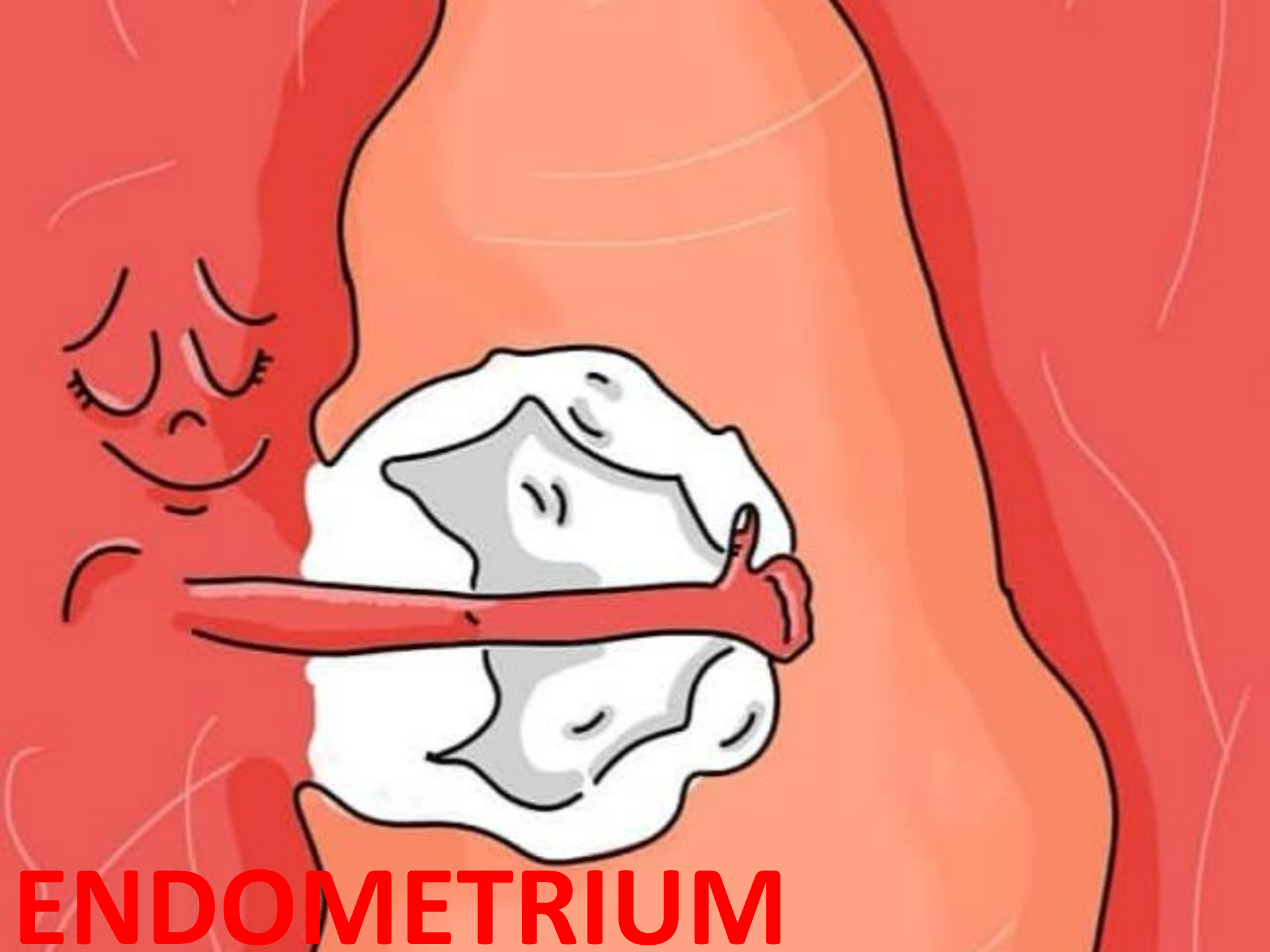
ENDOMETRIUM

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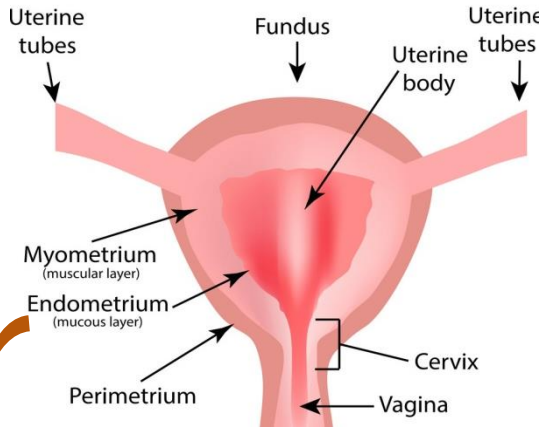
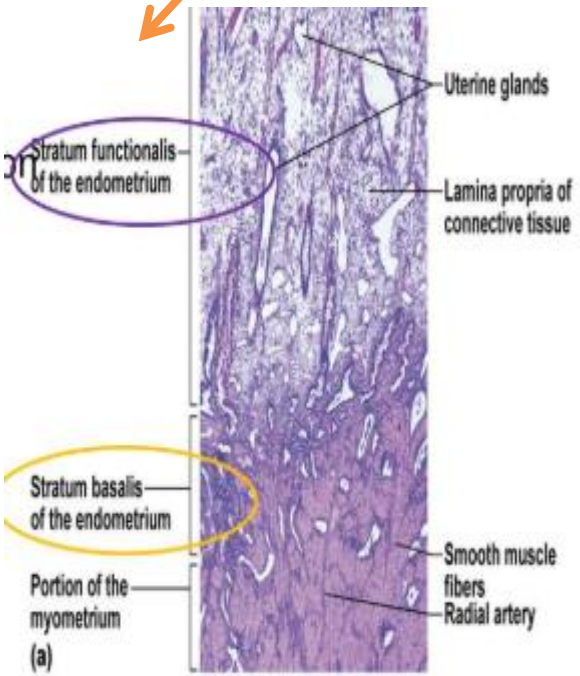


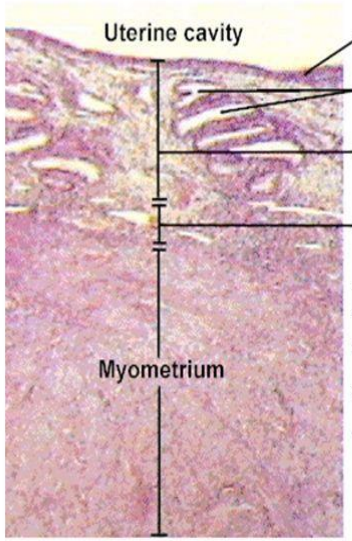
FUNCTION OF ENDOMETRIUM

- ✓ Ability to trigger process of its own shedding
- ✓ To protect itself from pathogen invasion
- ✓ To support implantation of the embryo

Stratum functionale
Stratum basale



Layers of Endometrium

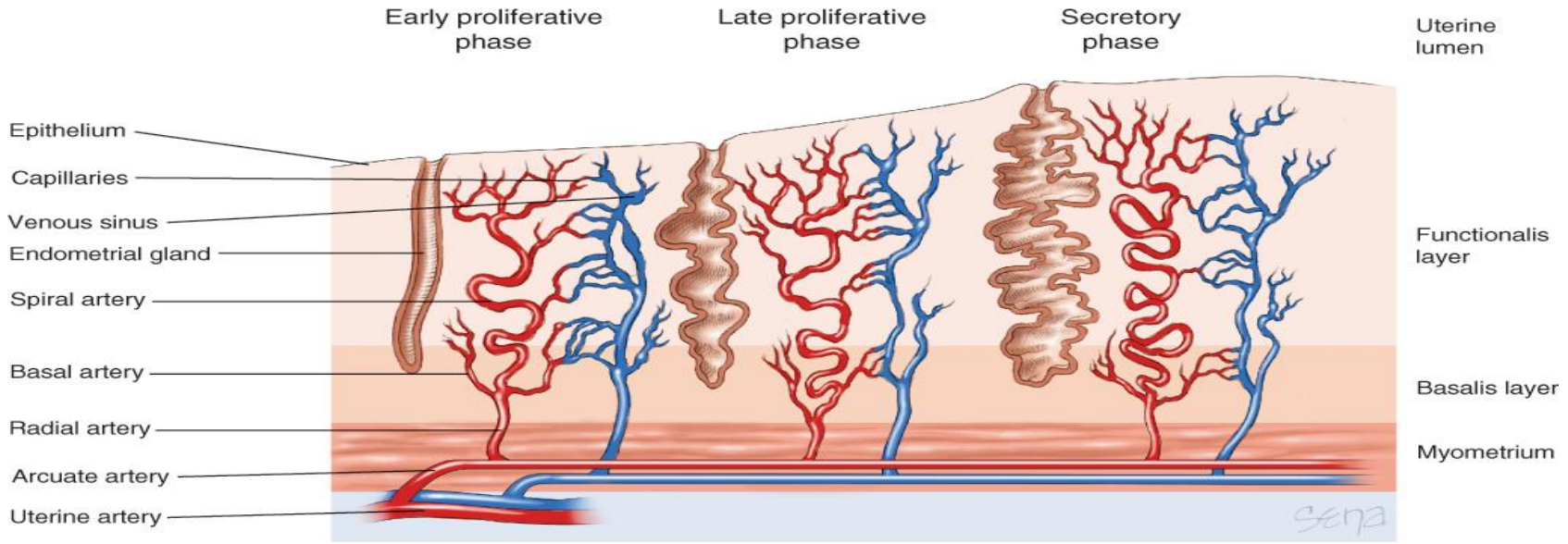


Functional Zone (stratum functionalis)
-contains endometrial glands and spiral arteries
-is shed each month

Basilar Zone (stratum basale)
-regenerative layer that creates functional zone

Endometrial structure

The human ENDOMETRIUM is a hormone-responsive mucosa that lines the uterine cavity. It is highly regenerative due to the fact that it grows up to 7 mm in thickness in response to cyclical changes of plasma sex steroid hormone levels

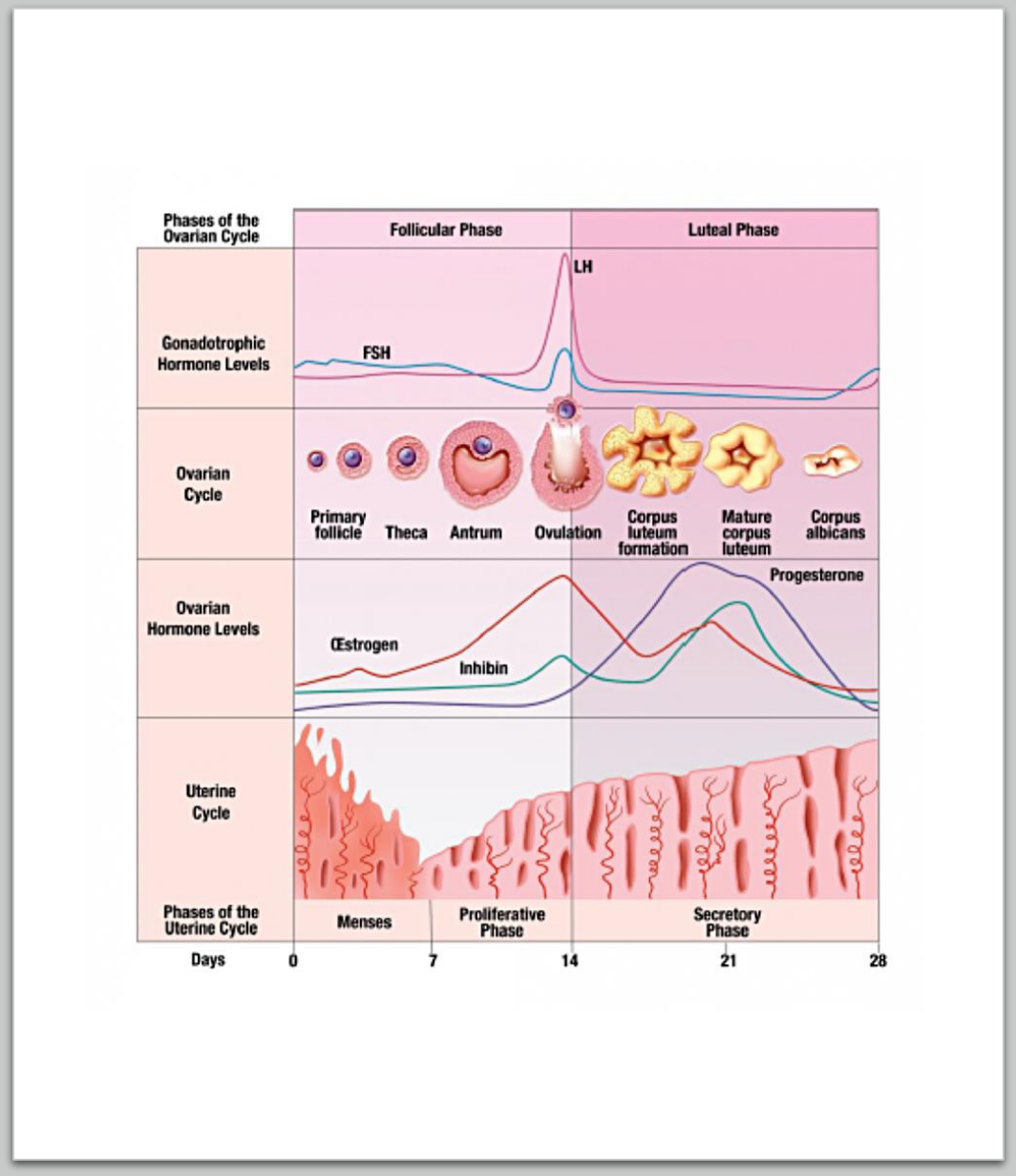


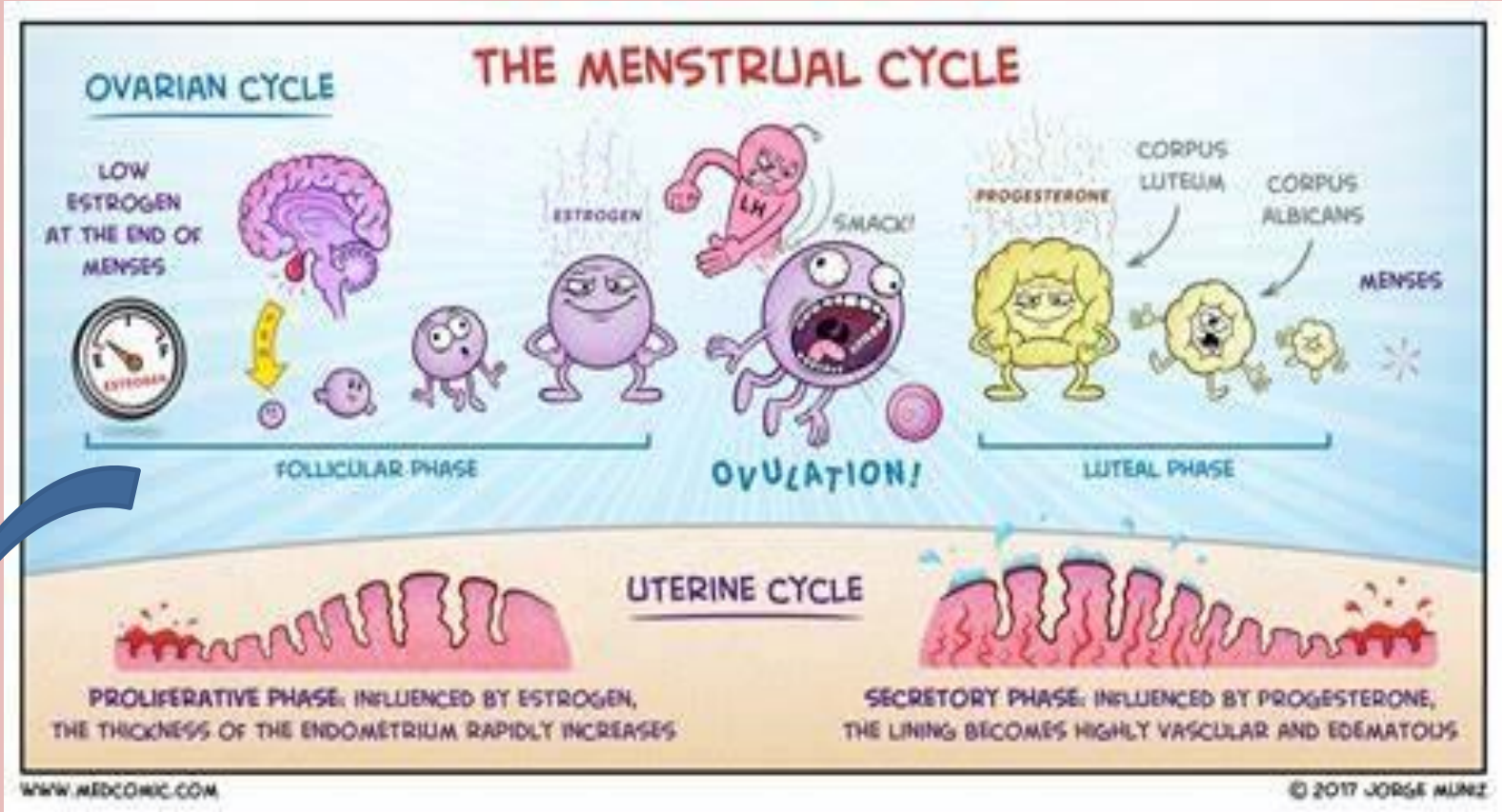
It is composed of numerous glands embedded within a supportive stroma. During a woman's reproductive years the superficial portion of the endometrium, the endometrium functionalis, moves through cycles of proliferation and secretion, followed by desquamation during the menstrual phase, in the absence of any ovum implantation.

The uterine cycle

- **Cyclic changes in endometrium are in responce to fluctuating ovarian hormon levels !**

- Three phases:
  - Days 1-5 menstrual phase
  - Days 6-14 proliferative (preovulatory) phase
  - Days 15-28 secretory (postovulatory) phase





Both the menstrual and proliferative phases occure before ovulation,and together they correspond to the FOLLICULARE PHASE of the ovarian cycle. The SECRETORY PHASE corresponds in time to the luteal phase of the ovarian cycle

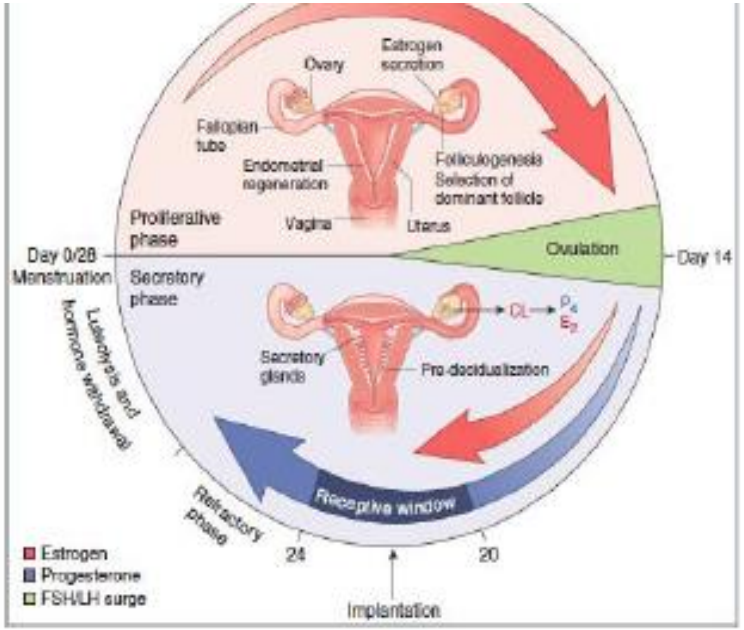
Behind the scenes, the main role is played by hormones

The PROLIFERATIVE PHASE is under the influence of rising estrogen levels from growing follicles, leading to proliferation of the epithelium, stroma and vascular endothelium. During the late proliferative phase, glands assume tortuous morphology.

At midcycle, there is a surge of **FSH** and **LH**, leading to **OVULATION** on day 14.

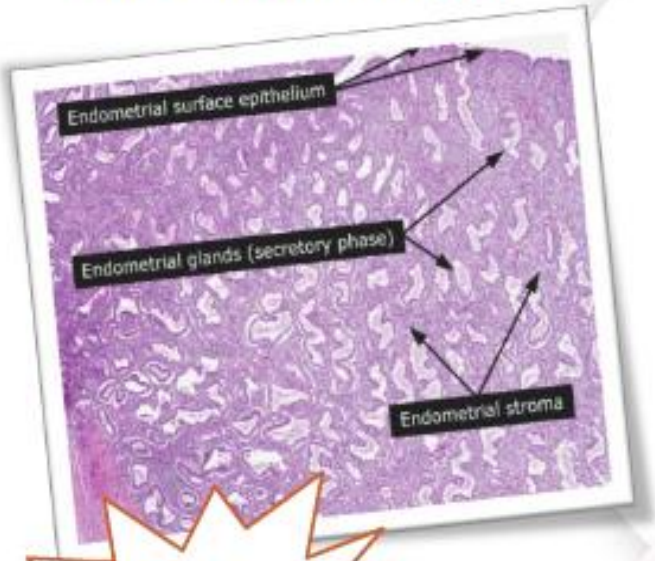
The early SECRETORY PHASE is marked by thickening of the endometrium and the formation of the **CORPUS LUTEUM** from the ruptured follicle and subsequent Progesterone secretion in preparation for implantation.

Rising estrogen levels superimposed on Progesterone define the **WINDOW OF RECEPTIVITY** during the midluteal phase (cycle days 19–24), conducive to implantation and pregnancy. .



The Actors of Human Implantation

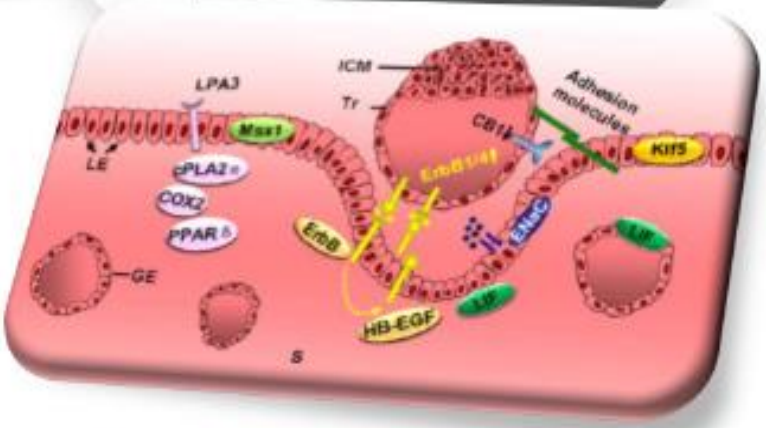
The ENDOMETRIUM



Endometrial Receptivity

The BLASTOCYST

Embryonic Competence

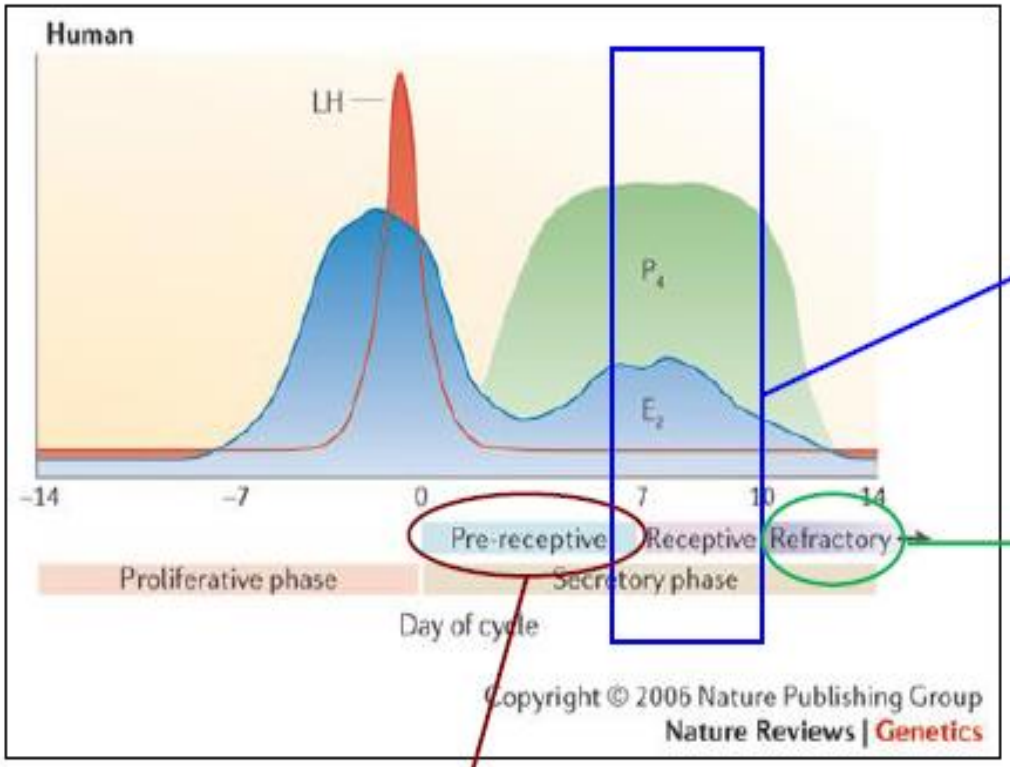


The crosstalk between the ENDOMETRIUM and the BLASTOCYST

Endometrial receptivity

The uterus is receptive to blastocyst implantation during a spatiotemporally restricted "window" (***Implantation Window*, WOI**) when the uterine environment is favorable for blastocyst implantation.

> The key is to time the embryo transfer in IVF to be in sync with the uterine lining for implantation to occur.
>
> - Dr. Marcy Maguire, RMA New Jersey



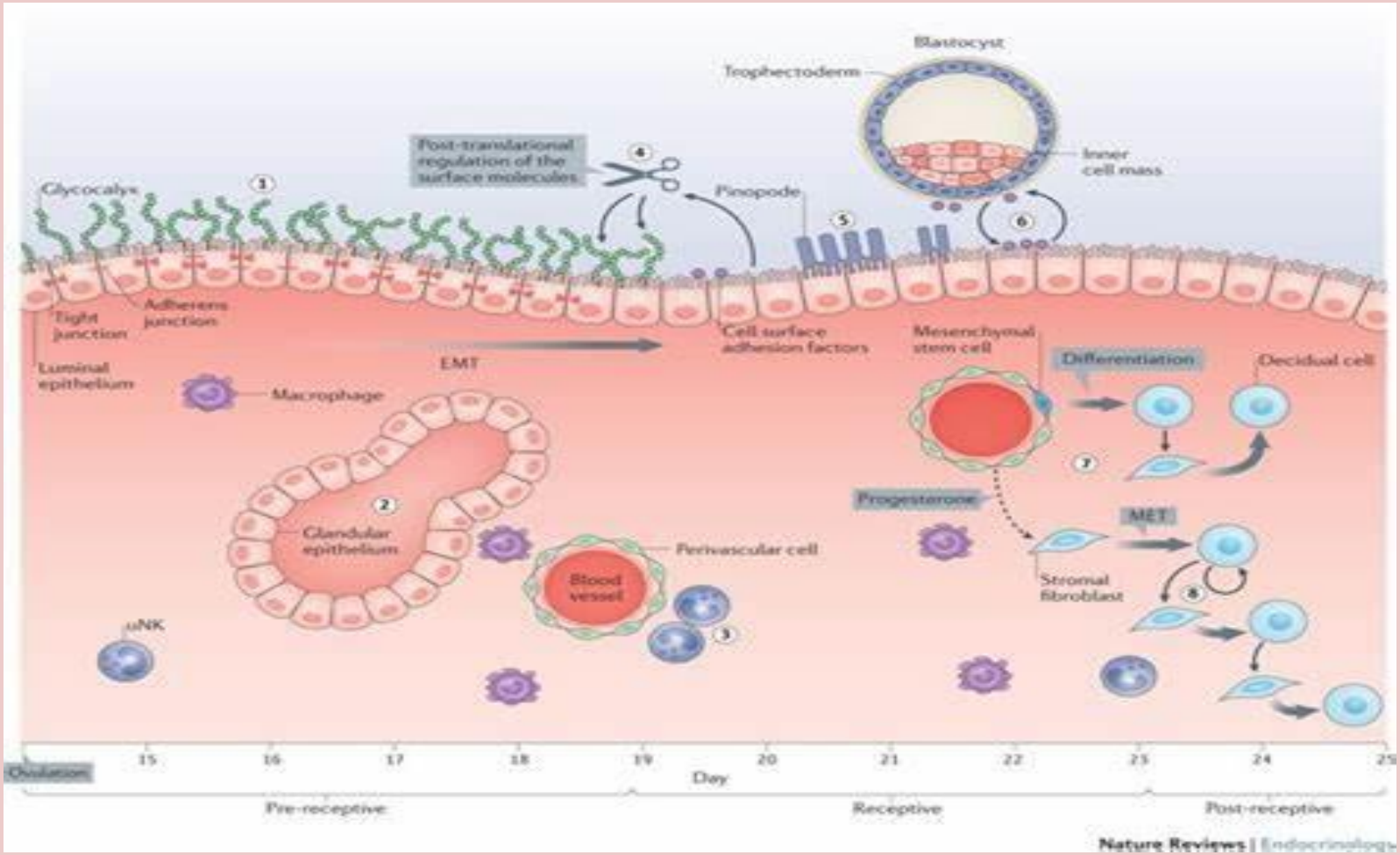
The uterus becomes receptive during the **Mid-Secretory Phase**, which spans 5–10 days after ovulation

The **Non-Receptive Phase** comprises the rest of the secretory phase.

During the **Secretory Phase**, the uterus is considered prereceptive for the first 5 days following ovulation (day 0).

For pre-receptive endometrium to become receptive, luminal epithelum it undergos some molecular modifications:

- ✓ Epithelial and blastocyst-secreted enzymes modify the glycocalyx
- ✓ Pinopodes appear on the surface
- ✓ Epithelium undergoes epithelial mesenchymal transition (EMT)

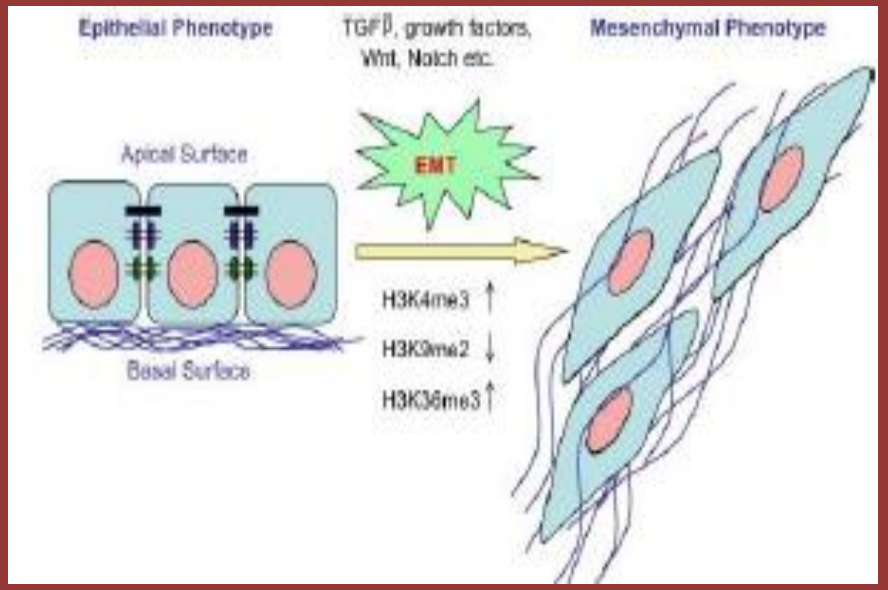


Epithelial-to-mesenchymaltransition (EMT)

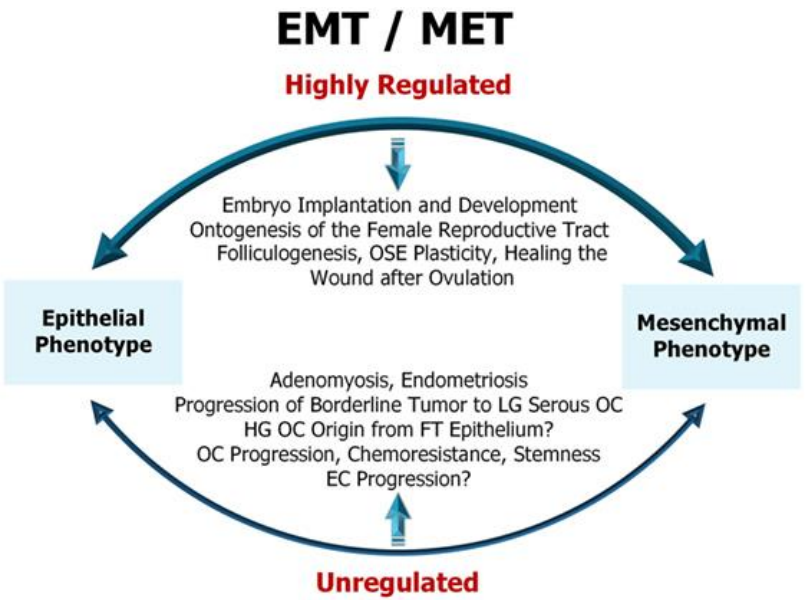
- Epithelial-to-mesenchymaltransition (EMT) is a physiological process, fundamental for embryo development, that is vital throughout the human lifespan.

During EMT, epithelial cells lose their epithelial cell characteristics:

- ✓ lose their apical basolateral polarity
- ✓ Lose their adhesion to adjacent cells and the basement membrane (dissolution of cell cell junctions, i.e. tight junctions (black), adherens junctions (blue) and desmosomes (green)
- ✓ acquire properties that promote migration and invasion
- ✓ Acquire a fibroblast like mesenchymal appearance, characterized by cell skeletonreorganization, motility, invasiveness and a heightened resistance to apoptosis
- ✓ Acquire cellular plasticity



THE ABILITY OF ENDOMETRIAL CELLS TO EXIST IN BOTH EPITHELIAL AND MESENCHYMAL PHENOTYPES BY UNDERGOING TIMELY SWITCHES BETWEEN EMT AND MET, ALLOWS THE ENDOMETRIUM TO ACQUIRE THE CELLULAR TRAITS NECESSARY TO DEVELOP HEALTHY GLAND ARCHITECTURE AND SUCCESSFULLY ACCEPT EMBRYOS FOR IMPLANTATION



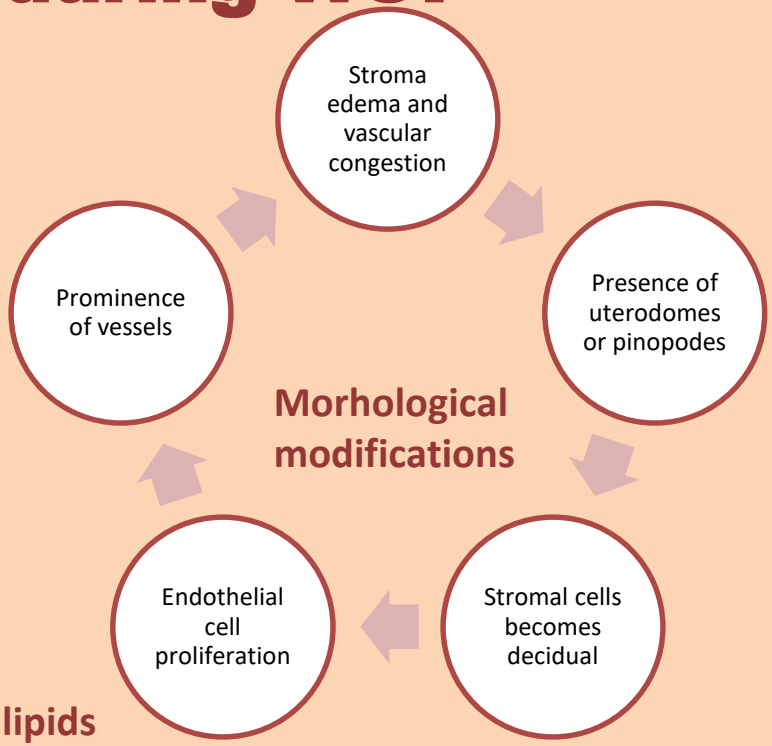
EMT DYSFUNCTION

Dysfunction of EMT in the stromal epithelial cells of the ovary and uterus resluts in pathological processes:

- Adenomyosis
- Ednometriosis
- Cancer initiation
- Progression
- Resistance to therapy

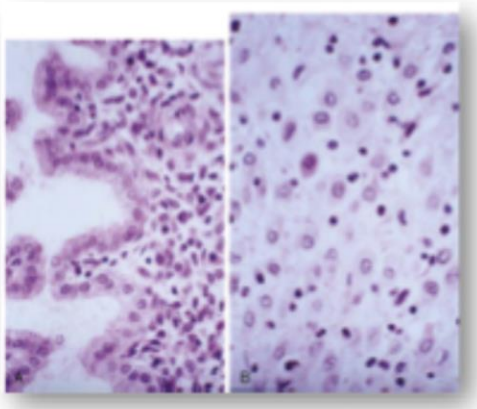
Endometrial modifications during WOI

- Morphological modifications
- Modification of adhesion molecules and their receptors
- Cytokine secretion



Steps of decidual reaction:

- ✓ **Endometrium becomes thicker and more vascular**
- ✓ **The endometrial glands become full of secretion**
- ✓ **The conncetive tissue cells enlarge due to accumulation of lipids**



A, Histological section through the endometrium during the late secretory stage of the endometrial cycle. A large uterine gland with an irregular epithelial border is on the left. On the right, note the stromal cells with compact nuclei and scanty cytoplasm.
B, Endometrial stroma, showing the decidual reaction. Note the expanded cytoplasm and less compact nuclei of the decidual cells. (Hematoxylin and eosin stain.)

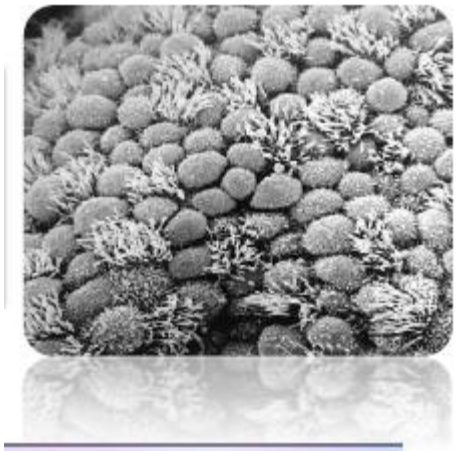
The decidual reaction spreads throughout stromal cells in the superficial layers of the endometrium

Morphological modifications during WOI

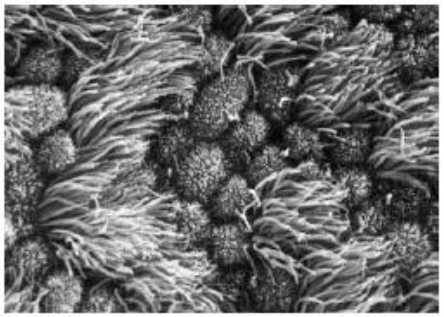
Uterine Epithelium

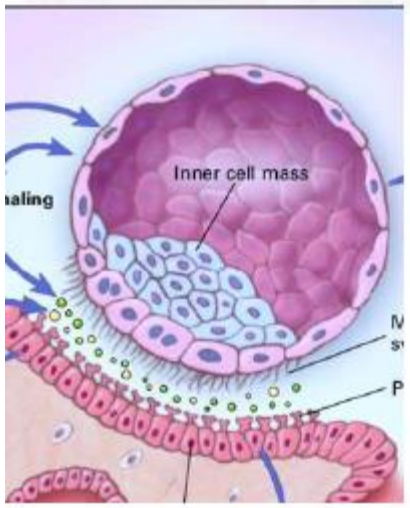
Uterine **epithelial cilia** are responsible for the initial movement of the embryo. In humans, this is during the first week of development. Uterine **epithelial microvilli** are involved with the implantation process.

ESTROGEN AND PROGESTERONE REGULATE BOTH CILIA AND MICROVILLI NUMBER AND STRUCTURE.



The differences in size and shape of cilia and microvilli are shown by scanning micrographs of the lumenal surface of the epithelium lining the mammalian uterine tube.

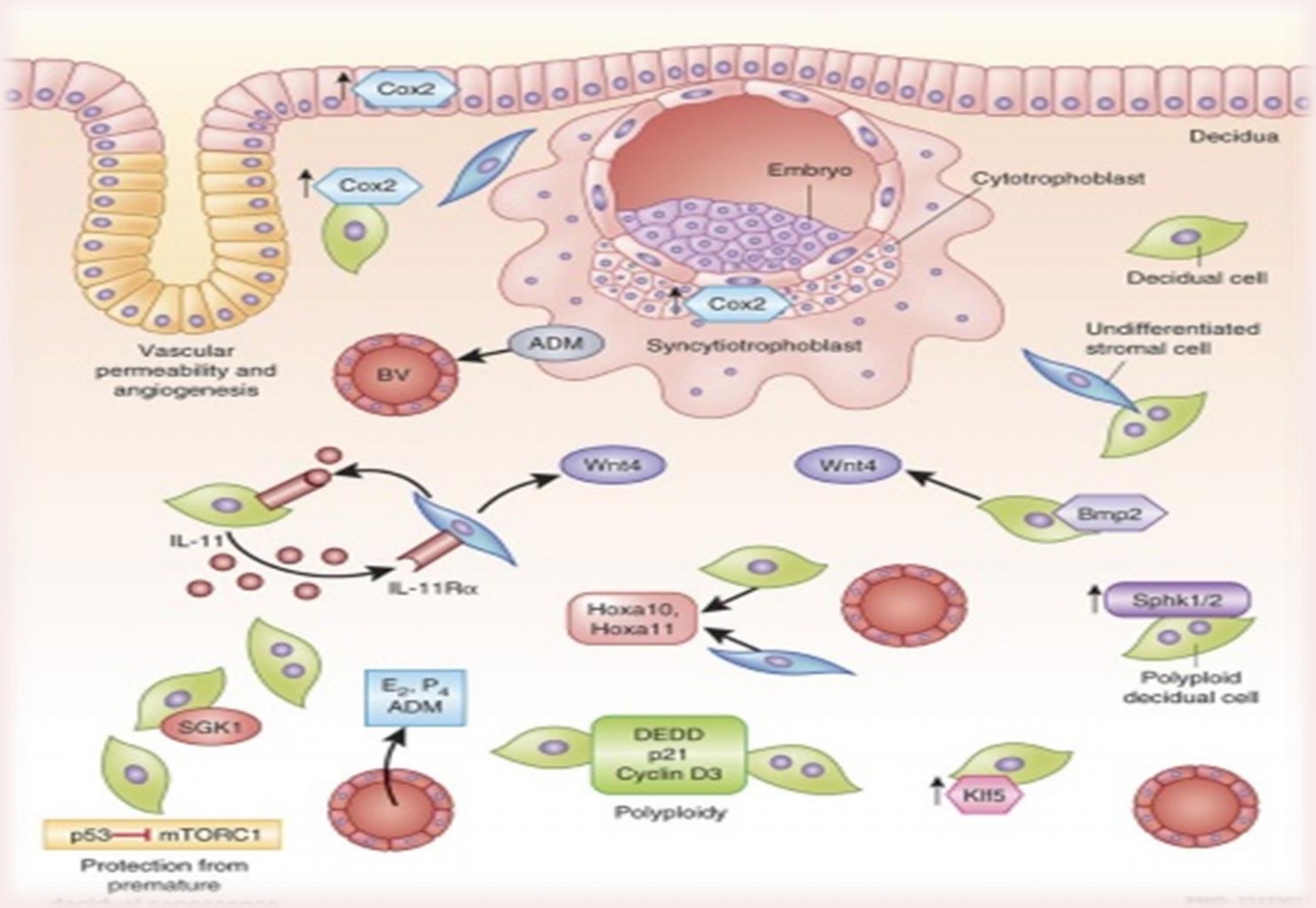




Uterodomes or Pinopods

Membrane protrusions on the apical surface of nonciliated endometrial epithelial cells during the secretory phase

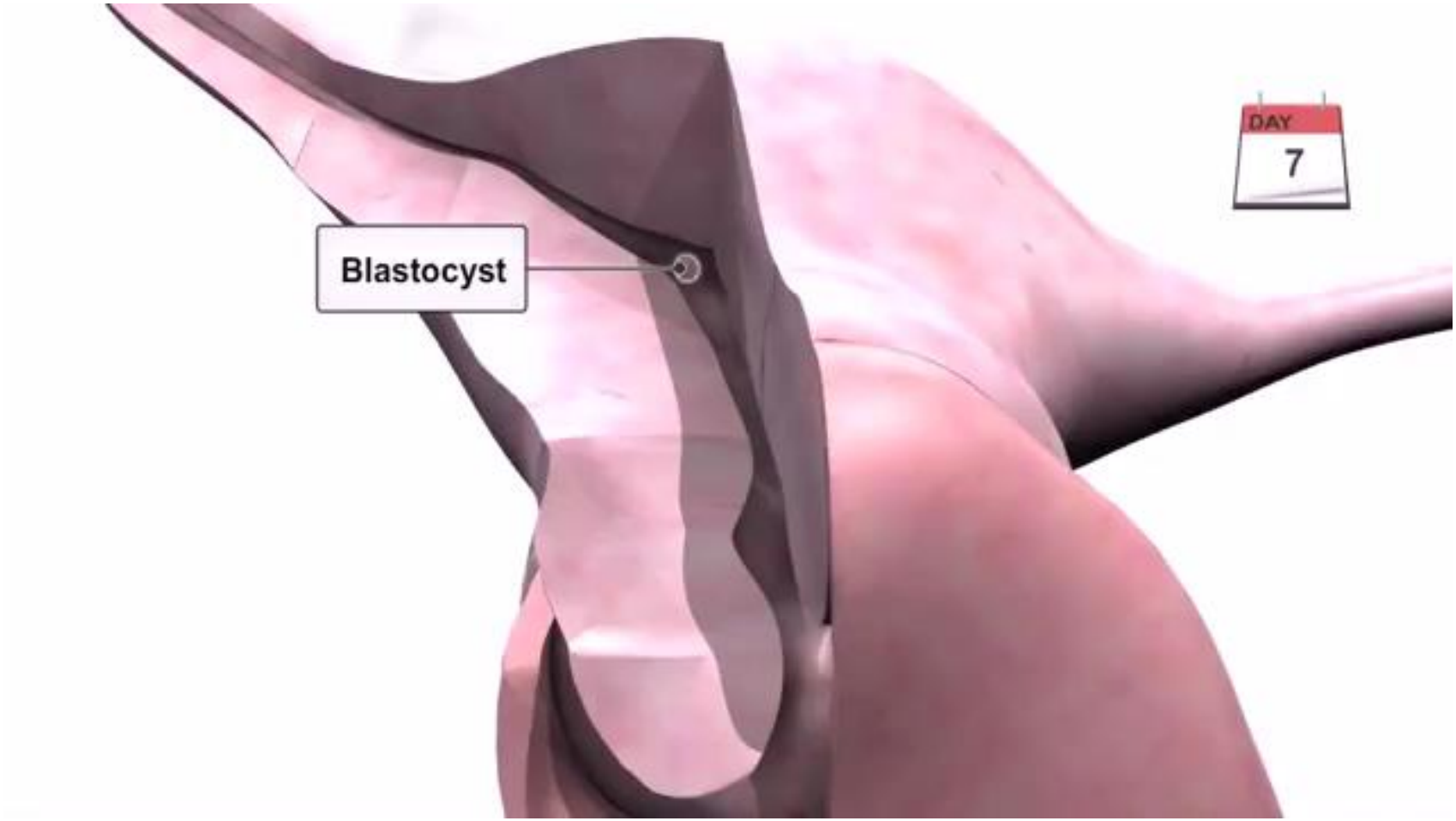
Signaling network in decidualization



Pro-invasive factors: IL-5,IL-6,IL-7,IL-8,IL-9,IL-15,EOTAXIN CCL11,IP-10 AND RANTES

ANTI-INVASIVE FACTORS:IL-10,IL-12 AND VEGF

ADM, adrenomedullin; BV, bloodvessel; DEDD, deatheffectordomain–containingprotein; IL-11Rα, interleukin11 receptorα; mTORC1, mammaliantarget of rapamycincomplex1; SGK1, serum-and glucocorticoid-induciblekinase1; Sphk1/2, sphingosinekinase1/2.

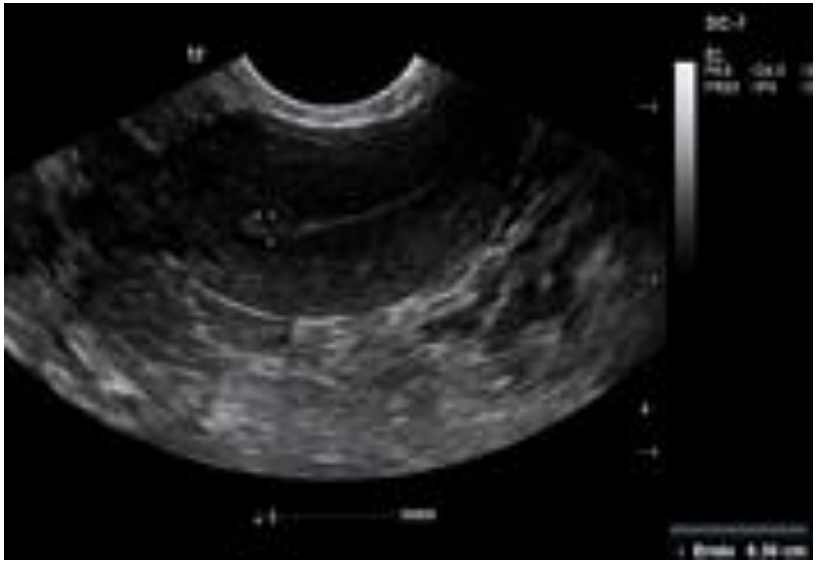

Blastocyst
DAY
7

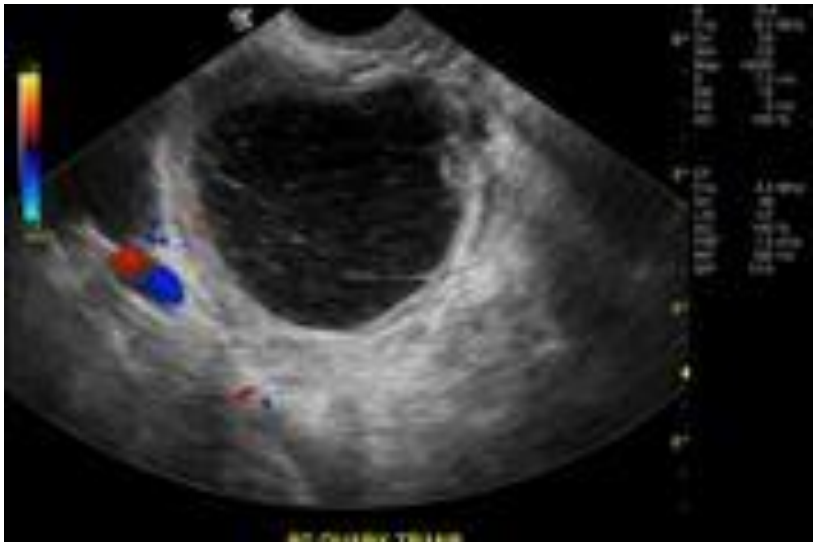
How can we measure endometrial receptivity??

- ✓ Ultrasaund technique
- ✓ Doppler technique
- ✓ ERA test

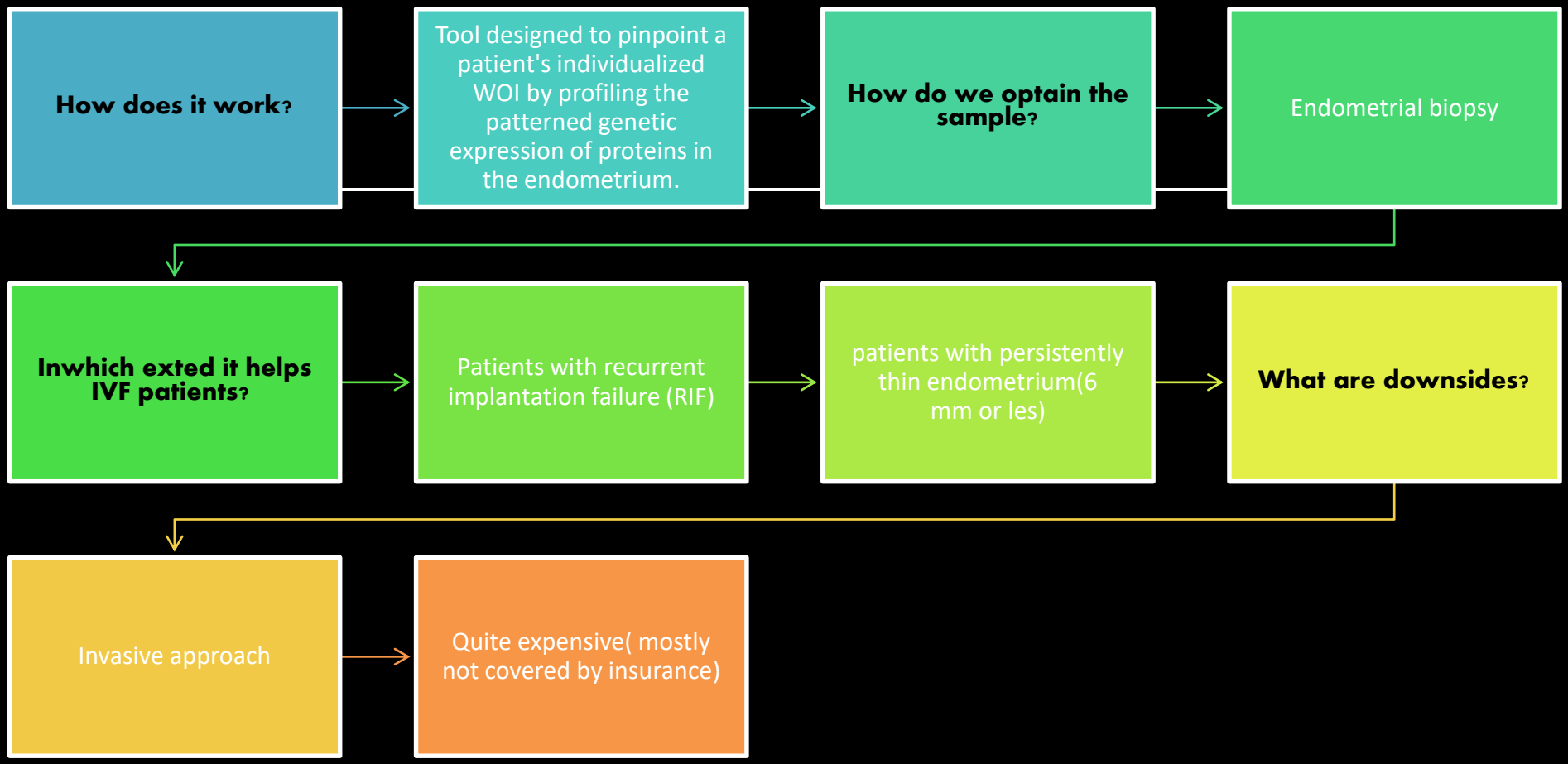
Ultrasound technique -it is possible to obtain information about the alterations in the endometrial morphology due to the hormonal setting (two endometrial echographic aspects change during the ovarian cycle: **thickness and echogenicity**

Doppler technique- measurements of uterine blood flow ,where high uterine vascular resistance implies a markedly reduced chance of fertilization.





ERA-Endometrial Receptivity Array?

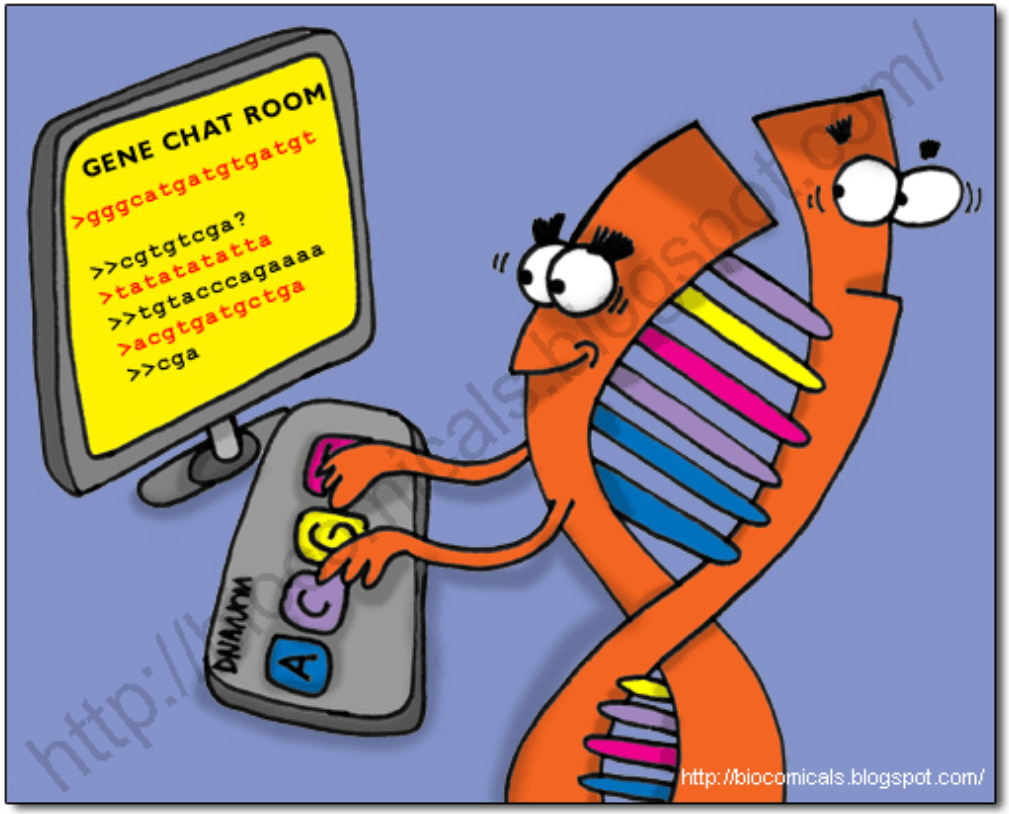


ERA

Different Endometrial Receptivity In Each Hemiuterus of a Woman with Uterus Didelphys and Previous Failed Embryo Transfers

- ✓ Profile: - 31-year-old patient with uterus didelphys (double uterus) ,and primary infertility,with no success in IVF treatment
  -no significant medical history or allergies
  -no hepatitis B or C, no HIV
  Male : beta-thalassemia minor
  Asthenozospermia
  8 % of spermatozoa with normal morphology
- ✓ Method: ERA test performed on both hemiuterus to assess endometrial receptivity
- ✓ Results: right-sided hemiuterus was receptive in 5 days since the beginning of progesterone administration while the left-sided hemiuterus was not receptive in that day.
- ✓ Conclusion: ERA analysis is a useful tool for IVF patients with uterus didelphys to choose the most appropriate hemiuterus and day to perform embryo transfer.

Endometrial signaling molecules

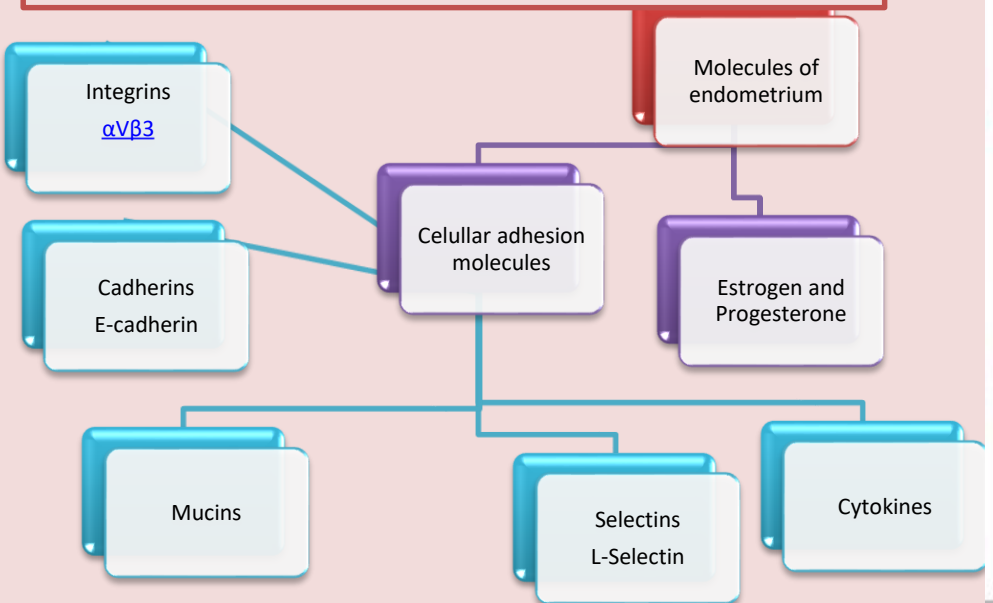


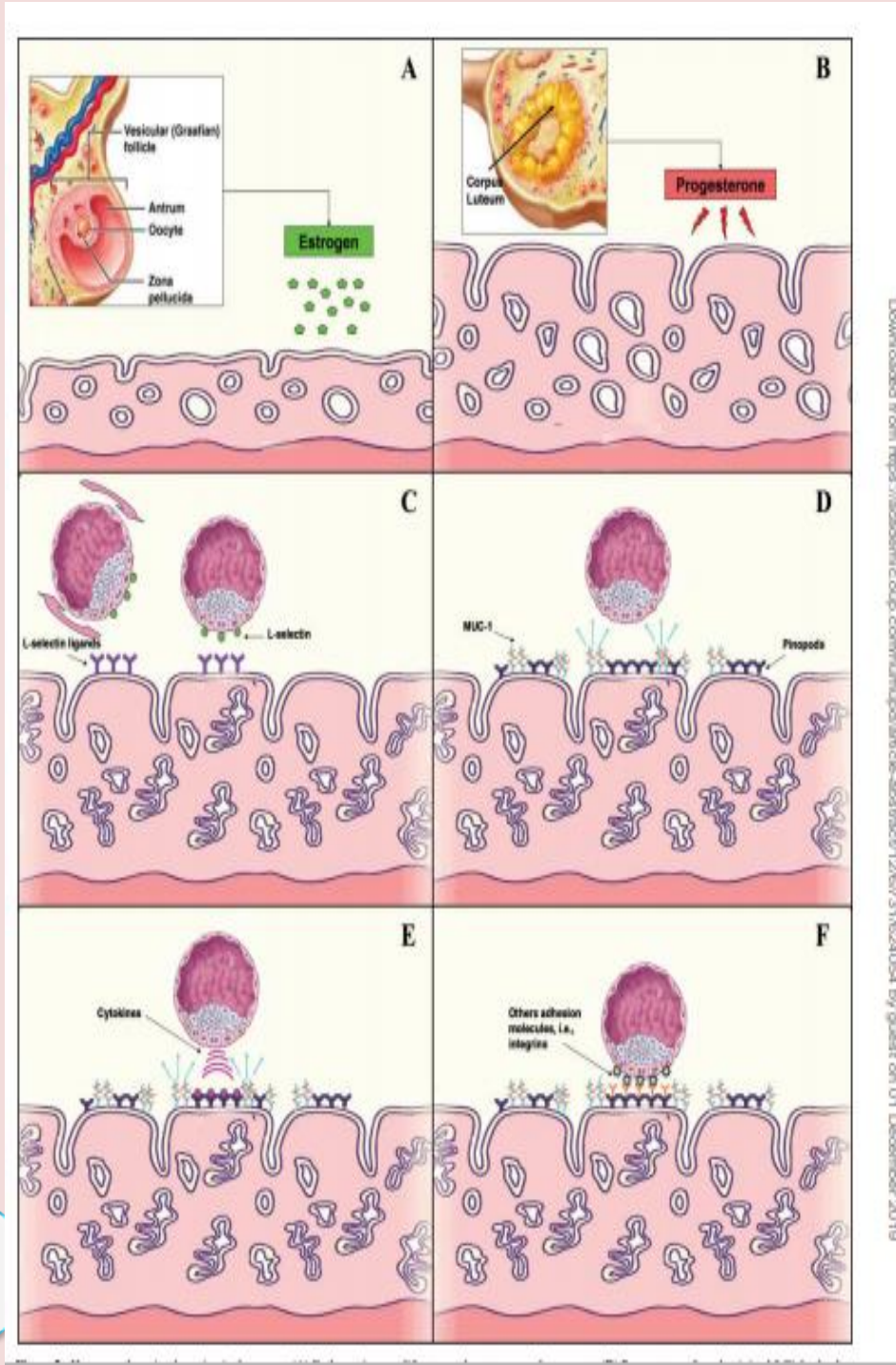
Human embryo implantation is a three-stage process :

- ✓ **Apposition**
- ✓ **Adhesion**
- ✓ **Invasion**

→ Synchronized **crosstalk between a receptive endometrium and a functional blastocyst.**

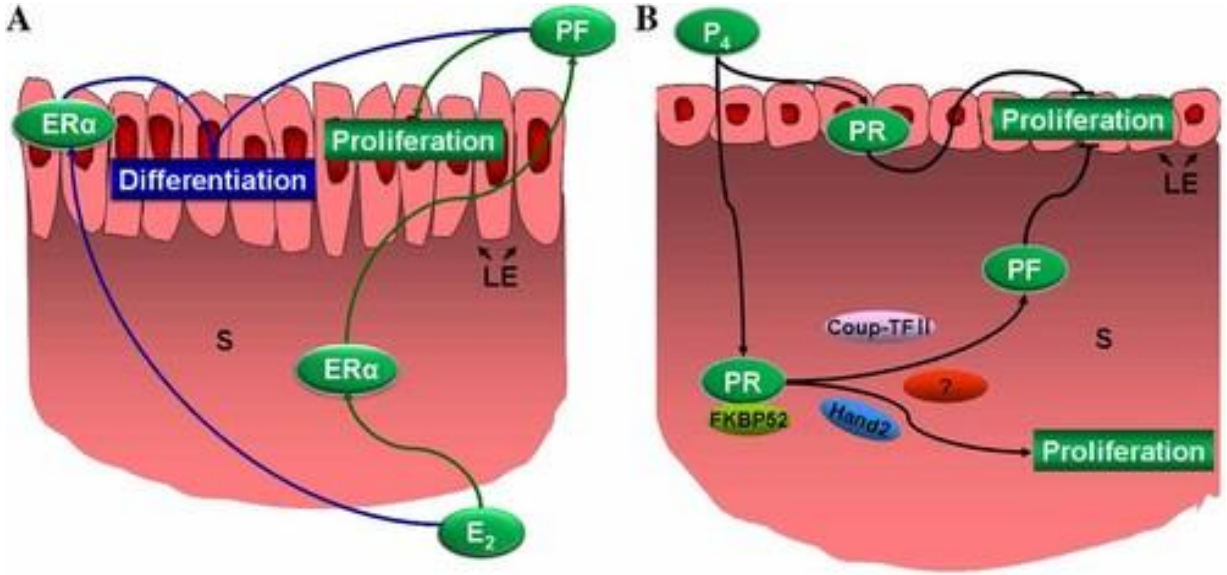
→ take place during the window of implantation, and involves a complex **sequence of signalling events and molecules.**





Putative mechanisms of uterine proliferation and differentiation in response to ovarian steroid hormones

- Synchronization of estrogen and progesterone directs the uterus into a receptive state that is accompanied by obvious morphological and functional changes in the epithelium.

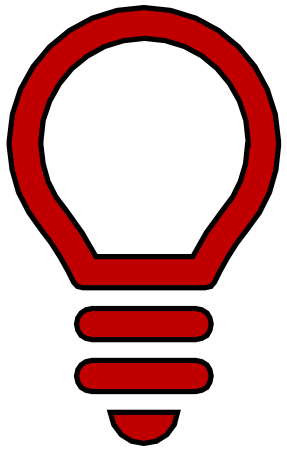


A: The proliferation of uterine epithelium in response to estrogen requires stromal estrogen receptor alpha (ERα) and occurs via paracrine factors whereas the differentiation of uterine epithelium requires both epithelial and stromal ERα and occurs in a paracrine/autocrine manner. B: Progesterone acts through stromal and epithelial PRs to inhibit estrogen-induced epithelial proliferation while inducing proliferation of the underlying stroma. This effect is mediated by numerous progesterone receptor (PR) target genes. COUP-TF II, chicken ovalbumin upstream promoter transcription factor II; Hand2, Heart and neural crest derivatives-expressed protein 2; E2, 17β-estradiol; ERα, nuclear estrogen receptor-α; FKBP52, FK506 binding protein-4; LE, luminal epithelium; P4, progesterone; PF, paracrine factor; PR, progesterone receptor; S, stroma.

TABLE 1. Molecules Associated With Endometrial Receptivity

Molecules		Potential role	References
Hormones	Estrogen Progesterone	Coordinate proliferation and differentiation of endometrial, stromal, and epithelial cells	Huet-Hudson et al. (1989), Lydon et al. (1995)
Adhesion molecules	MUC1 L-selectin cadherins integrins	Facilitate blastocyst capture and attachment; promote interaction between the epithelium and trophectoderm	Stewart et al. (1992), Meseguer et al. (2001), Horne et al. (2005)
Cytokines	LIF IL6 IL11	Regulate functions of endometrial cells and embryo–maternal interactions during attachment and decidualization	Stewart et al., (1992), Salamonsen et al., (2009), Menkhorst et al. (2011)
Growth factors	HB-EGF IGF TGFβ	Locally mediate the hormone's effects on uterine cell proliferation and differentiation	Paria et al., (2001a), Chen et al. (2005), Kurita et al. (2005), Zhu and Pollard (2007)
Homeobox gene	HOXA10 HOXA11	Determine the early reproductive tract development and regulate post-implantation uterine development	Wang and Dey (2006), Lim and Wang (2010), Daikoku et al. (2011), Nallasamy et al. (2012)
	MSX1/2	Maintain uterine readiness to implantation; Regulate uterine luminal epithelial cell polarity	
Lipids	cPLA2 COX2 PPAR LPA3	Regulate prostaglandin production and mediate prostaglandin intracellular function, increase vascular permeability, promote implantation, promote adhesiveness of uterus	Lim et al. (1997, 1999), Song et al. (2002), Wang et al. (2004, 2007), Ye et al. (2005)
Other factors	MMPs	Degenerate the components of extracellular matrix for uterine remolding	Kao et al., (2002), Skrzypczak et al. (2007), Rashid et al. (2011), Pabona et al. (2012)
	DKK1	Mediate epithelial-embryo and/or epithelial-stromal interactions for preparation of uterine receptivity	

The molecular basis of uterine receptivity and crosstalk between the blastocyst and the uterus during implantation remains largely unknown. Many defined genes that are expressed in an implantation-specific manner and appear to be important for implantation cannot be studied in depth because deletion of these genes often results in embryo developmental defects.



Endometrial microbiota-new player in the city

Challenging the classic dogma of 'sterile womb'?

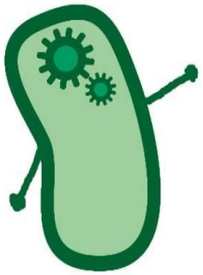
- ✓ Microbiota and mammals depend on their symbiotic relationship.
- ✓ Metagenomic analysis **revealed the presence of microbiota at body sites that were previously considered to be sterile.** This includes the upper reproductive tract and placenta, challenging the classic dogma of a ‘sterile womb’ as coined by Henry Tissier more than a century ago.
- ✓ Due to the earlier limitations in microbial characterization and challenges in sample acquisition, **the significance of endometrial bacteria may have been missed or overlooked**

What is the evidence of endometrial microbiota?

- Microbiome dana are produced: -culture based
  -sequencing based technology

Dana from the HMP and other studies using these techniques have revealed that sites in the body historically thoughts to be sterile, such as the uterine cavity and the placenta, are in fact colonized with their own unique microbiome .

HOW??



16S rRNA's High Conserved Sequence

Conserved Sequence	Species
GTCGACAACAGAGTTTGATCCTGGCTCAC	Most *Fungis*
GTCGACAACAGAGTTTGATCATGGCTCAC	Gut flora
GTCGACAACAGAGTTTGATCCTGGCTTAG	*Spirochetes*
GTCGACAACAGAATTTGATCTTGGTTCAG	*Chlamydia*
TCCAAGCTTACGGATACCTTGTTACGACTT	*Fusobacterium*

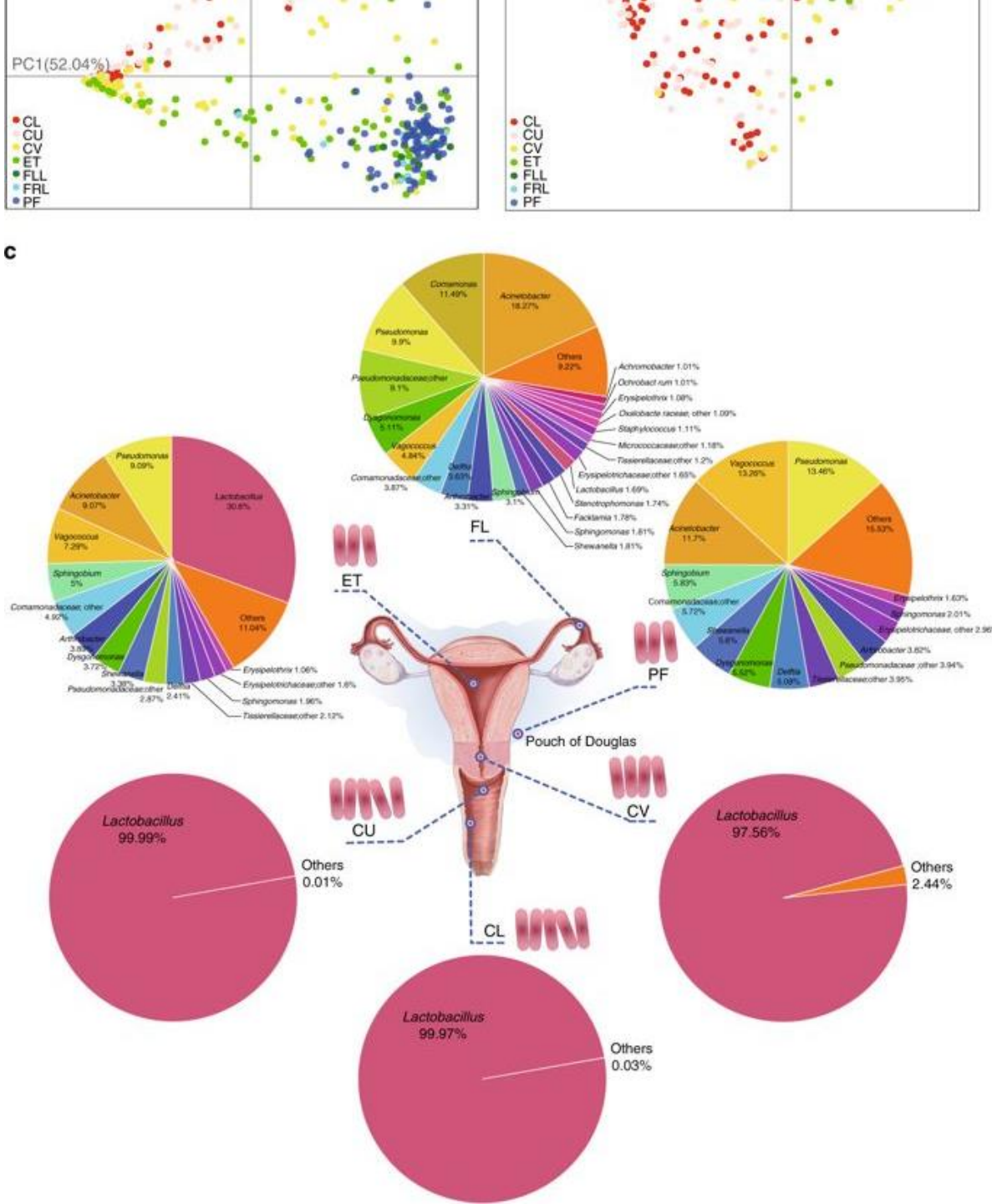
These molecular techniques take advantage of the **16S rRNA gene** that is unique to bacteria and contains a number of hypervariable regions that serve as unique identifiers for a genus or species of bacterium

Composition of female reproductive tract

- **Endometrial and vaginal microbiota are not identical**

Vaginal microbiome in healthy women:

- ✓ Infancy (aerobic and anearobic bacterial populations
- ✓ Puberty (Lactobacilli)



Physiological Endometrial microbiome in reproductive age women

Like the normal vaginal microbiota, the endometrium of healthy and asymptomatic women is often dominated by Lactobacilli

Similarity between the bacterial taxa found in endometrial and vaginal samples supports the hypothesis that the colonization of the uterine cavity mainly comes from bacteria ascending from the vagina.

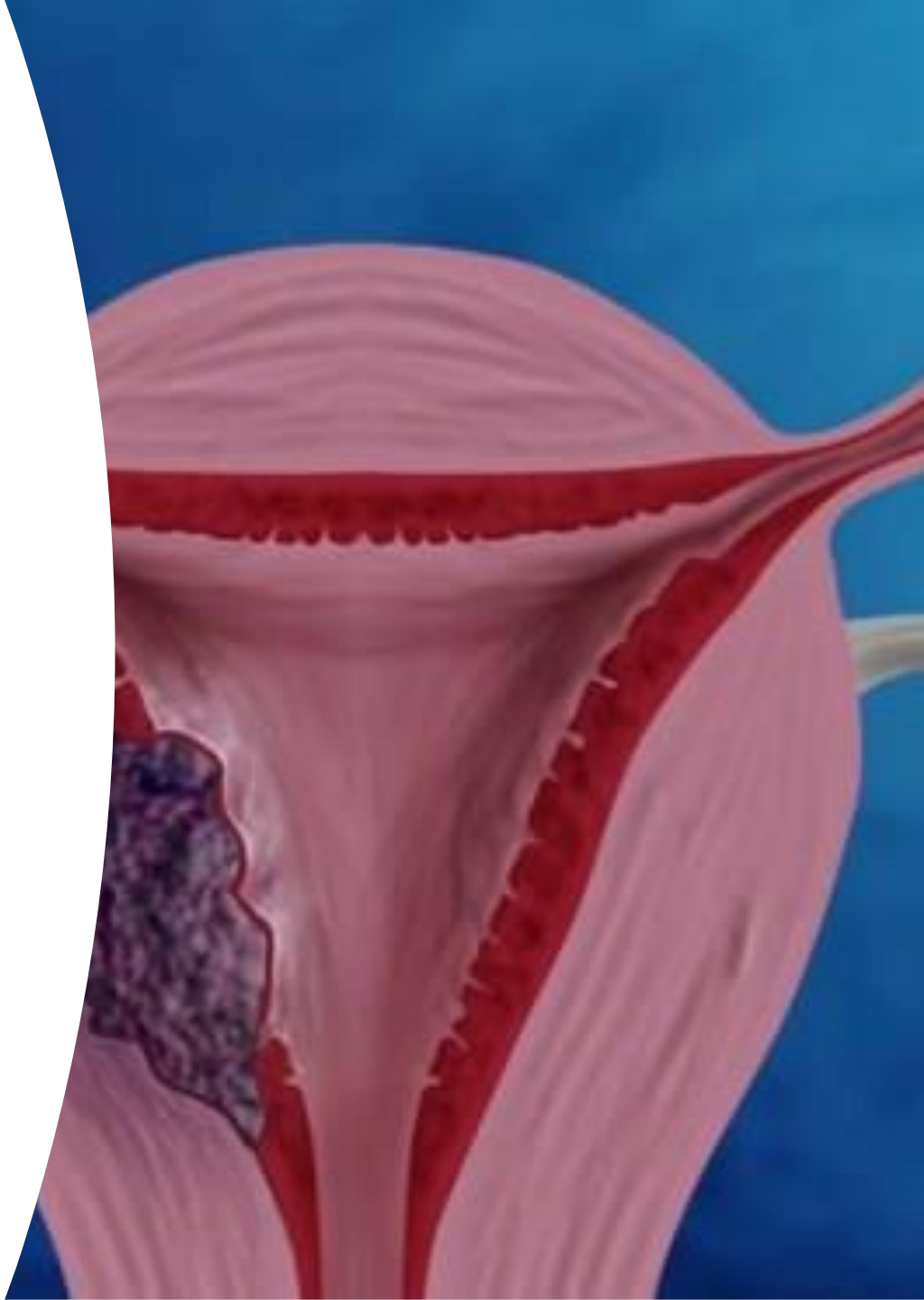
Some women experiencing other physicochemical or biological conditions present in the uterus may cause the bacterial populations inhabiting the endometrium to differ significantly from the ones in the vagina.

Altered Endometrial Microbiome

- ✓ **Chronic Endometriosis** –prominent example of pathology caused by altered endometrial microbiota .Caused by continuing inflammation of endometrial mucosa.

- ✓ **Endometriosis** – existence of endometrial epithelial and stromal tissue outside of endometrium .Although the orgin of endometriosis is not known, bacterial contamination of endometrium is possible cause. (menstrual blood – E.Coli; endometrial smears Gardnerella)

- ✓ Other Gynecological conditions :colorectal cancer ,pelvic inflamatory disease,cervical intraepithelial neoplasia



Possible impact of microbiota on various levels

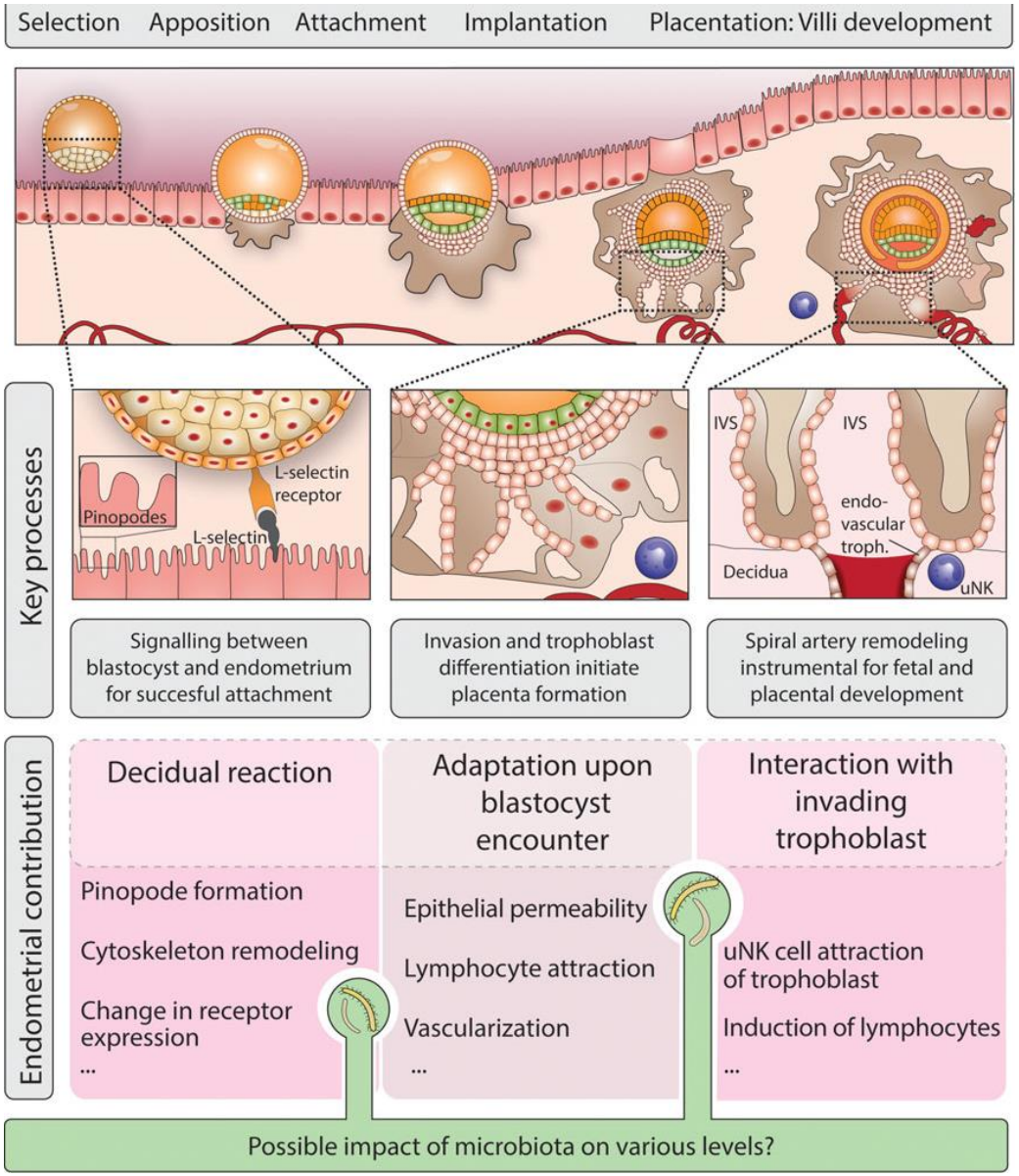
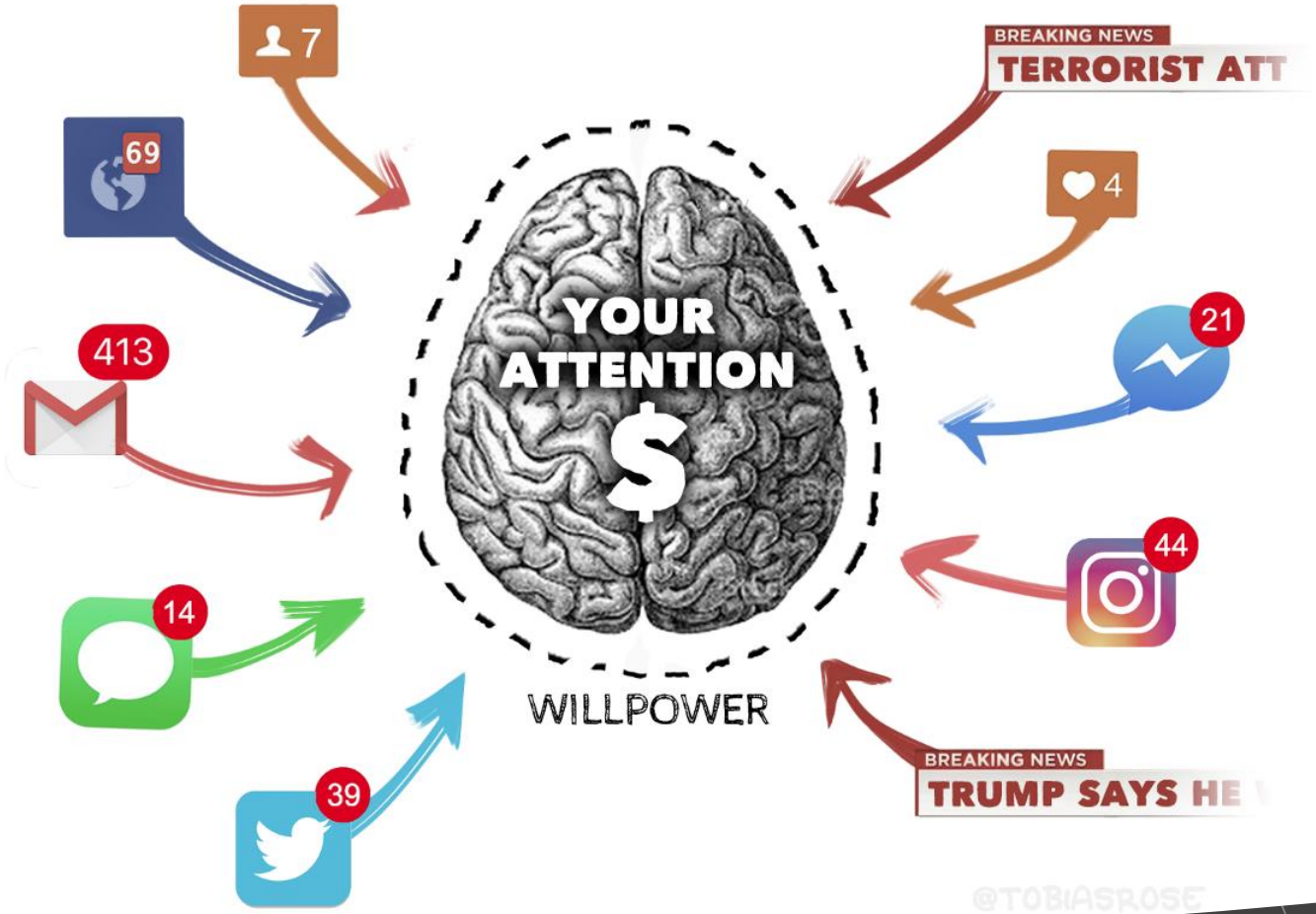


Illustration of key elements in blastocyst–endometrium interaction needed for a receptive endometrium. Due to its important contributions during early placentation, the endometrium is key to healthy pregnancy. During the window of opportunity (Days 19–24 of the menstrual cycle), the endometrium resides in a receptive state that allows selection, apposition and attachment of a healthy blastocyst. Implantation is marked by invasive growth and differentiation of trophoblast cells. Proper interaction of invading trophoblast and local immunity is needed to achieve correct villi development and a healthy placenta. All these highly regulated properties of the endometrium needed in the initial phase of pregnancy are possibly affected by uterine microbiota. IVS, intervillous space; uNK, uterine natural killer cell.



Thank You, any questions?