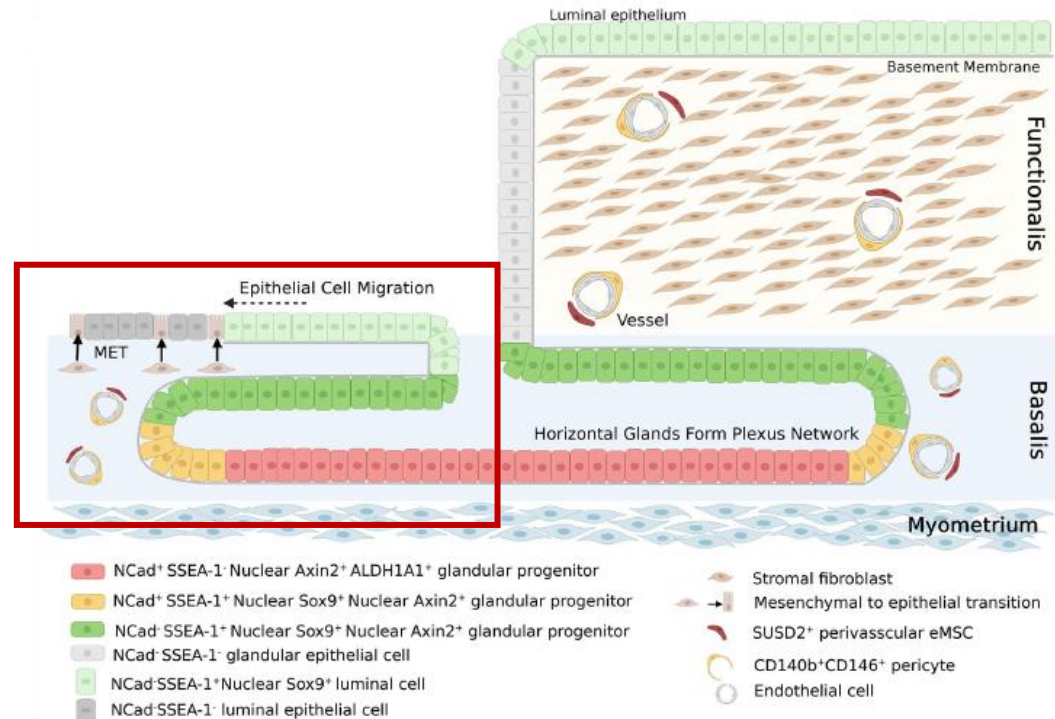
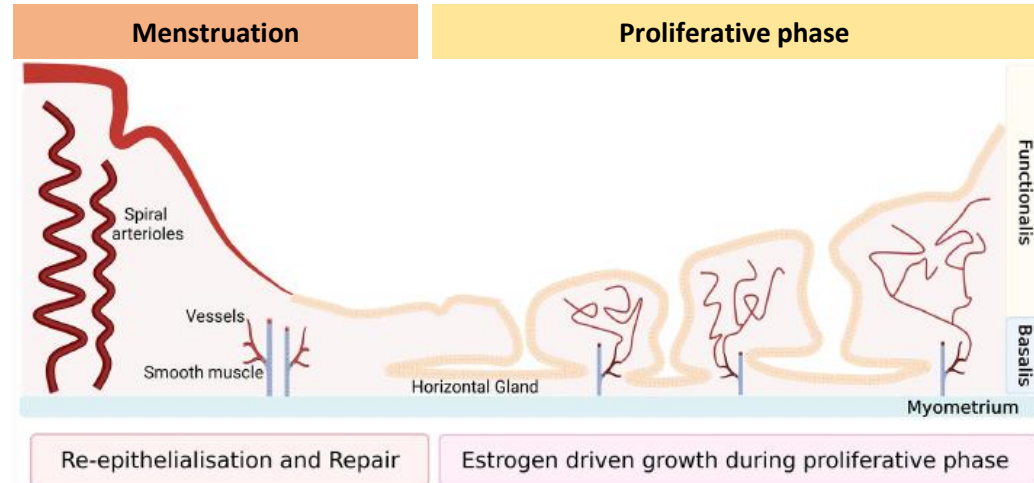
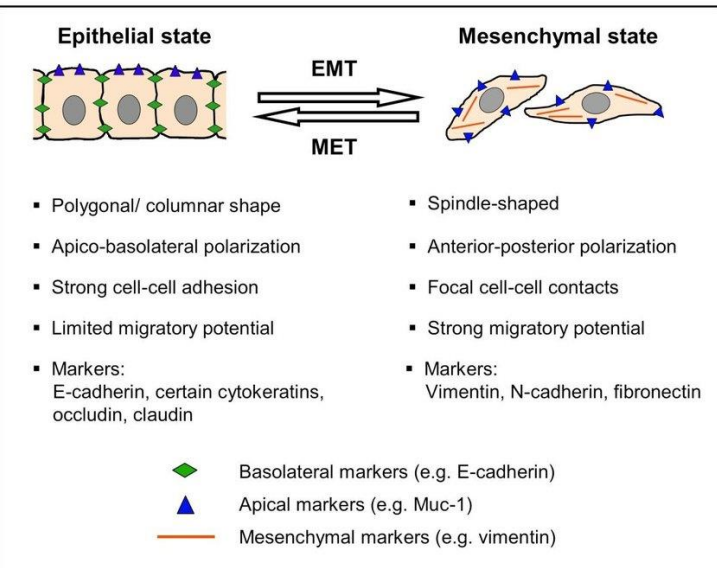
Menstrual phase

❖ ENDOMETRIAL REPAIR

Luminal epithelium resurfacing

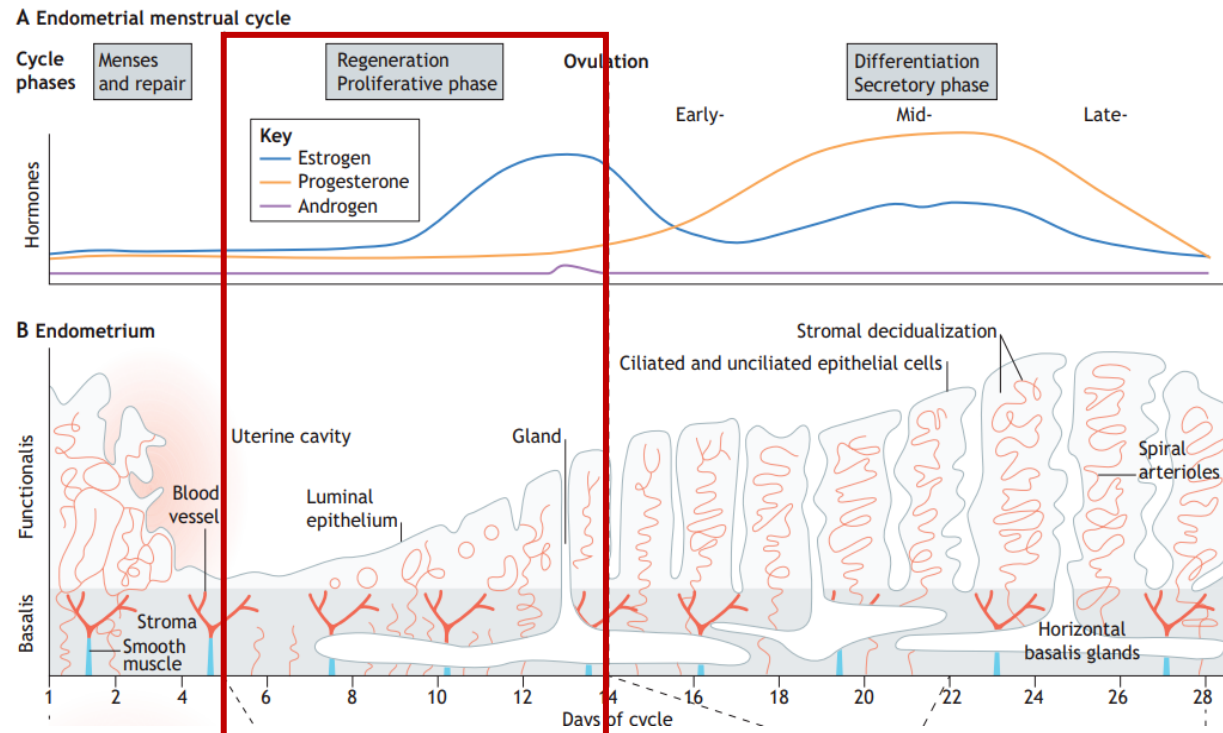
← migration of epithelial cell progenitors from exposed horizontal endometrial glands in *basalis*

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



Proliferative phase

- post-menopausal period (starts ~ day 4, last ~ 10 days)
- increase of endometrial lining 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**



Proliferative phase

- E2-dependent

- E2-receptor present in epithelial and stromal part

+ role of androgens

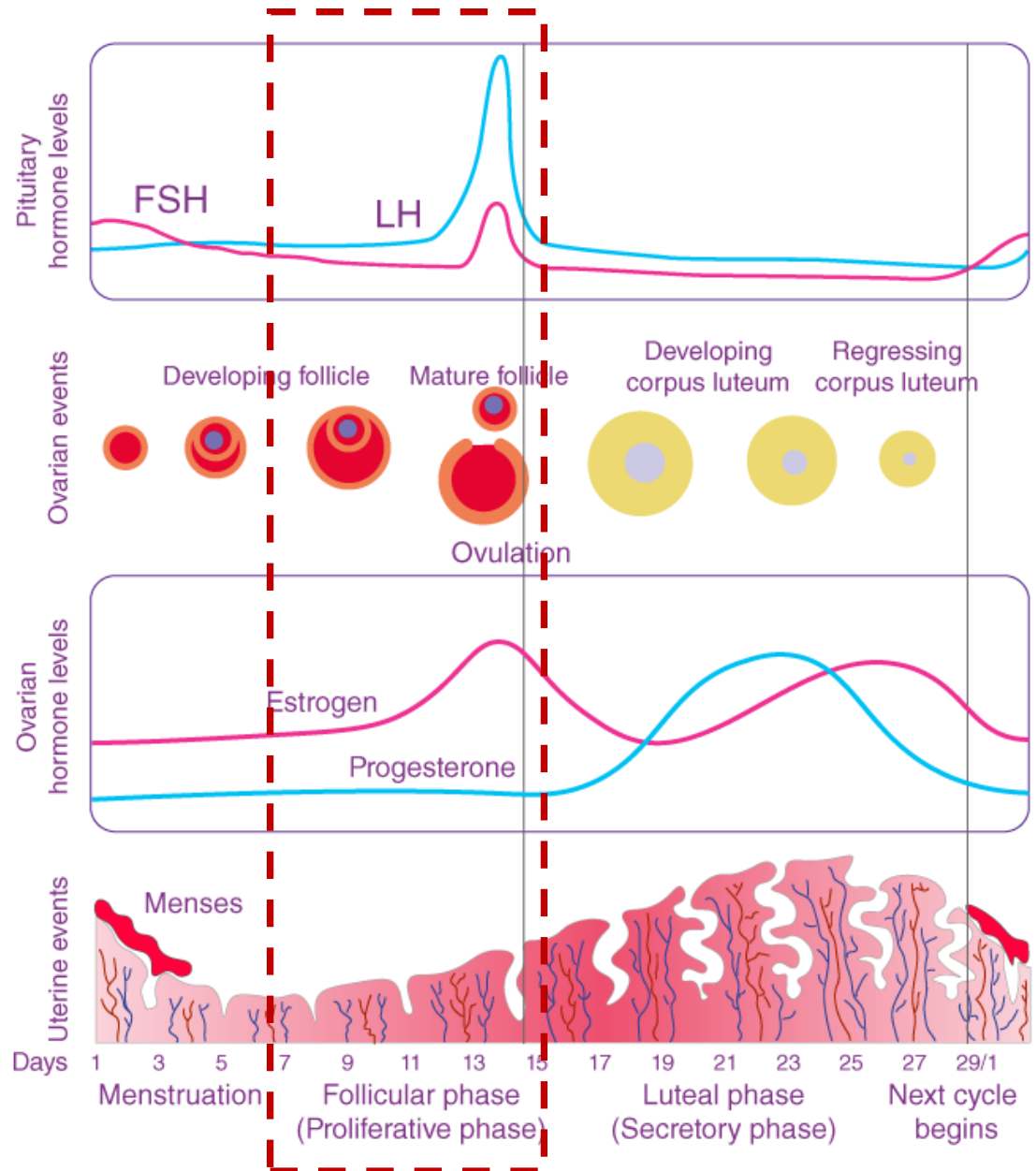
(initiate gland reformation)

- E2 induces cell proliferation and expression of P4 receptor

→ endometrium

thickening and priming

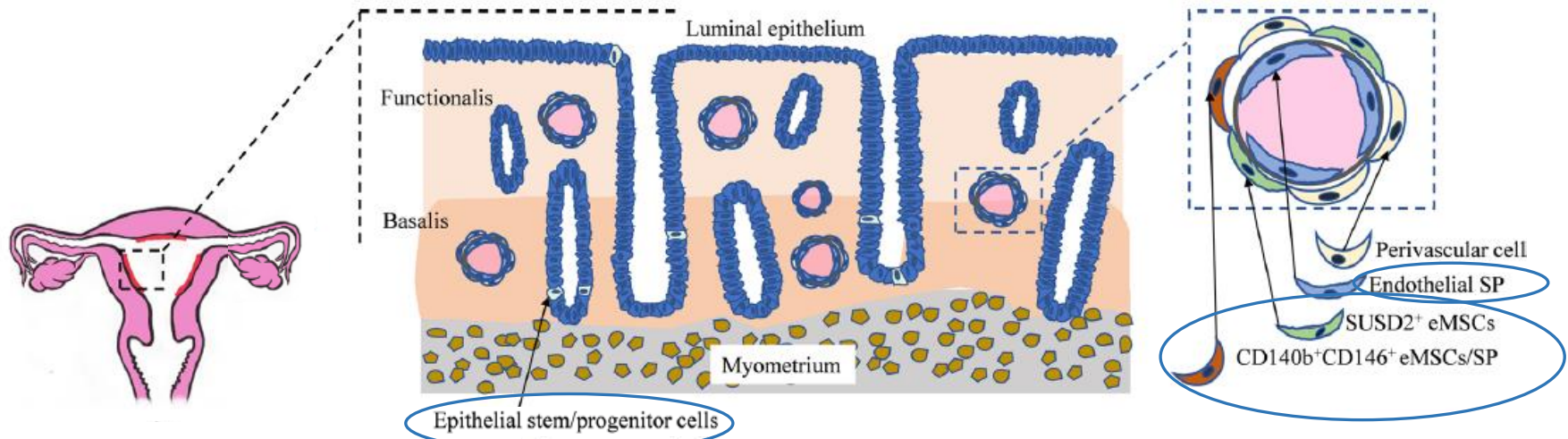
for the structural changes that will undergo during the secretory phase



Proliferative phase

❖ UTERINE STEM CELLS

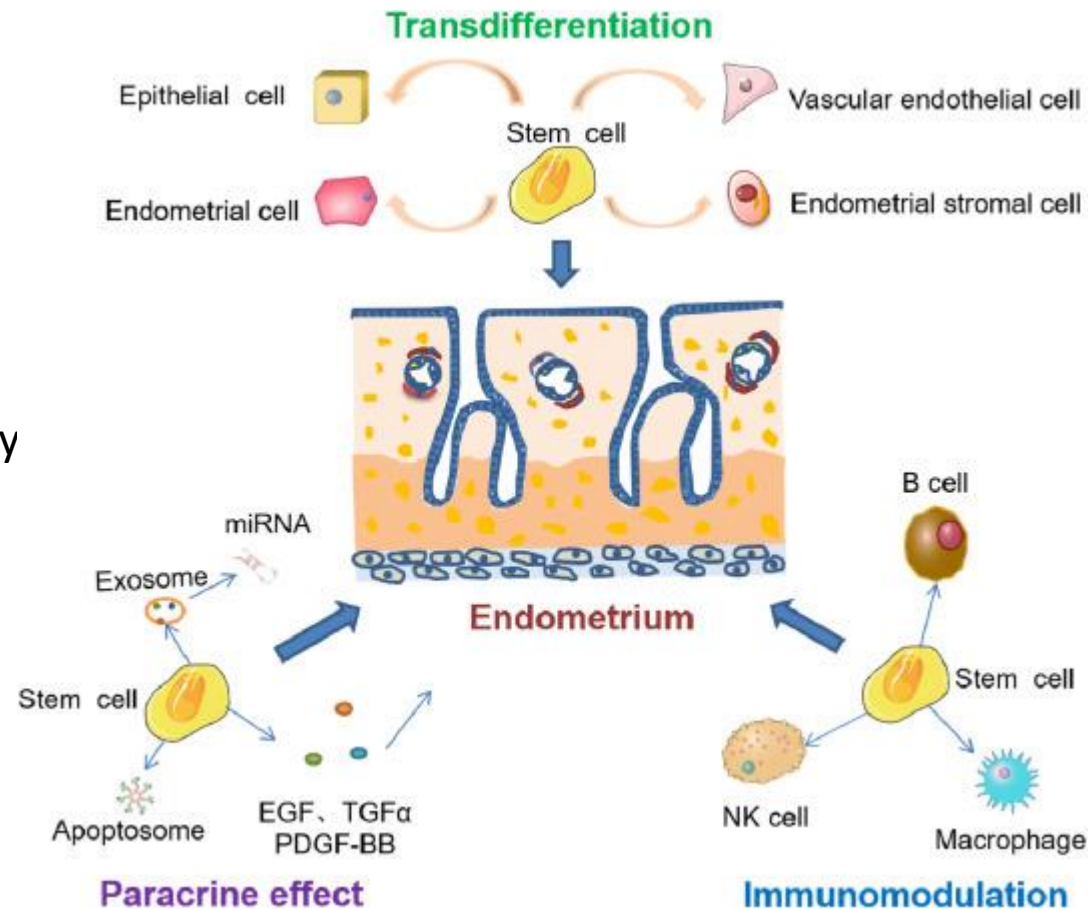
- rare clonogenic population with high proliferative potential
- **capable of self-renewal and differentiation** to one or more lineages of specialized tissue cells
- reside predominantly in **basalis endometrial layer** (epithelial + stromal compartment)
 - Epithelial progenitors → glandular epithelium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



Proliferative phase

❖ UTERINE STEM CELLS

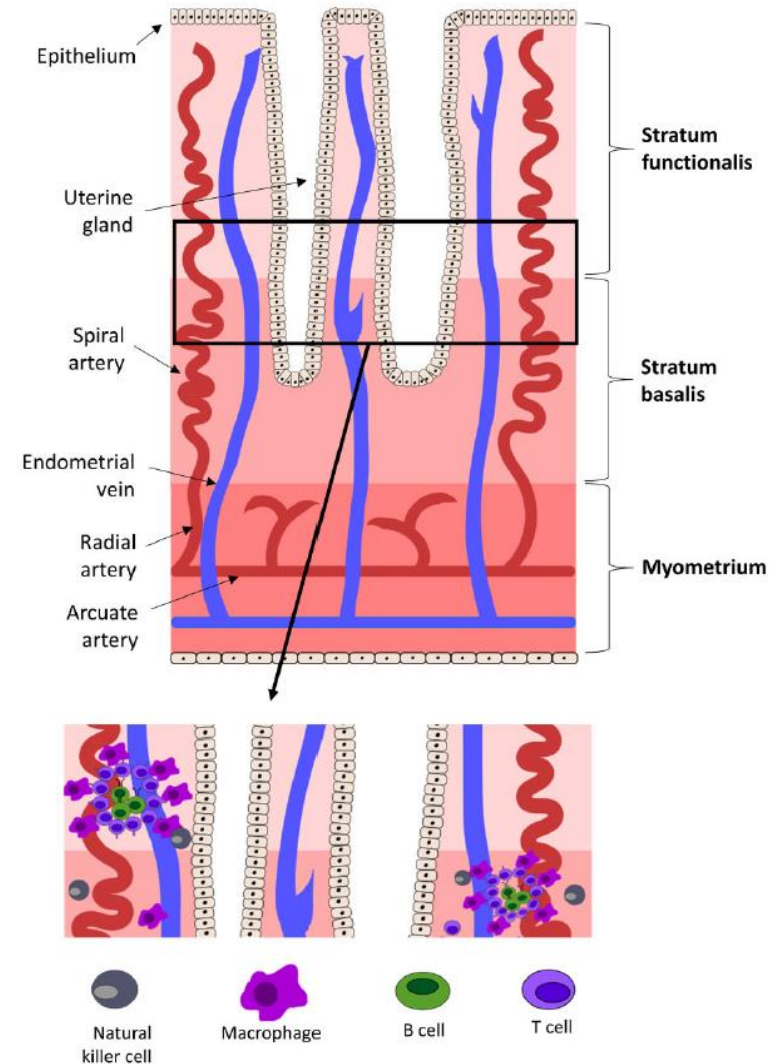
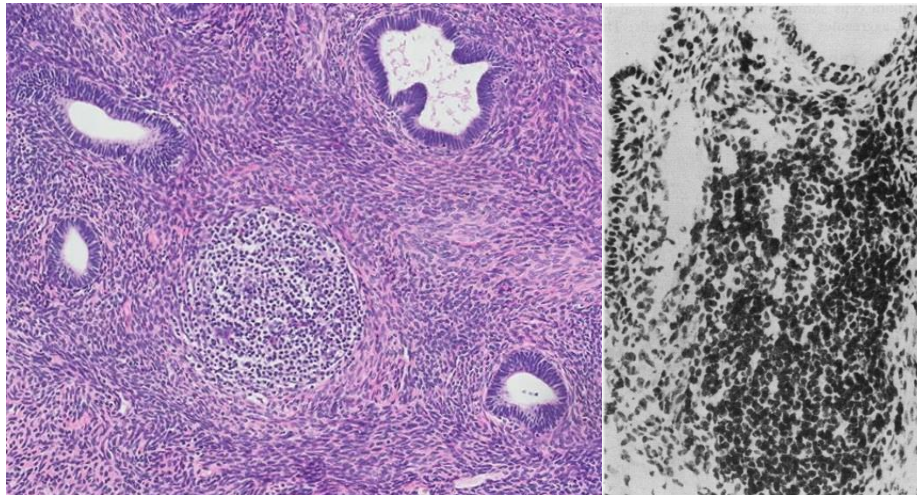
- essential for endometrial, stromal and vascular regeneration following menstruation and parturition
- distinct populations giving rise to different endometrial cell types, multizonal differentiation hierarchy
- repopulate *functionalis* and generate proangiogenic and paracrine factors promoting angiogenesis and immunosuppression
- dysregulated function leads to cancer



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



Proliferative phase

- cellular specification and tissue patterning in endometrial tissue

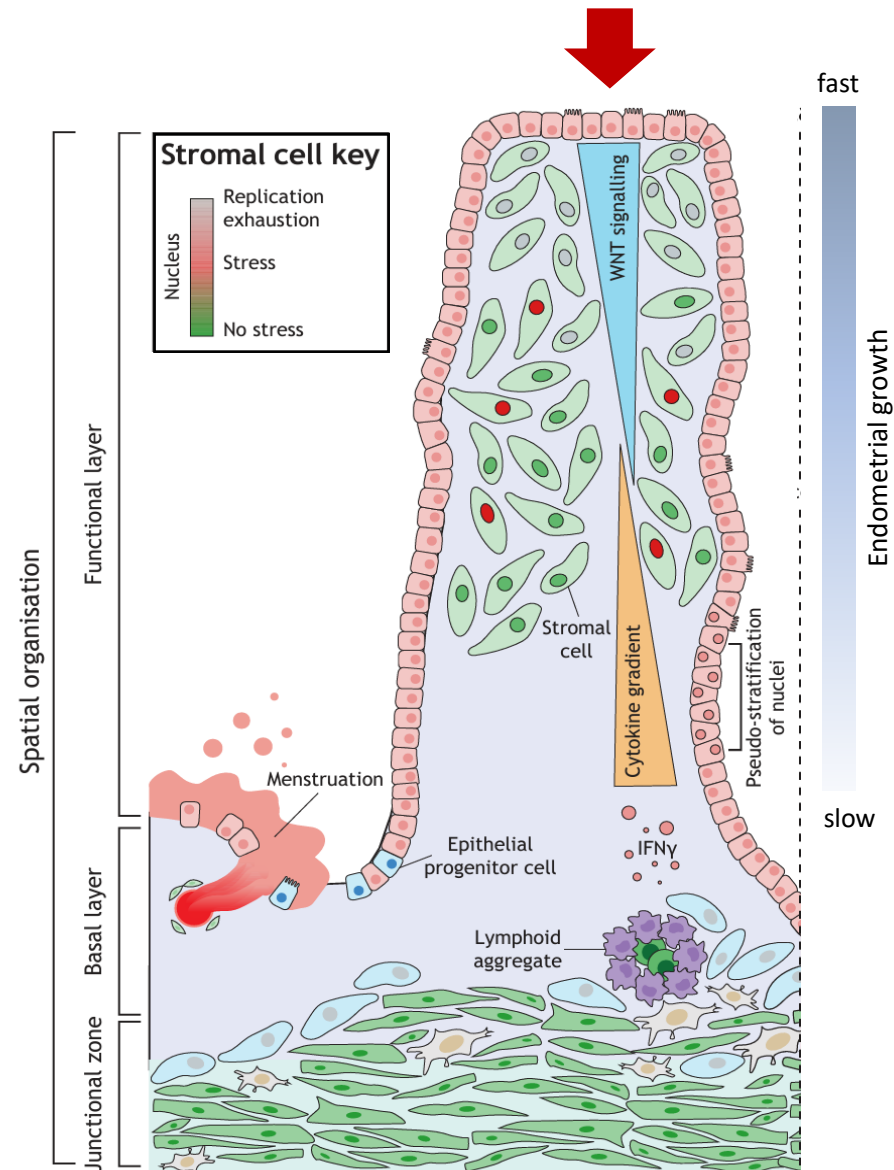
Cytokine and morphogen gradient:

- **INF γ** 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

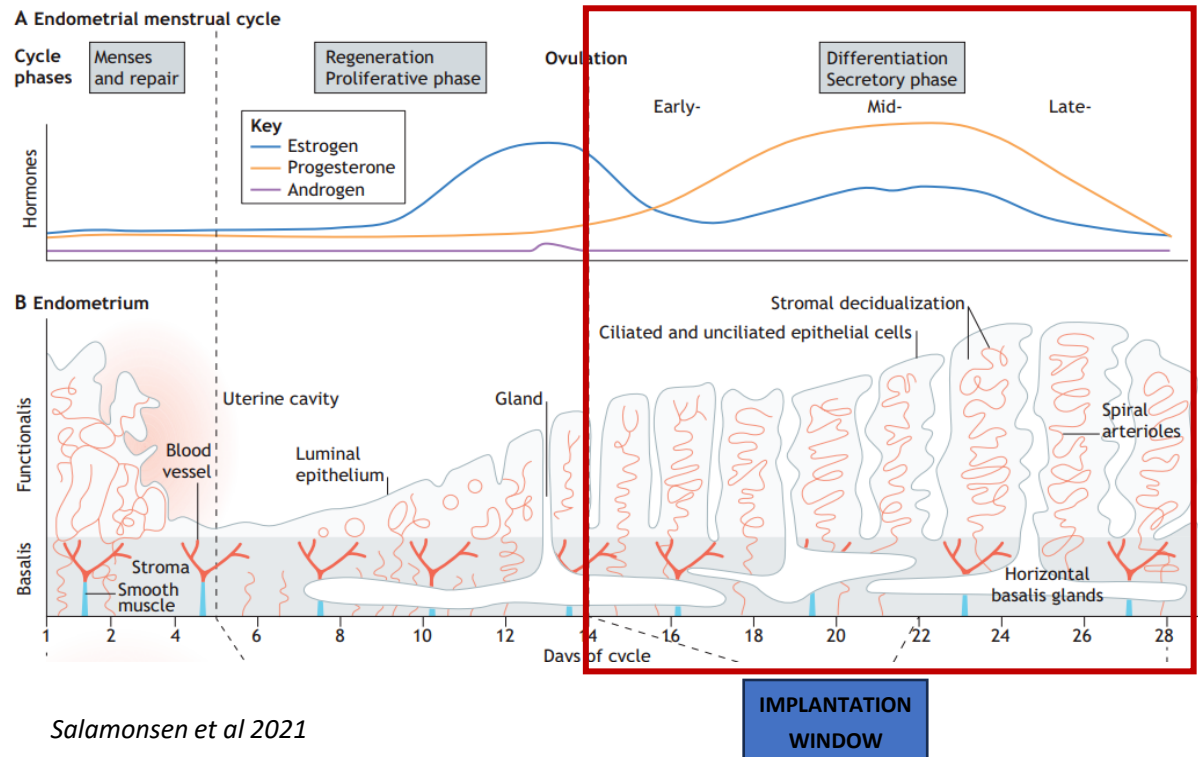


Secretory phase

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

Features:

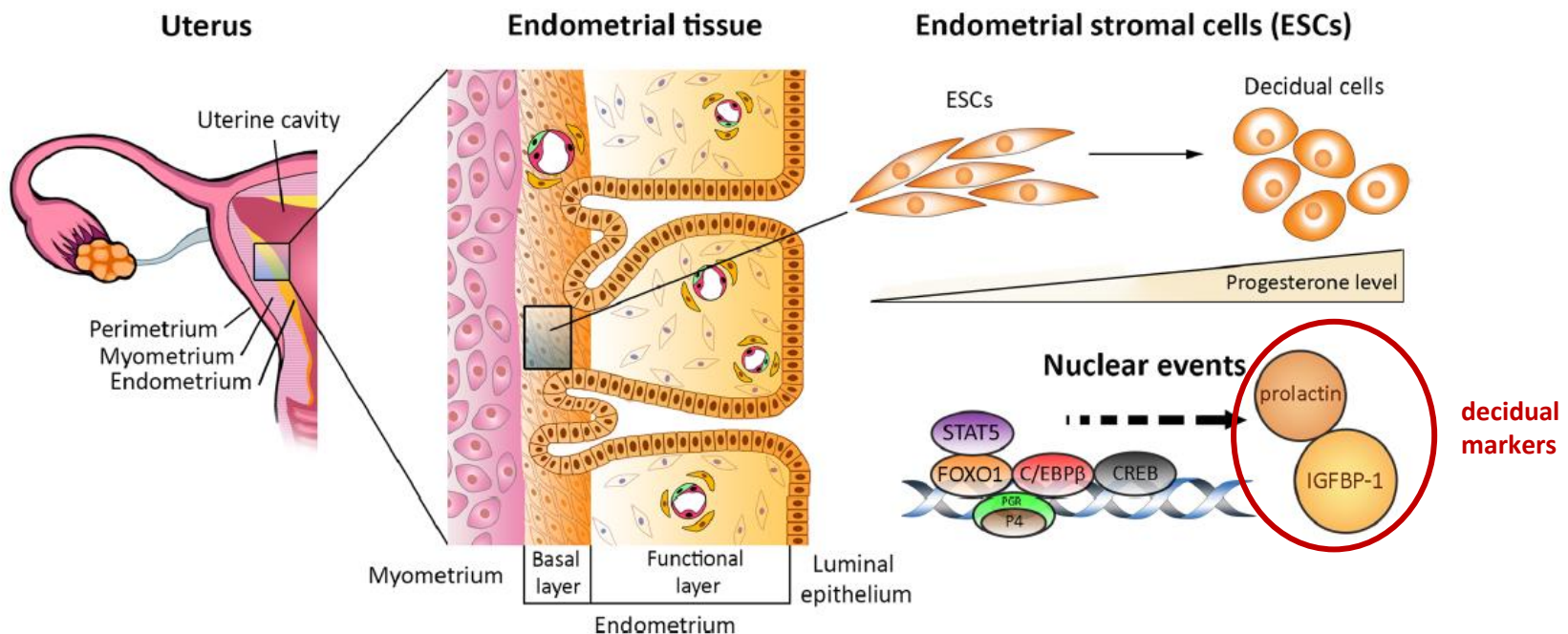
- ↓ endometrial cell proliferation
- ↑ adhesive capacity of epithelial cells
- ↑ glandular secretion
- differentiation of stromal cells
- development of spiral arteries
- stromal edema
- stem cell recruitment
- influx of immune cells



Secretory phase

❖ DECIDUALIZATION (= DECIDUAL REACTION)

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



Secretory phase

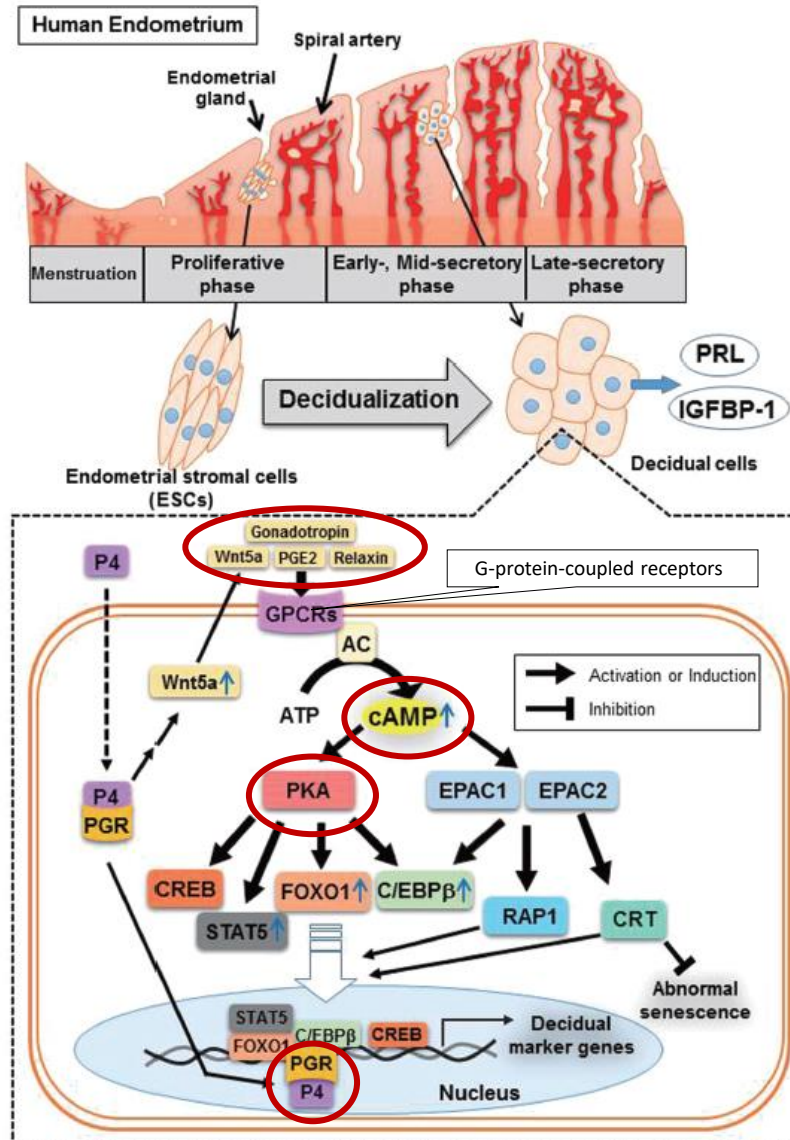
❖ DECIDUALIZATION

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 inducers of decidualization *in vitro*
- pharmaceutical modulation of cAMP signalling pathway can affect implantation efficiency

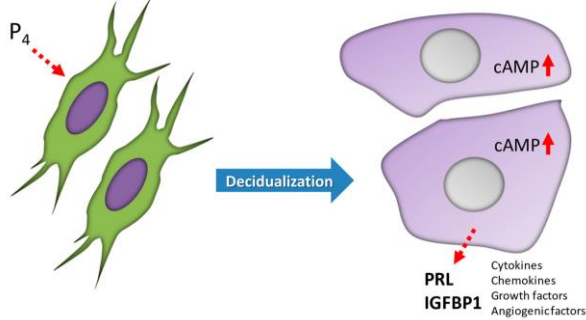


Jan Brosens

Secretory phase

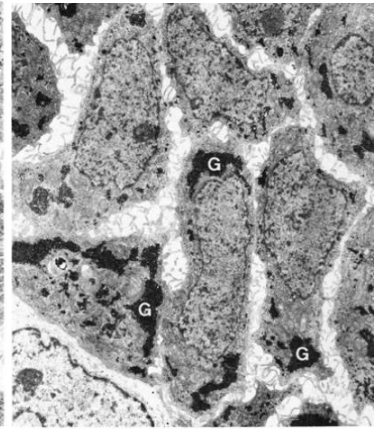
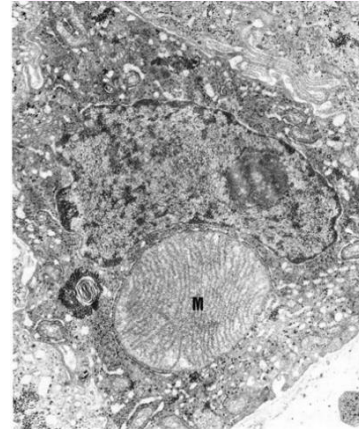
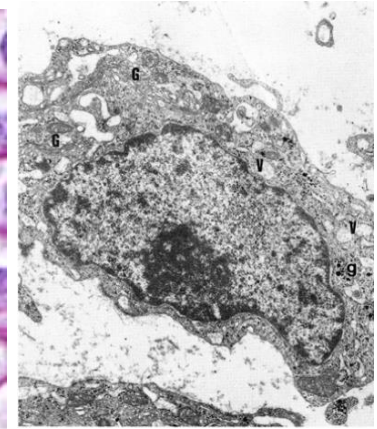
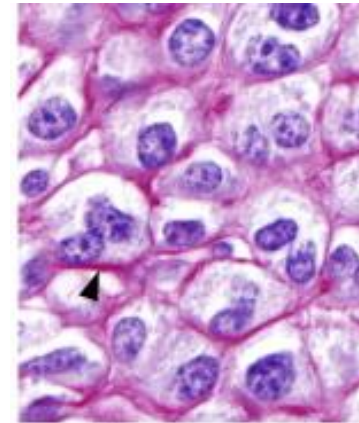
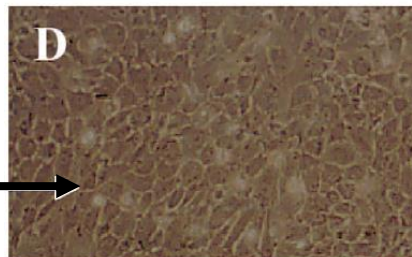
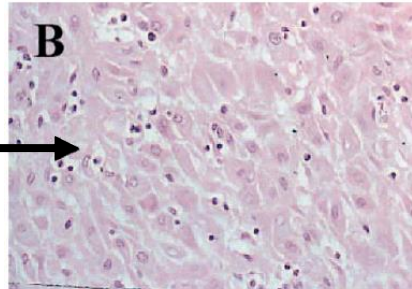
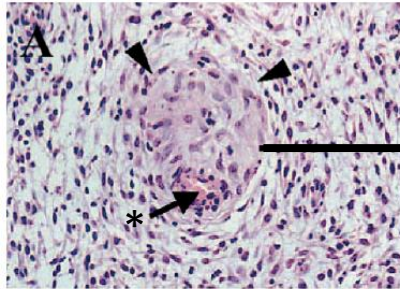
❖ DECIDUALIZATION

- morphological change:



fibroblastic-phenotype
Spindle-shaped cells

large rounded cells with large nuclei
and abundant cytoplasm



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

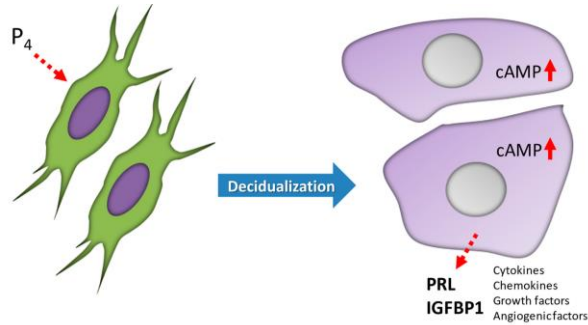
* decidualization starts around spiral arteries

Gellersen and Brosens 2003

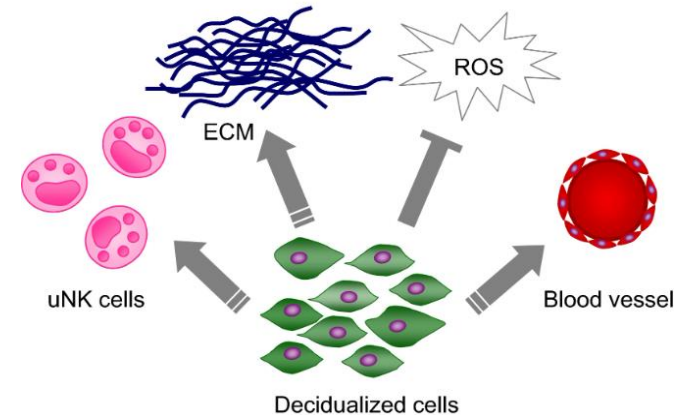
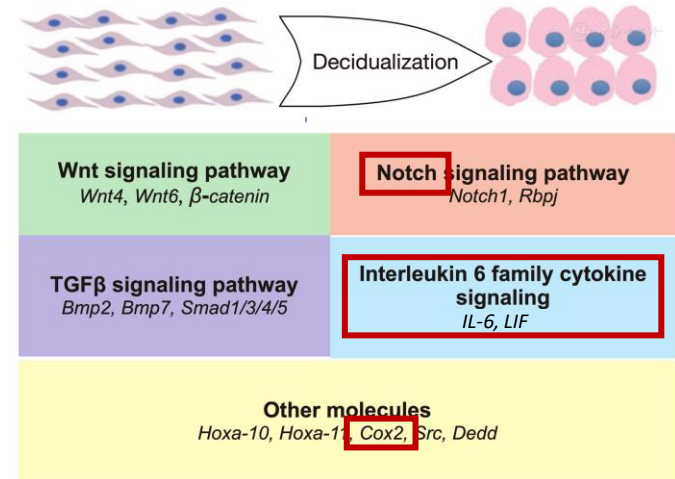
Secretory phase

❖ 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 proinflammatory regulators
(particularly in decidual-like senescent cells damaged by replication stress in the proliferation phase)
- accumulation of uterine NK cells (uNK)

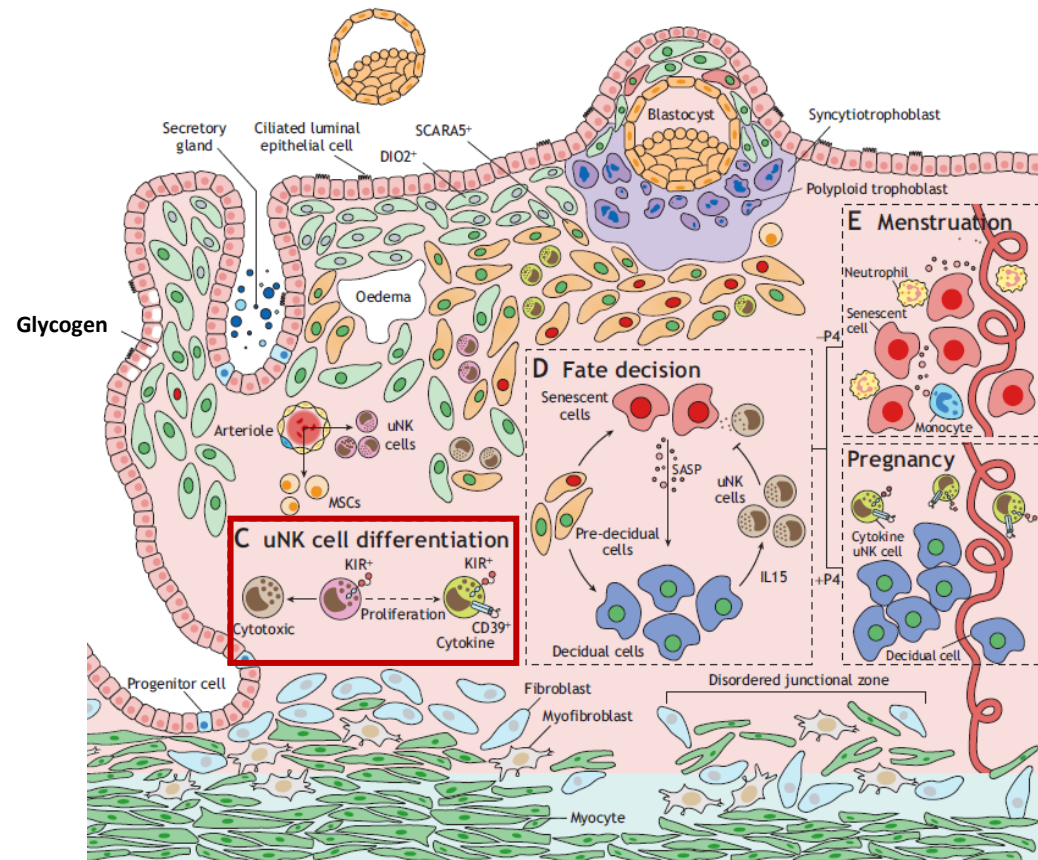


Secretory phase

❖ 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

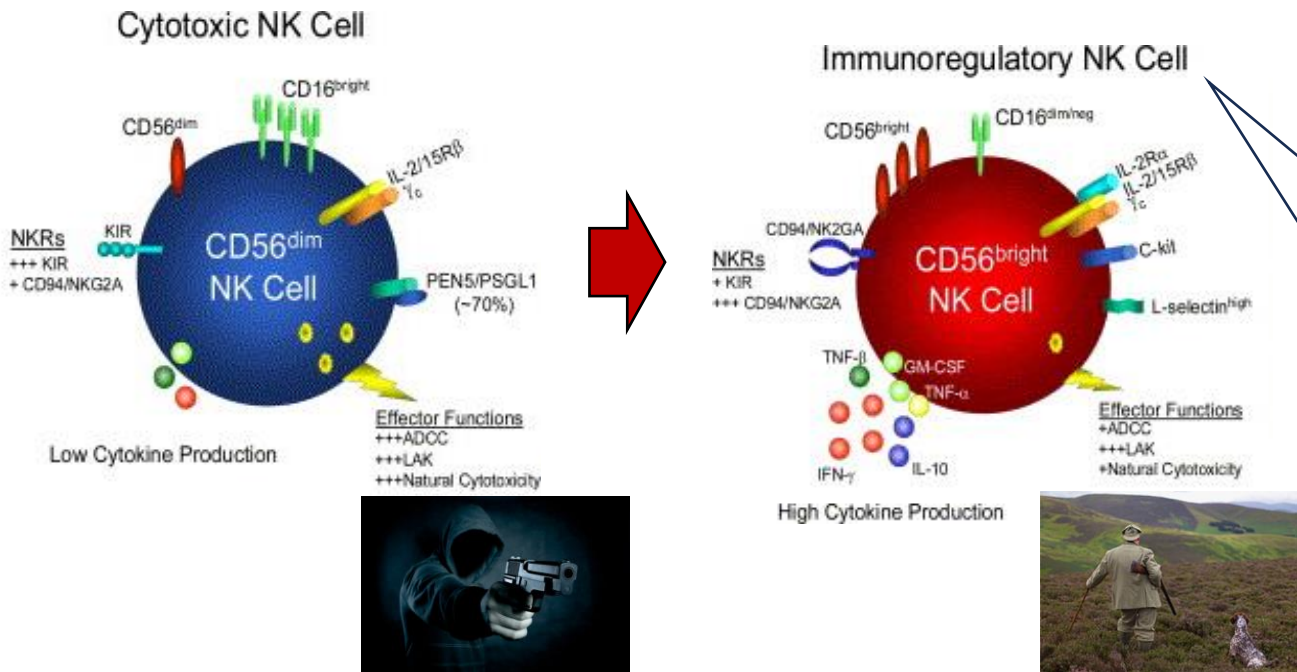


Secretory phase

❖ DECIDUALIZATION

• Uterine Natural Killer cells (uNK)

- switch from pro-inflammatory phenotype to immunomodulatory, cytokine-producing, and angiogenic phenotype

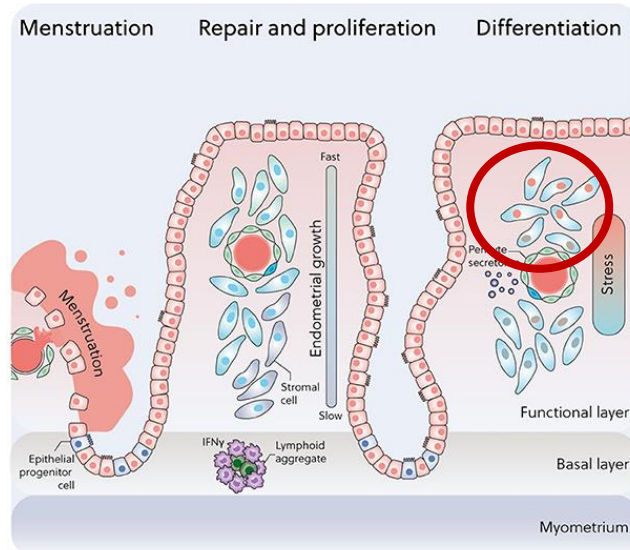


KIRs+ (killer cells immunoglobulin-like receptors)
CD56^{bright} CD16⁻
CD39⁺ CD9⁺ CD49a⁺ CD57⁻
Ki67 nuclear proliferation marker

Farag and Caligiuri 2006

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

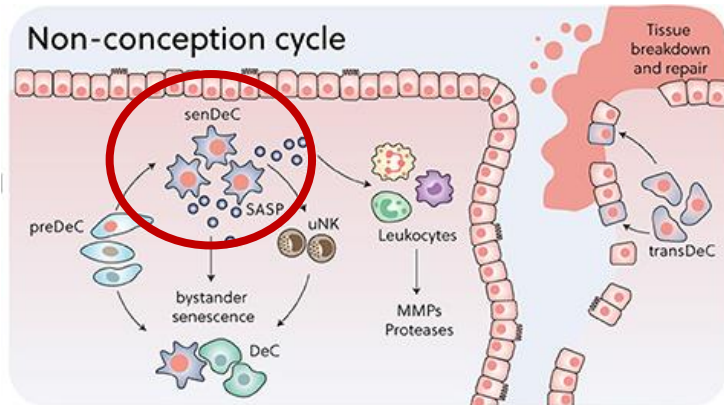
Menstruation versus pregnancy



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

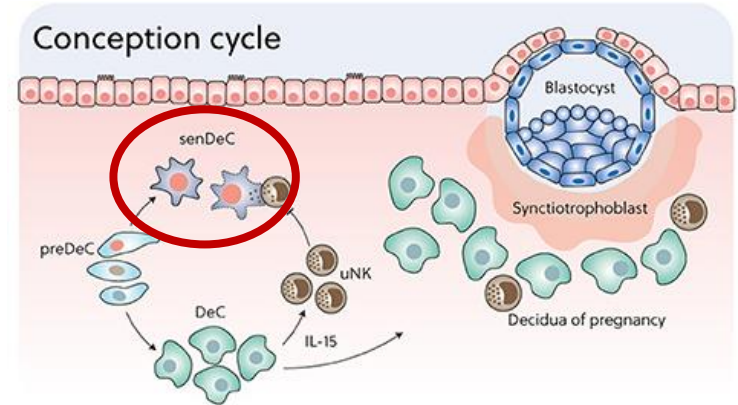
↓ P4



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

Endometrium breaks down → menstruation

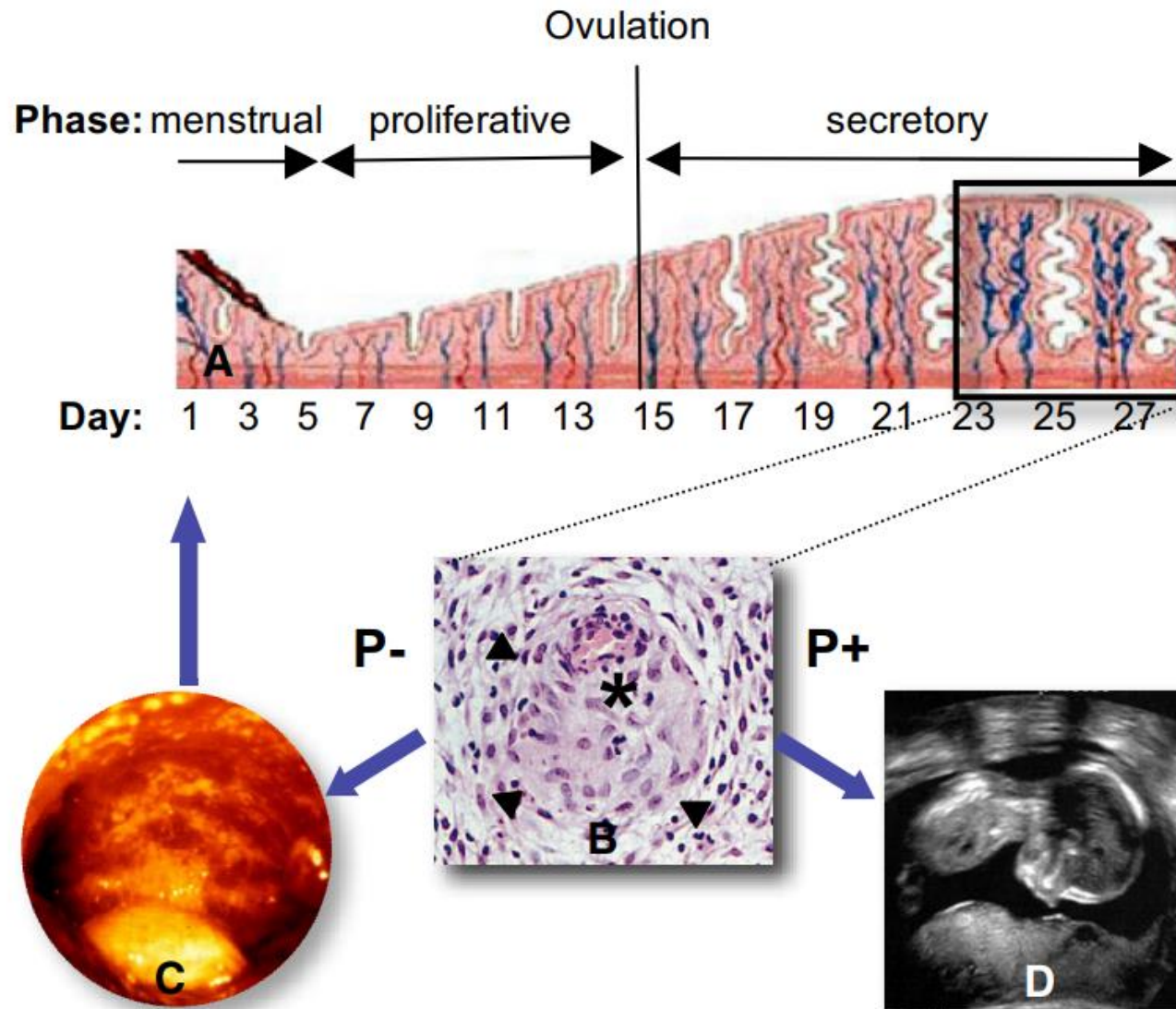
↑ P4



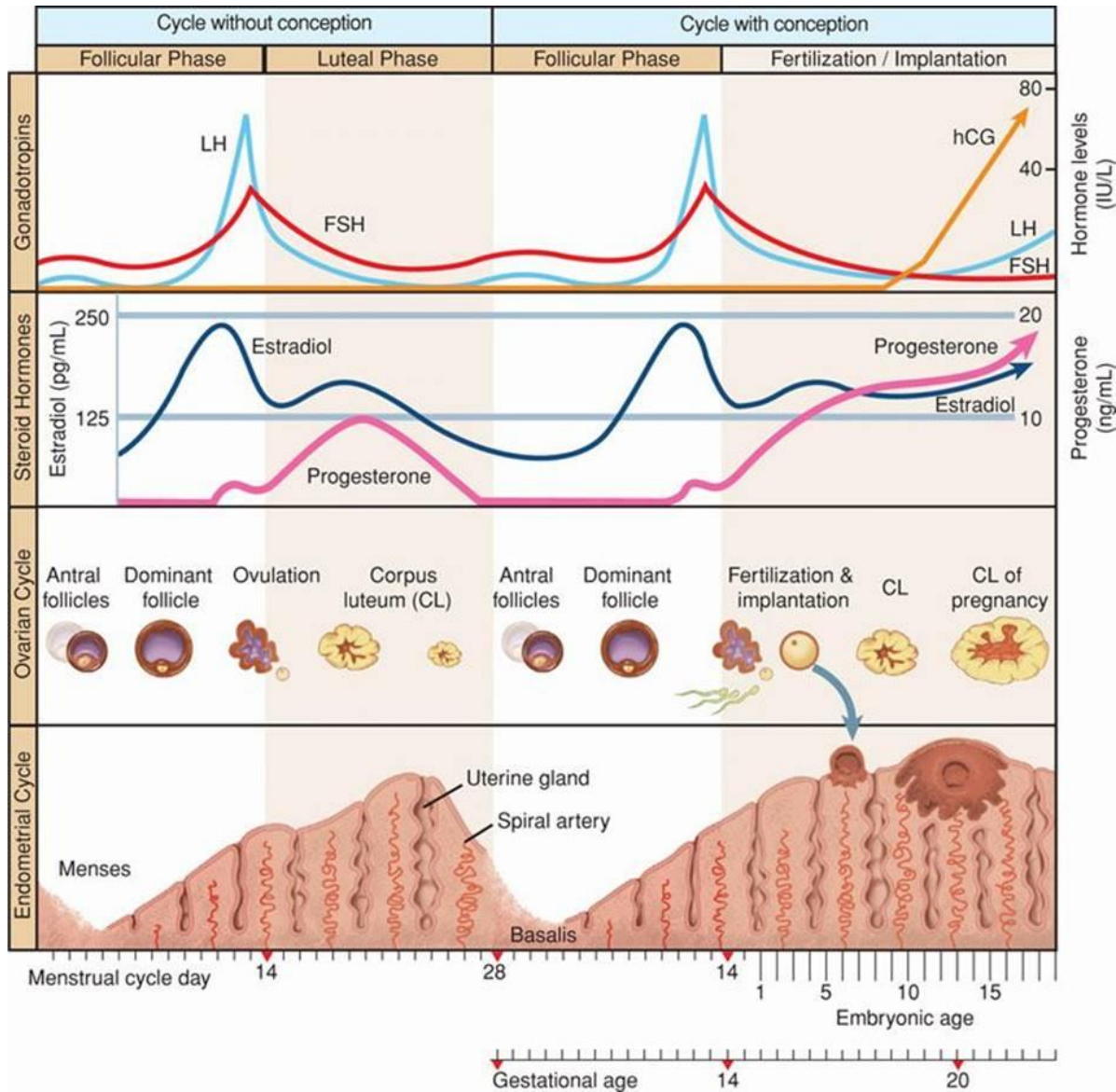
- P4 sensitive decidual cells engage uNK to eliminate senescent cells

Endometrium → decidua bed of pregnancy

Menstruation versus pregnancy

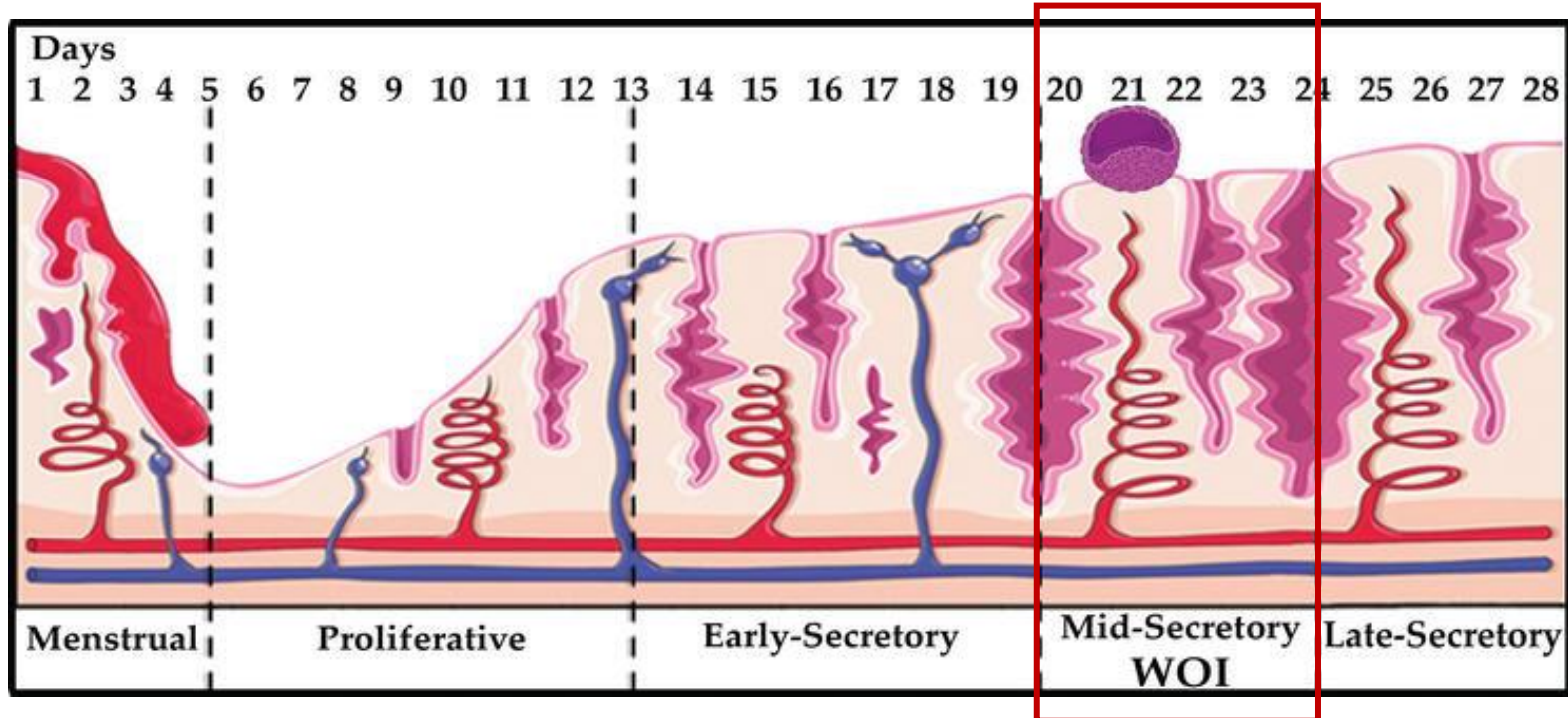
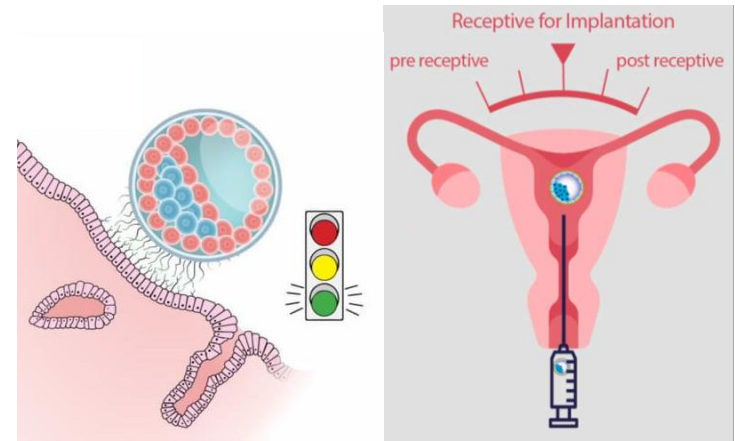


Menstruation versus pregnancy



Endometrial receptivity

- uterine lining preparation for an embryo implantation
- **WINDOW OF IMPLANTATION (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

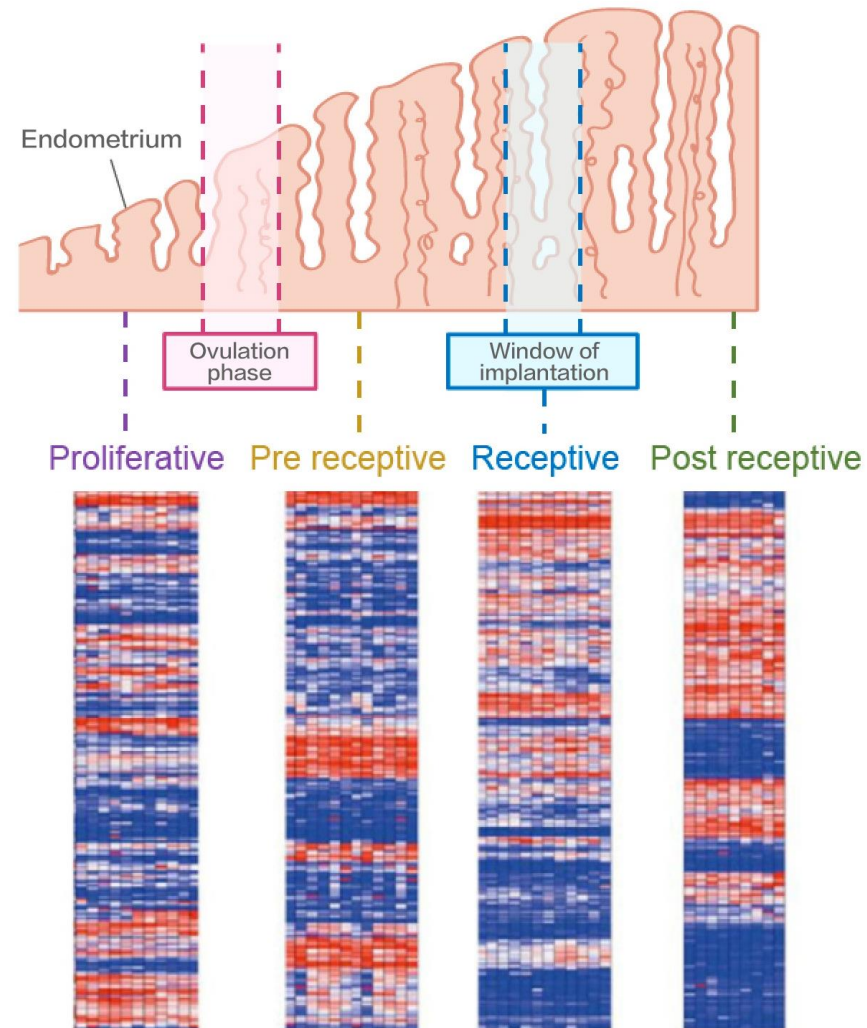
✓ ENDOMETRIAL RECEPTIVITY ARRAY (ASSAY/ANALYSIS) – „ERA“

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



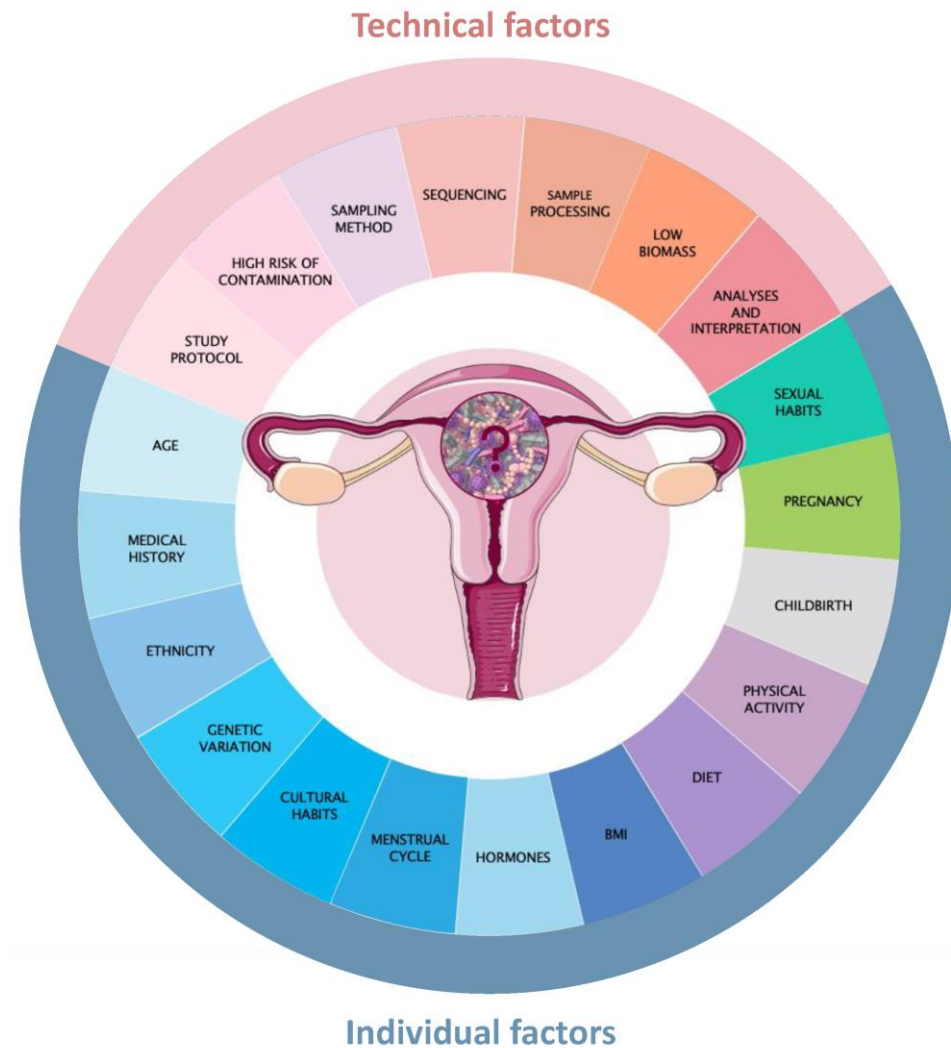
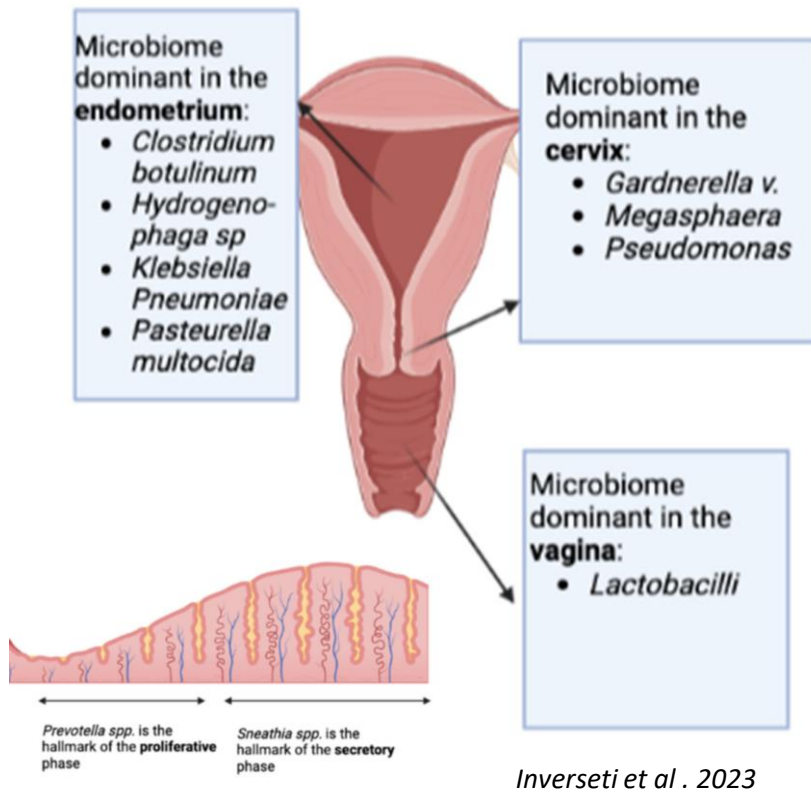
identification of patient's WOI for personalized embryo transfer

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



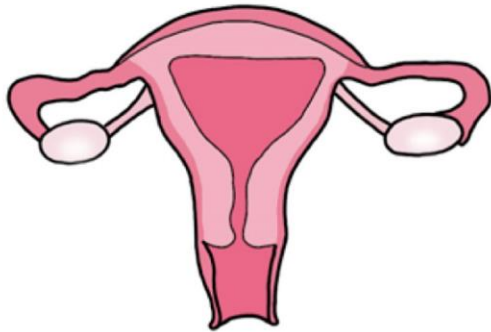
Uterine microbiome

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



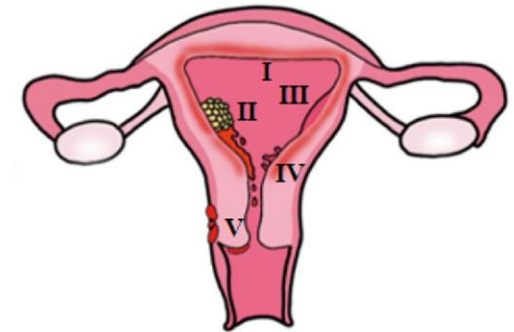
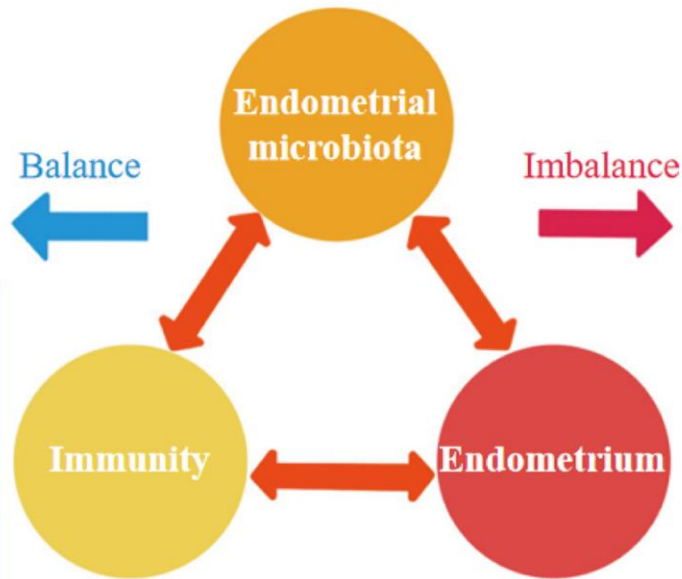
- dysbiosis associated with adverse reproductive outcomes
- ideal composition?
- benefits of species diversity?

Uterine microbiome



Balance of uterine microecology

- ① Mucosal barrier function ↑
- ② Risk of endometrial pathological changes (eg, inflammation, polyps, hyperplasia, endometriosis and cancer) ↓
- ③ Uterine functions (eg, endometrial immune tolerance, endocrine function) ↑



Imbalance of uterine microecology

- ① Mucosal barrier function ↓
- ② Risk of endometrial pathological changes (eg, inflammation, polyps, hyperplasia, endometriosis and cancer) ↑
- ③ Uterine functions (eg, endometrial immune tolerance, endocrine function) ↓

Uterine microbiome

❖ Uterine microbiome diagnostic tests

EndomeTRIO

The endometrium matters

ENDOMETRIAL RECEPTIVITY ANALYSIS
Expression of 248 genes to guide pET*

+

COMPLETE MICROBIOME ANALYSIS
Percentage of Lactobacilli, pathogens and dysbiotic bacteria
Microbiological counselling for a personalised treatment

+

CHRONIC ENDOMETRITIS
Pathogenic bacteria related to CE
Microbiological counselling for a personalised treatment

ERA®

Endometrial Receptivity Analysis

ENDOMETRIAL RECEPTIVITY ANALYSIS
Expression of 248 genes to guide pET*

EMMA

Endometrial Microbiome Metagenomic Analysis

COMPLETE MICROBIOME ANALYSIS
Percentage of Lactobacilli, pathogens and dysbiotic bacteria
Microbiological counselling for a personalised treatment

+

CHRONIC ENDOMETRITIS
Pathogenic bacteria related to CE
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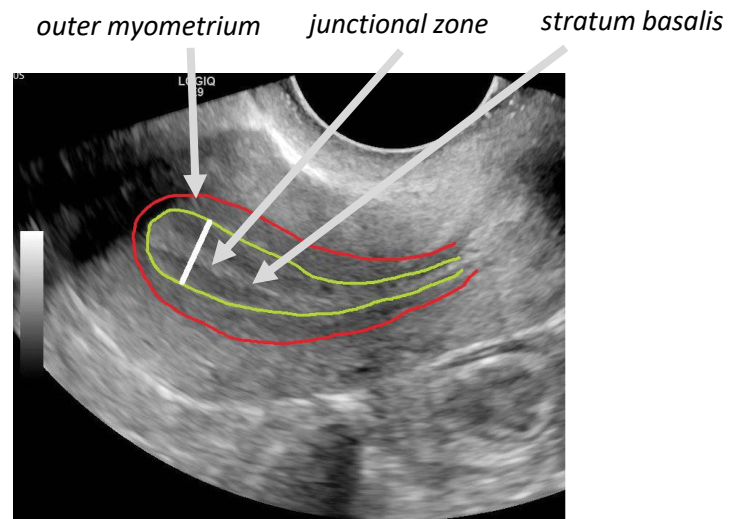
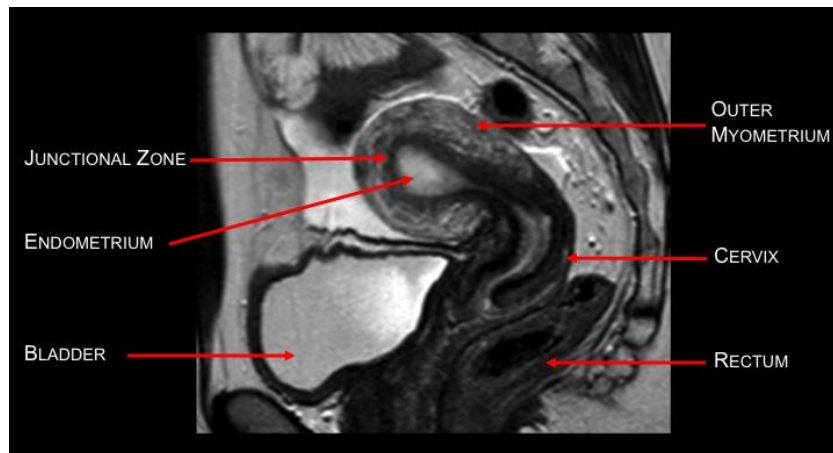
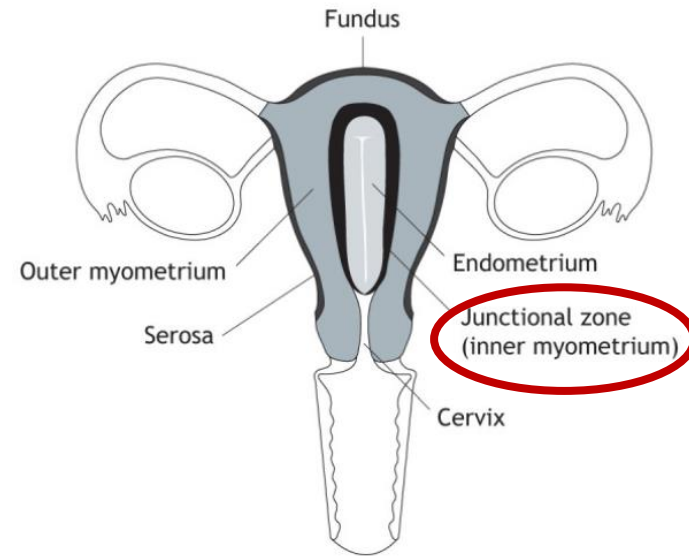
ALICE

Analysis of Infectious Chronic Endometritis

CHRONIC ENDOMETRITIS
Pathogenic bacteria related to CE
Microbiological counselling for a personalised treatment

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
- undergoes hormone-dependent **contraction and remodelling** during the menstrual cycle
 - trans-differentiation stromal fibroblast to myocytes
 - cervico-fundal contractions facilitate → sperm transport during the fertile window; fundo-cervical contractions → flow of effluent during menstruation

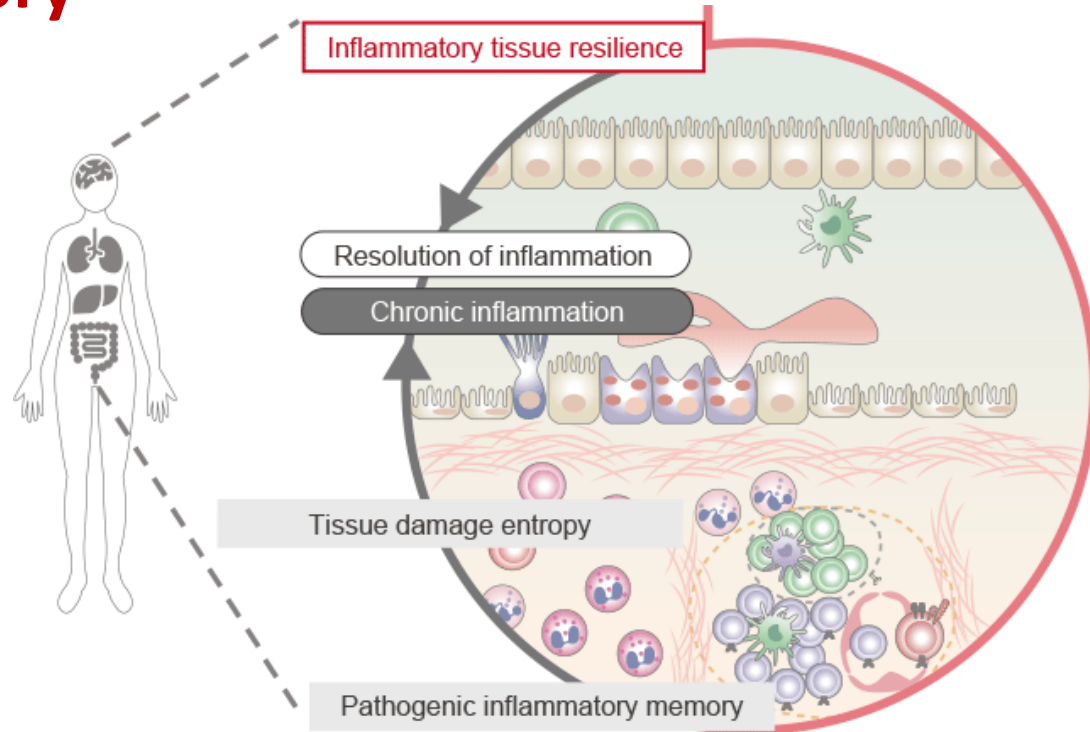


Triple line measurement

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

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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



Menstrual disturbances

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



Menopausal amenorrhea

= fall in estrogen production due to ovarian reserve exhaustion → **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

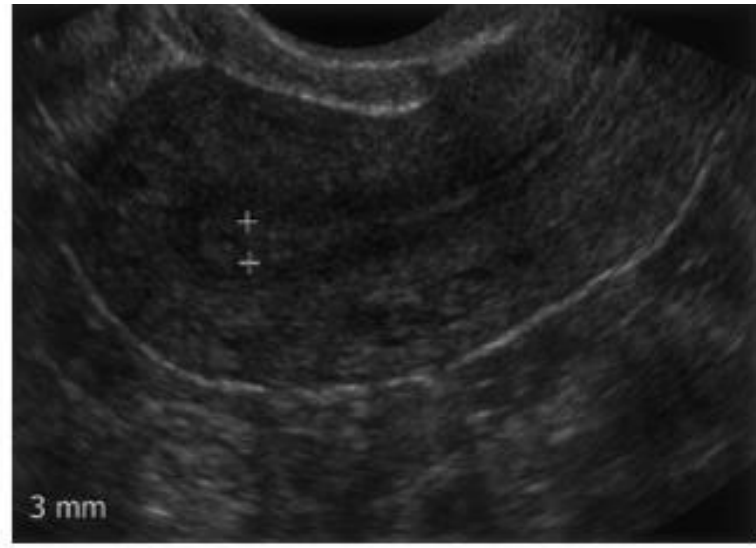
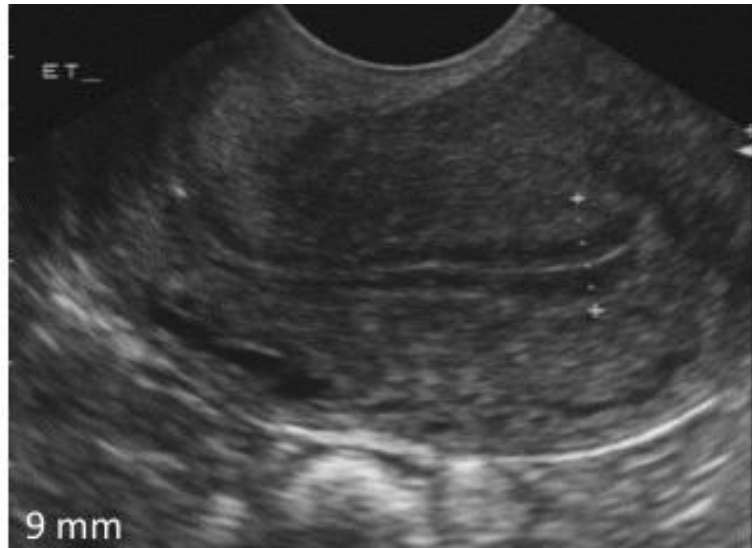
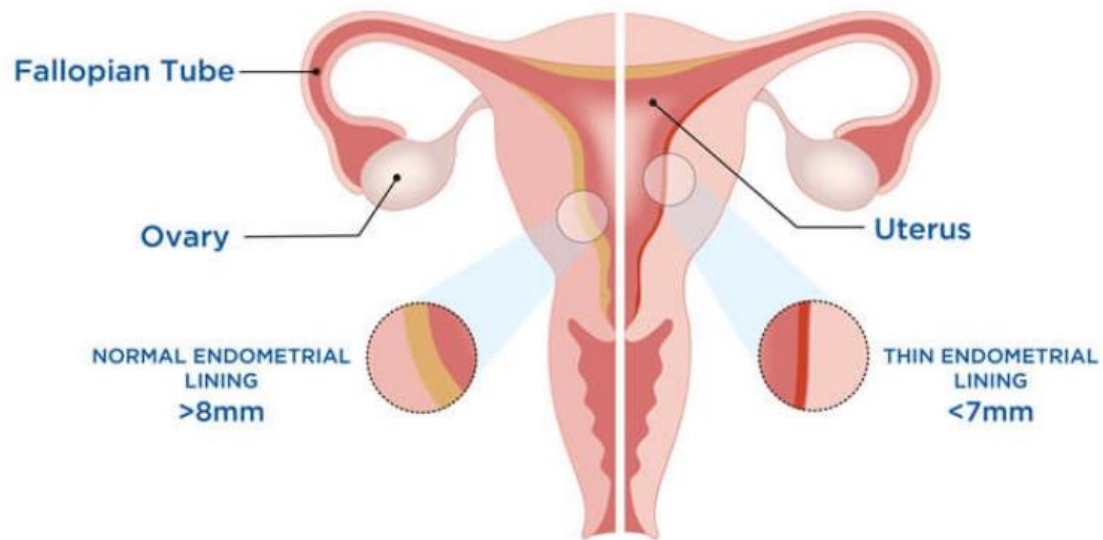


normal endometrium
(late proliferative phase)



postmenopausal endometrium

Thin endometrium



Triple line ultrasound measurement

Thin endometrium

Possible Causes

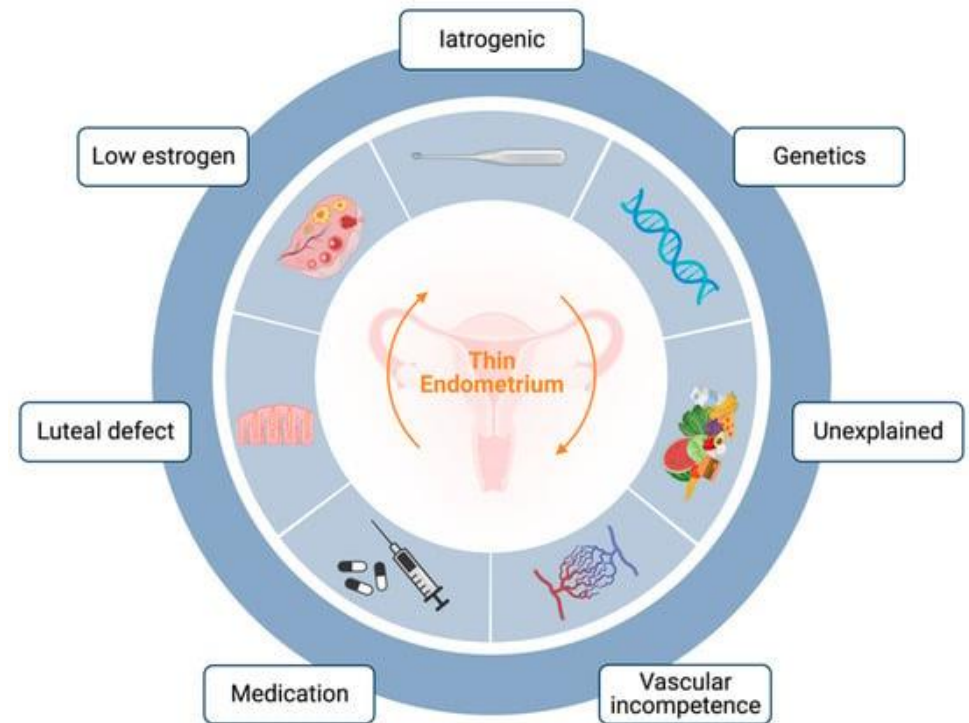
- Low estrogen levels
- Luteal defects
- Advanced age
- Fibroids
- Genetic factors
- Intrauterine Adhesions
- Poor blood flow
- Pelvic surgeries/inflammation
- Iatrogenic effect

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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EMBRYO TRANSFER IN THIN ENDOMETRIUM

Live birth after embryo transfer in an unresponsive thin endometrium

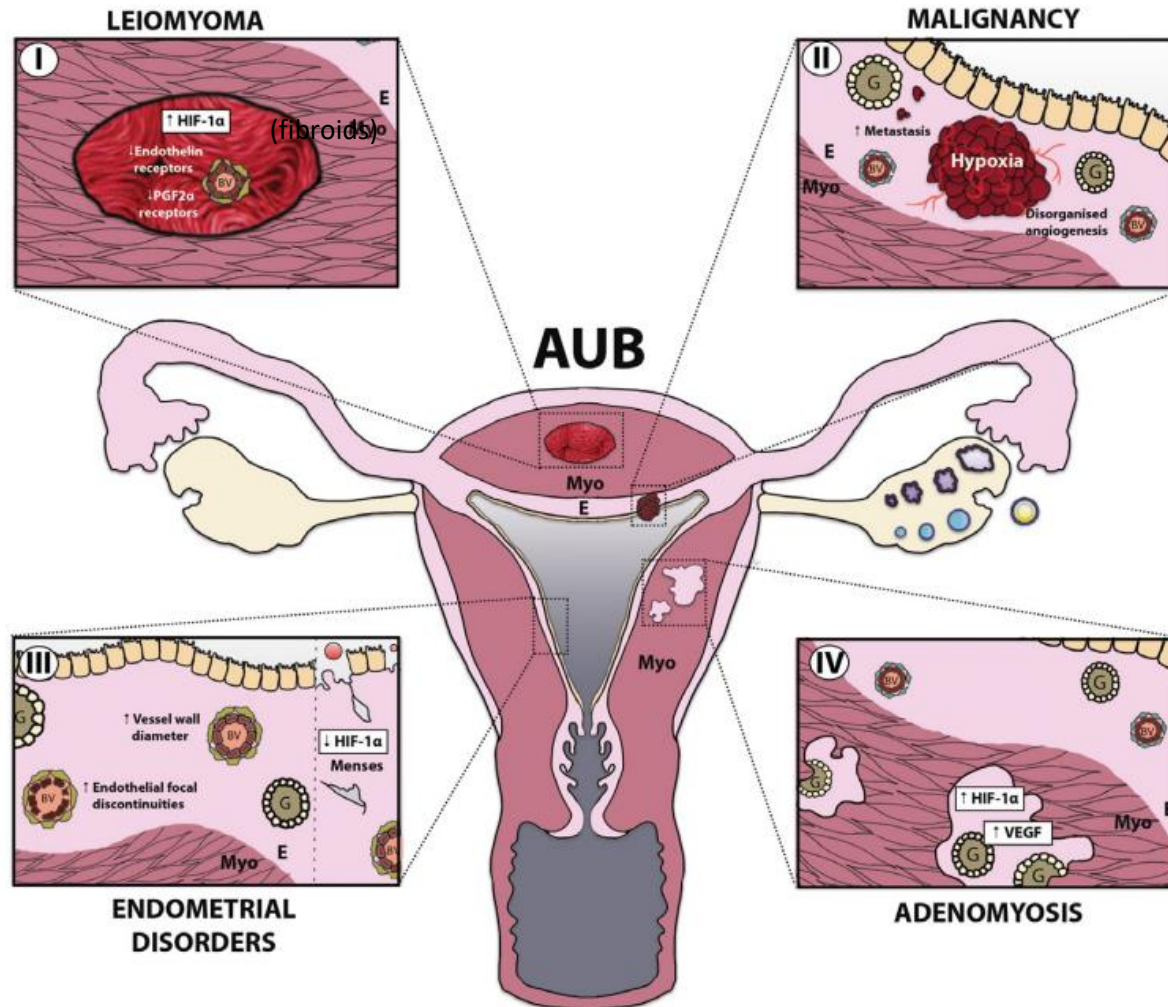
Fábio Cruz¹ and José Belver^{1,2,3}

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



Abnormal uterine bleeding (AUB)

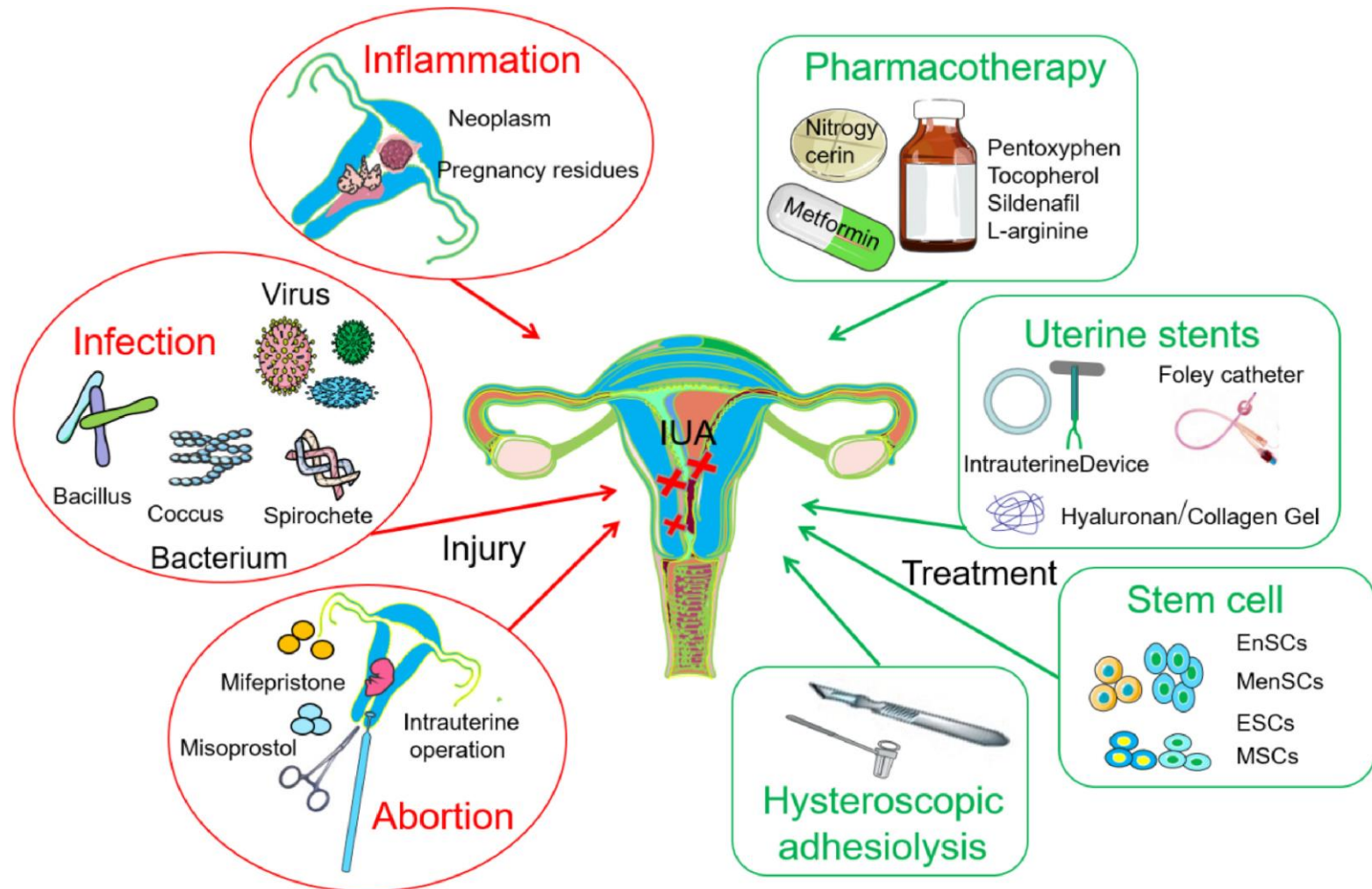
- altered tissue oxygenation and HIF-regulation are suspected to underlie AUB conditions



Intrauterine adhesions (IUA)

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

→ failure of adequate repair and regeneration



Intrauterine adhesions (IUA)

❖ STEM CELLS/PROGENITORS CELLS THERAPY

- non-invasive harvest from the menstrual fluid



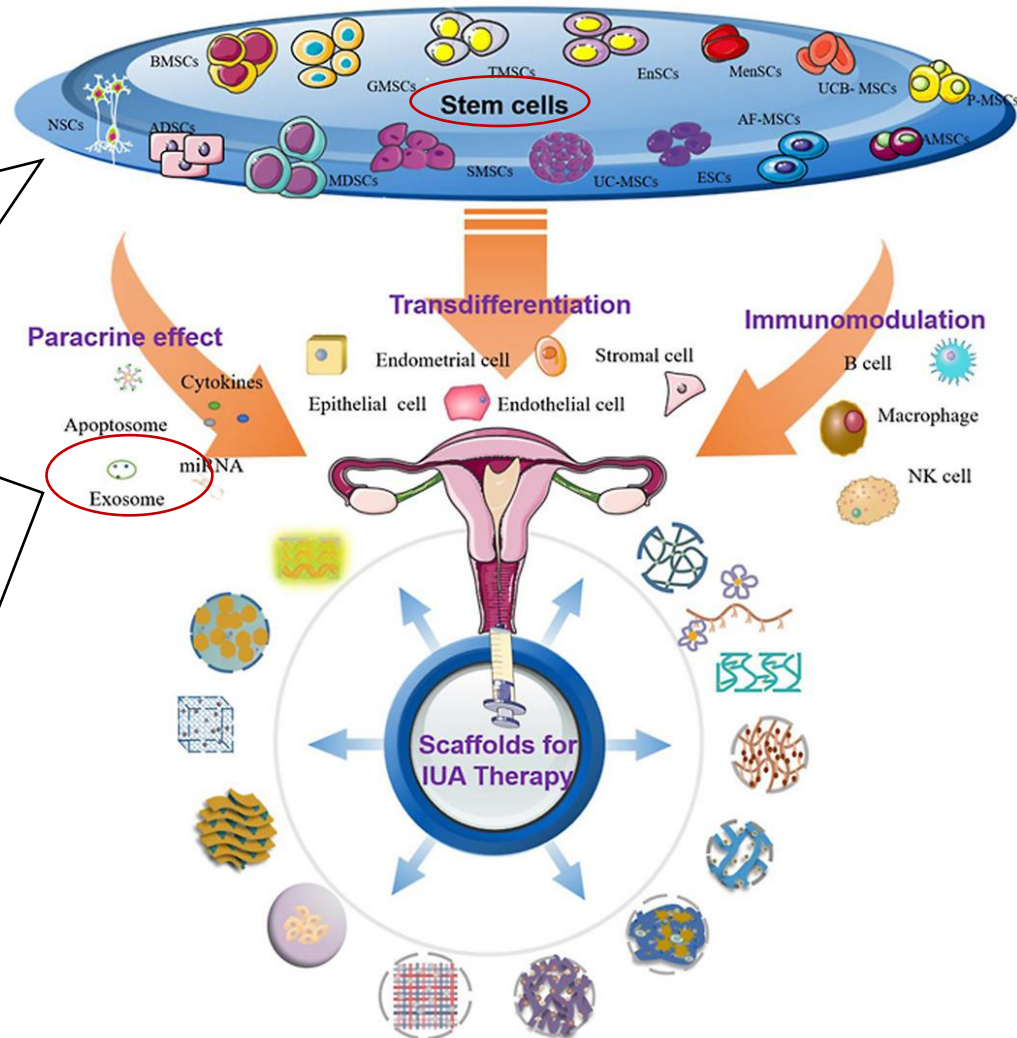
- short-lived
- risk of off-target migration and tumor growth

MSC-secreted exosomes

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

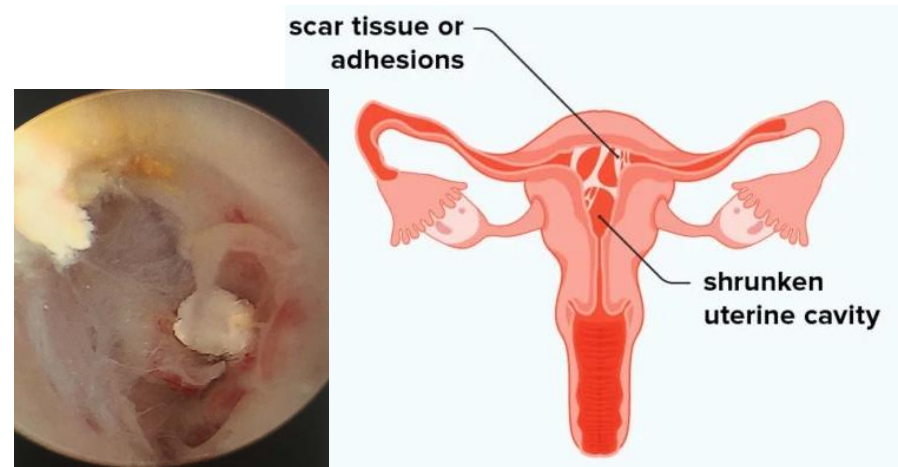
- ↑ epithelisation and wound healing
- ↑ collagen maturity
- ↓ scarring

- wound healing therapeutical potential



Asherman syndrome

- excessive intrauterine adhesions, scarring and synechiae
- causes dysmenorrhea, 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 cell demonstrated in clinical trials



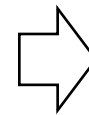
Carlos Simon

Endometriosis

= presence of cycling endometrial tissue outside of the uterine cavity



Chronic inflammation



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

Endometriosis lesions origin

✓ retrograde menstruation

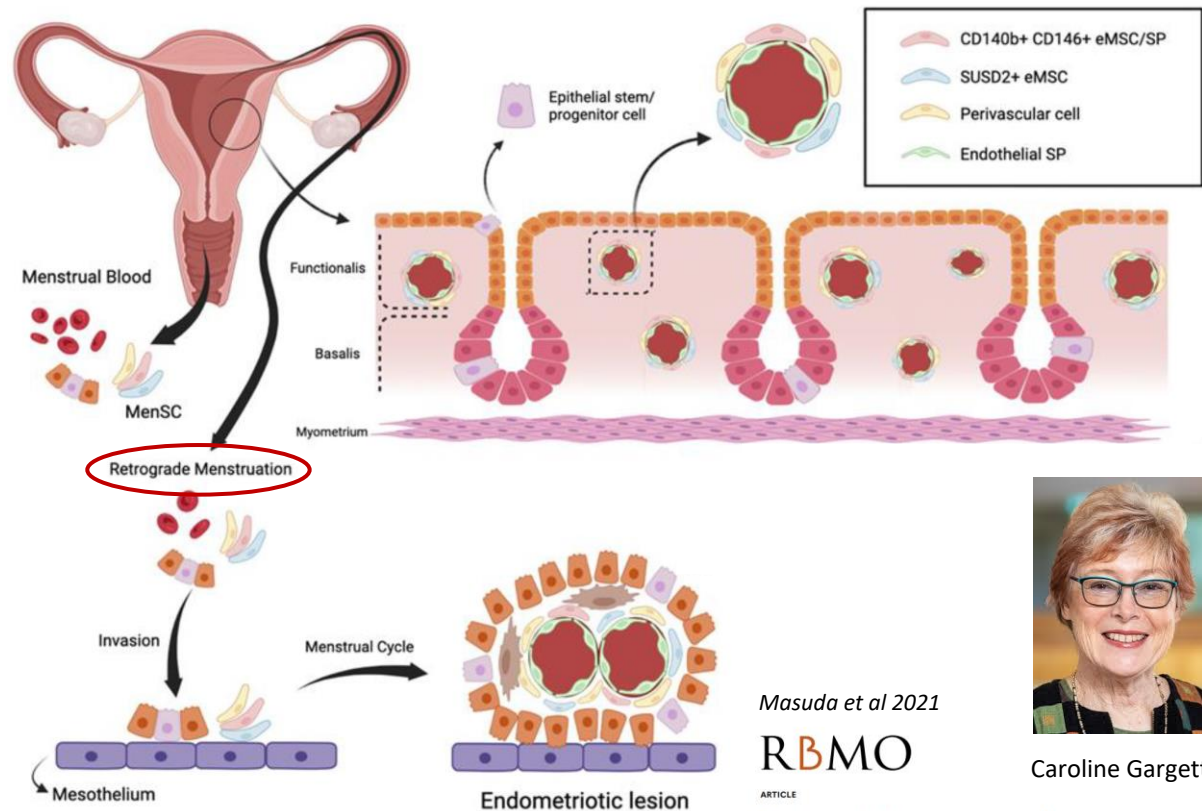
(shedding fragments of endometrium containing uterine stem cells into the Fallopian tube and pelvic cavity)

✓ 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 lesions

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



Masuda et al 2021

RBMO

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

Hirota Masuda^{1,2,*}, Kjana E. Schwab^{1,*}, C.E. Filby^{1,2,*}, Charmaine S.C. Tan^{1,2}, Jim Tsaltas², Gareth C. Weston^{2,3}, Caroline E. Gargett^{1,2,*}

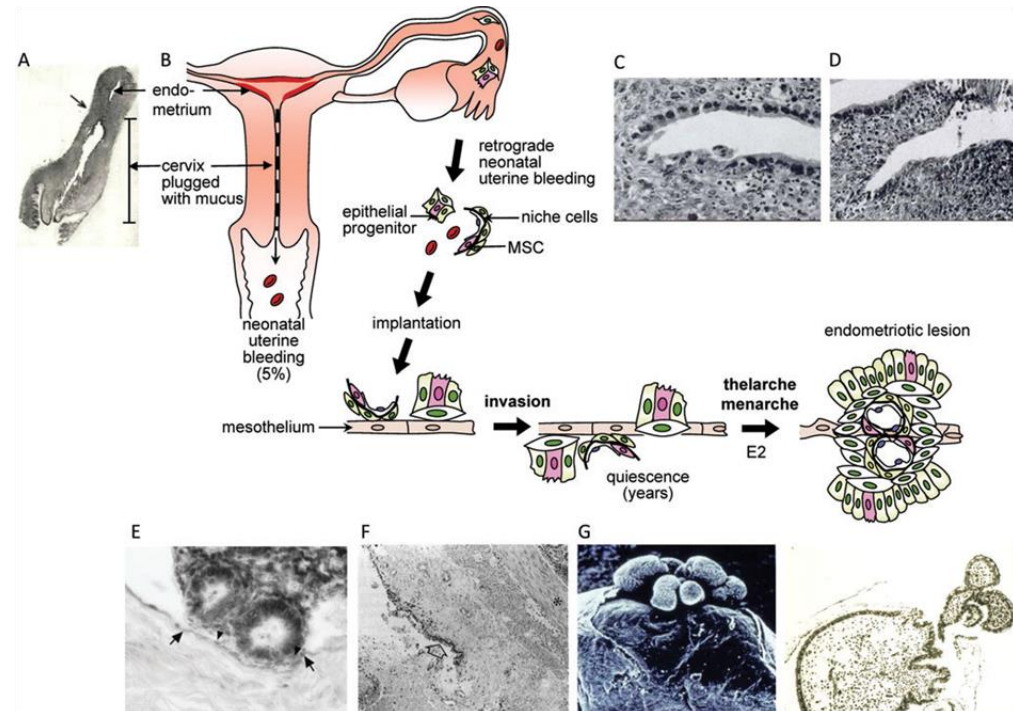


Caroline Gargett

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



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

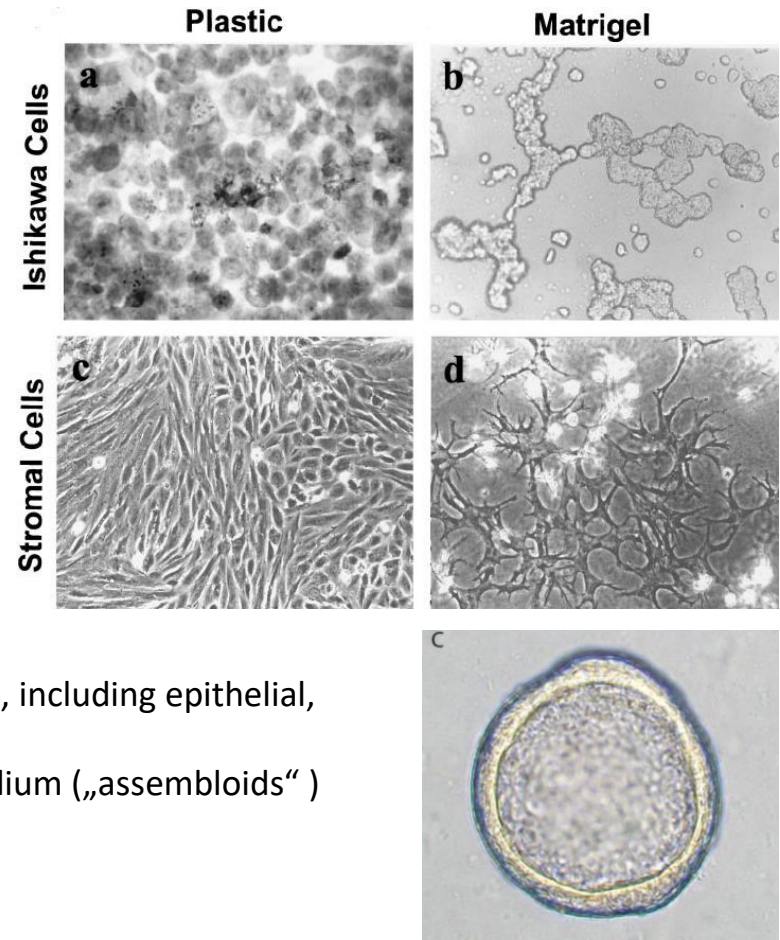
- hollow spherical structures consisting of multiple cell types, including epithelial, stromal, glandular, and vascular cells
- spontaneous self-organization in a defined serum-free medium („assembloids“)
 - ← primary endometrial cells from **biopsies**
 - ← endometrial cells isolated from **menstrual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



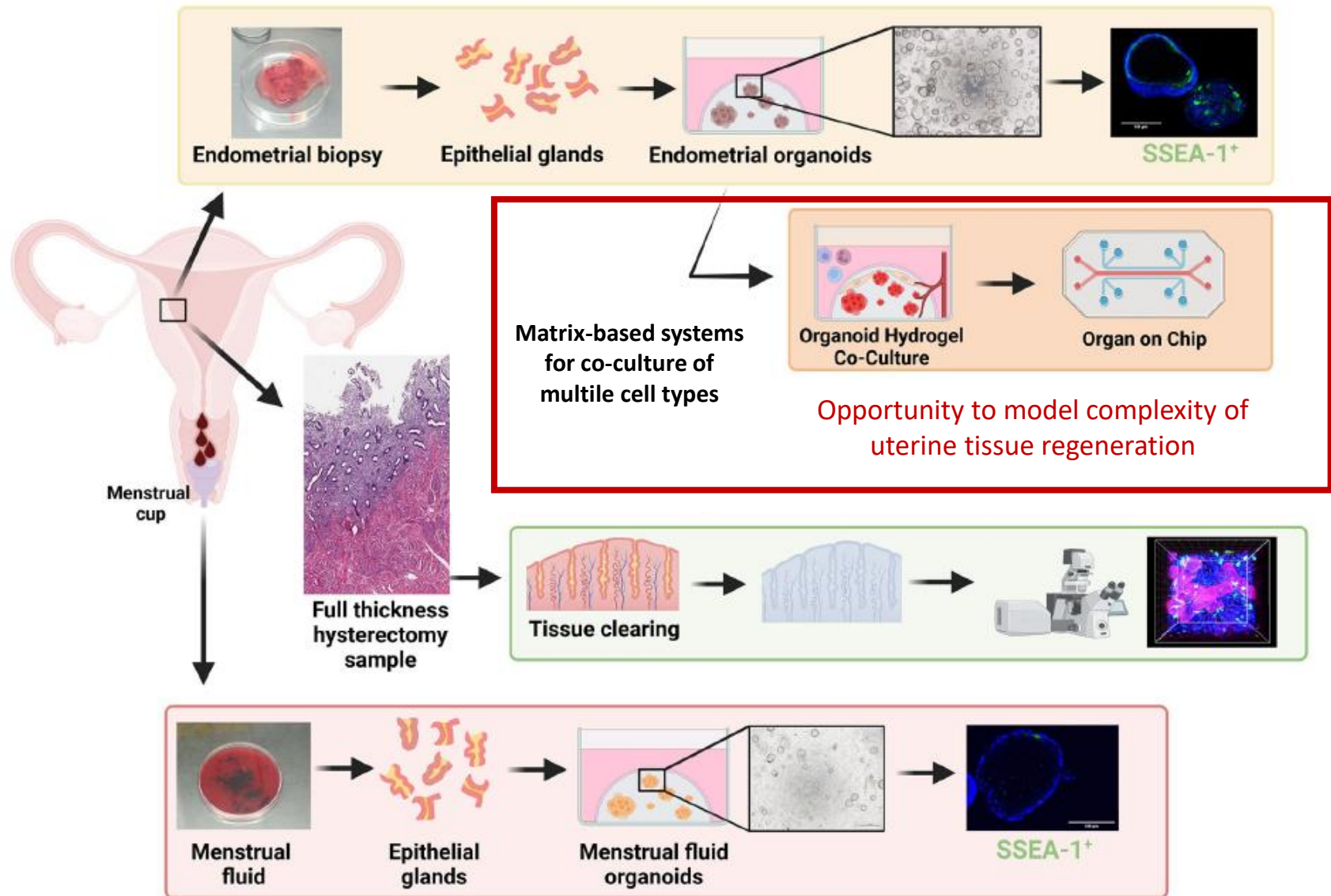
Disease modeling

Drug screening

Embryo-endometrial cross talk research



Research of endometrial physiology



**The uterus ensures that
a "good" embryo lands
safely**

