Endometrial Receptivity and Early Pregnancy

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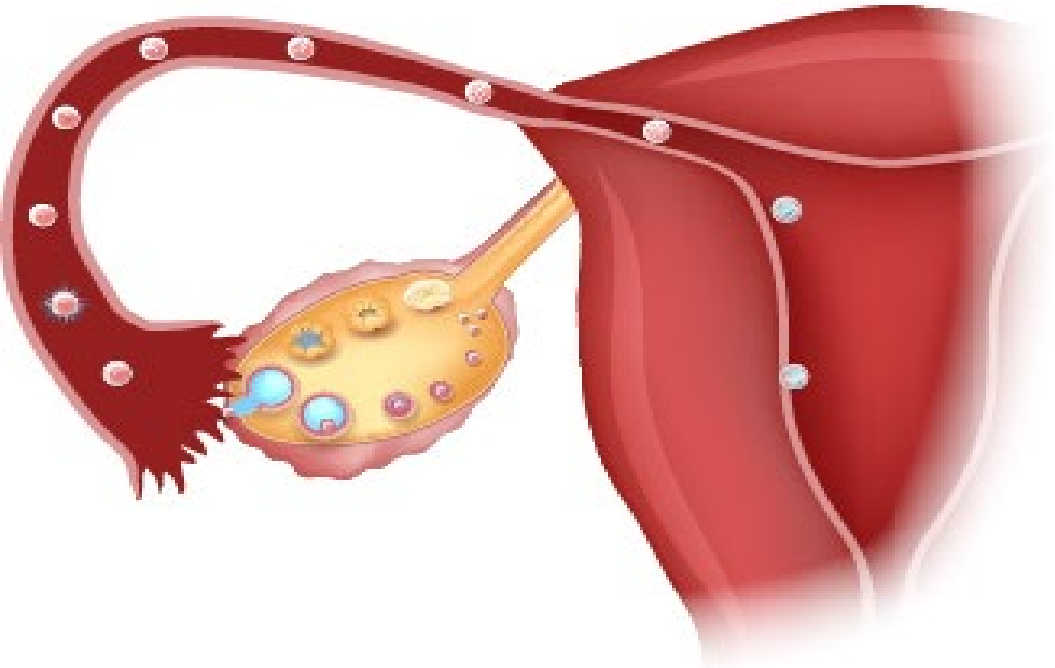
Objectives

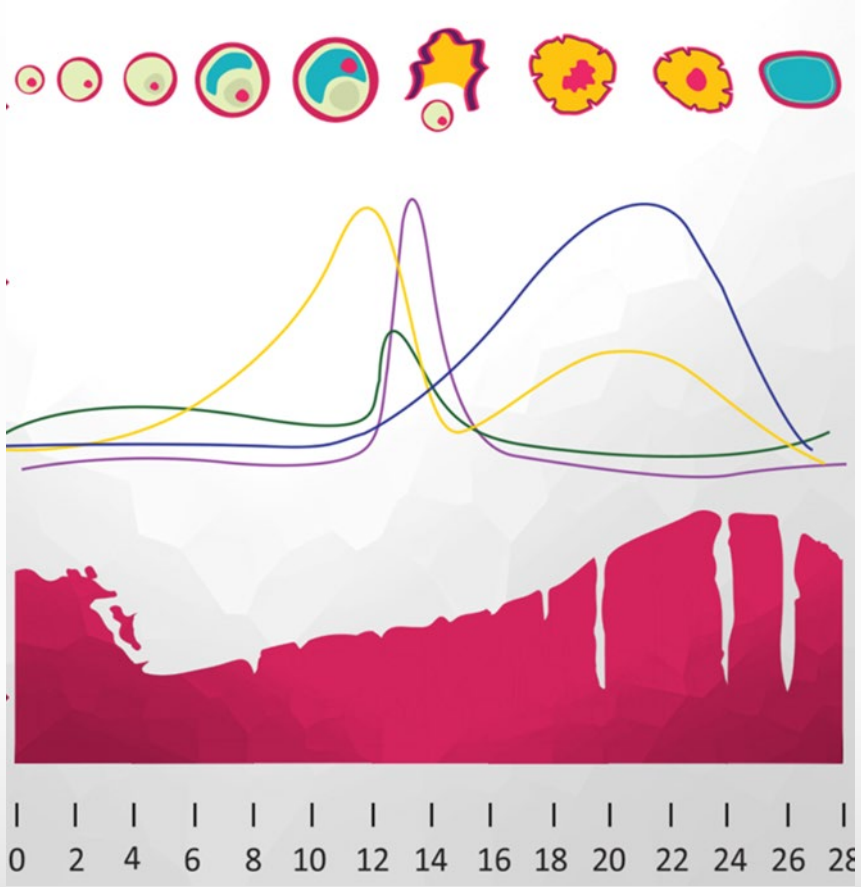
- Discuss importance of the window of implantation and define ‘uterine receptivity’.
- Explain the role of hormones in pregnancy
- Examine the stages of implantation
- Describe the effect of stimulating medications on the uterus
- Identify appropriate uterine structures in early pregnancy
- Differentiate between viable and non-viable pregnancies, their diagnoses and treatments



Uterine and Ovarian Synchrony is Key to Implantation

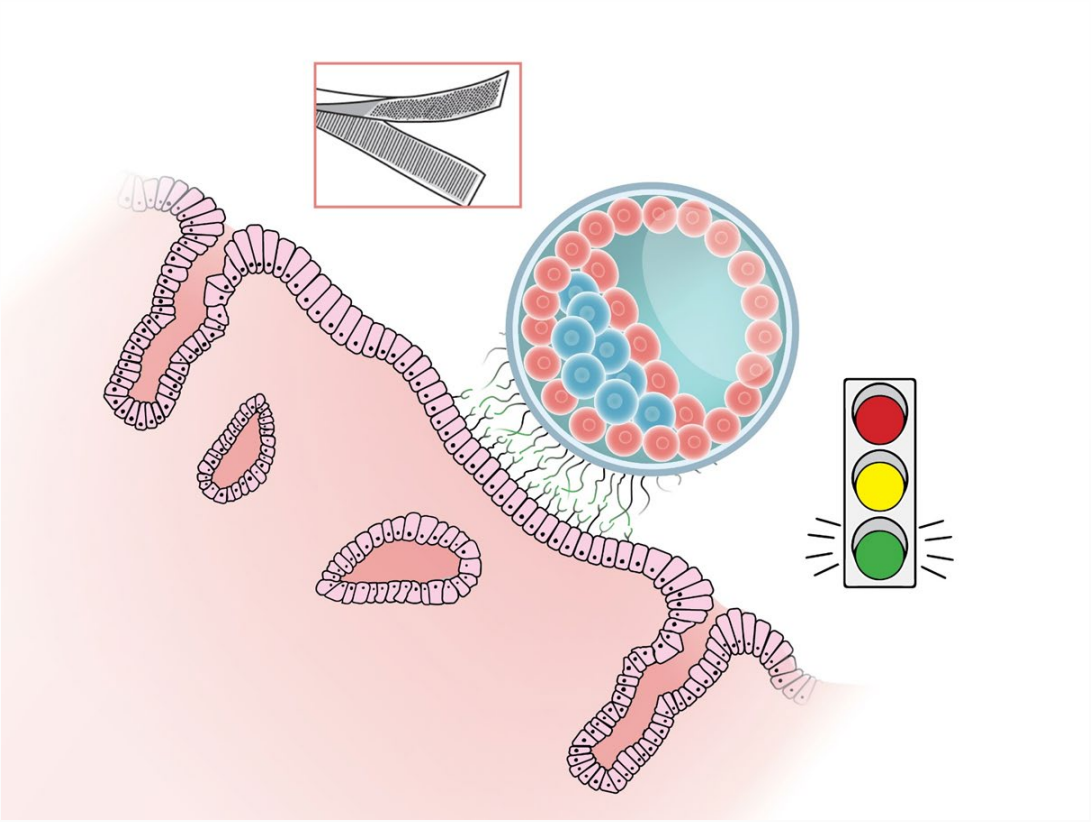
Window of Implantation-Phases of Menstrual Cycle

- Lining undergoes changes that prepare it for implantation
- Proliferative phase: lining grows due to increasing estrogen levels
- Secretory phase: production of P converts lining to a secretory one, changing the cells to prepare for implantation
  - A process called differentiation



Window of Implantation

- About 48 hours
  - Conventionally assumed that everyone has the same WIO (8-10 days after ovulation) but theory recently challenged
- Embryo is at blastocyst stage
  - While blastocyst is floating around, starts a dialog with endometrial lining
    - Signals uterine lining to “accept it”.
  - hCG must appear by the 10th day after ovulation to rescue the CL, so the blast must implant and secrete hCG within a narrow window



Endometrial Receptivity

- How do we test for it?
  - Endo biopsy-variable and subjective
  - Appearance by ultrasound?-Proliferative phase adequacy only
  - New methods now allow us to identify what the uterine lining looks like (in terms of epithelial changes and proteins secreted) for the lining to be receptive to implantation.
    - ERA test-1 out or 4 patients with RIF had a ‘displaced’ WOI.
    - Specific genes are involved to create the proteins necessary for implantation to take place
    - Identify these genes, analyze them, determine the patient’s personalized WOI and pET.
    - Receptive vs non-receptive
    - Some study design flaws prevent using it for all patients at this time.

Implantation Basics

Implantation Basics

- Defined as the process by which the embryo attaches to the endometrial surface of the uterus and invades the epithelium, then the maternal circulation to form the placenta.
- Time and location specific-crosstalk can only occur between a **receptive endometrium** and a **competent blastocyst** during a limited time span called the “window of implantation”.
- Uterus goes from non-receptive to receptive (5-10 days after LH surge) back to non-receptive.

Implantation Basics

- Defined as the process by which the embryo attaches to the endometrial surface of the uterus and invades the epithelium, then the maternal circulation to form the placenta.
- Organized but complex series of steps required for successful implantation.
- Embryo spends about 72 hours in the uterine cavity before implanting
  - Relies on nourishment in the uterine cavity as opposed to maternal bloodstream.
- Fetus is “in charge”.
  - Influences its own growth and development
- Implantation is initiated when blast comes into contact with uterine wall
  - Assisted hatching

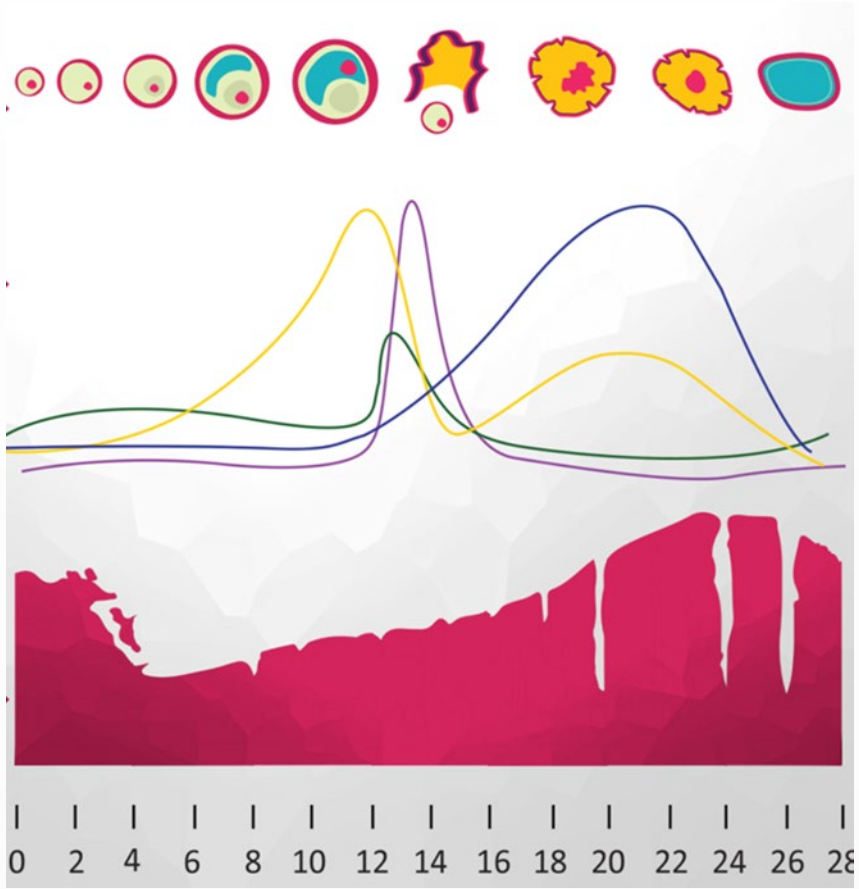
Implantation Basics

- Pregnancy needs to adhere to uterine wall
  - Hormones need to “prime uterus”. Estrogen and progesterone (vascularizes uterus and increases secretions).
  - Secretions provide nourishment to growing blast
  - Other hormones which are generated by uterus in response to progesterone are cholesterol, steroids, adhesion molecules, surface receptors.
  - Hormones might cause a “swelling” that helps press the blastocyst against the uterine cavity
  - Other molecules have important roles to loosen the decidual cells permitting invasion of the embryo into the uterus
- Following ovulation, the endometrium becomes a secretory organ

Role of Pregnancy Hormones

hCG

- Role of hCG is to support the CL and prevent its demise.
- hCG mostly synthesized by syncytiotrophoblast cells
- Levels peak at 8-10 weeks gestation
- Serum hCG levels assist with pregnancy monitoring until ultrasound



Estrogen

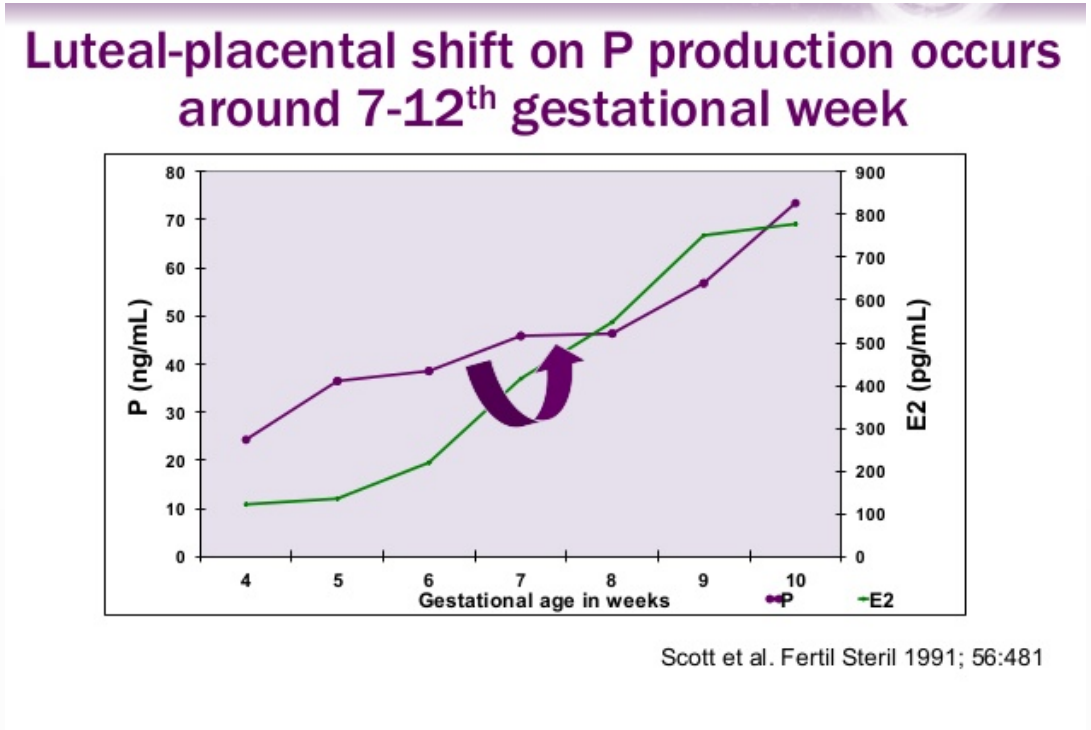
- Production dependent on fetal and placental communication
  - So measurement of estriol (estrogen primarily derived from fetus) level can be reflective of fetal well-being
- Estradiol produced by CL until weeks 6-8 when placental production starts-converts fetal androgens through process of aromatization
- Influences progesterone production, uteroplacental blood flow
- Regulates the maternal cardiovascular adaptations that are necessary in pregnancy, such as increased blood volume and vasodilatory effect

Progesterone

- Produced by corpus luteum until about 10 weeks gestation. Fetus entirely dependent upon corpus luteum production until 7 weeks.
- Placenta becomes major source of P production
- Majority of P derived from maternal CHO conversion from blood stream to trophoblast cells (not fetal contribution)
- Has a role in suppressing the maternal immunologic response to fetal antigens (to prevent maternal rejection of fetus).
- Prepares and maintains the endometrium to allow implantation (differentiation)

Luteal-Placental Shift

- Until 7 weeks gestation, survival of the pregnancy is contingent upon a properly functioning CL
- The placenta gradually takes over from 7-10 weeks with full placental function by 10 weeks.



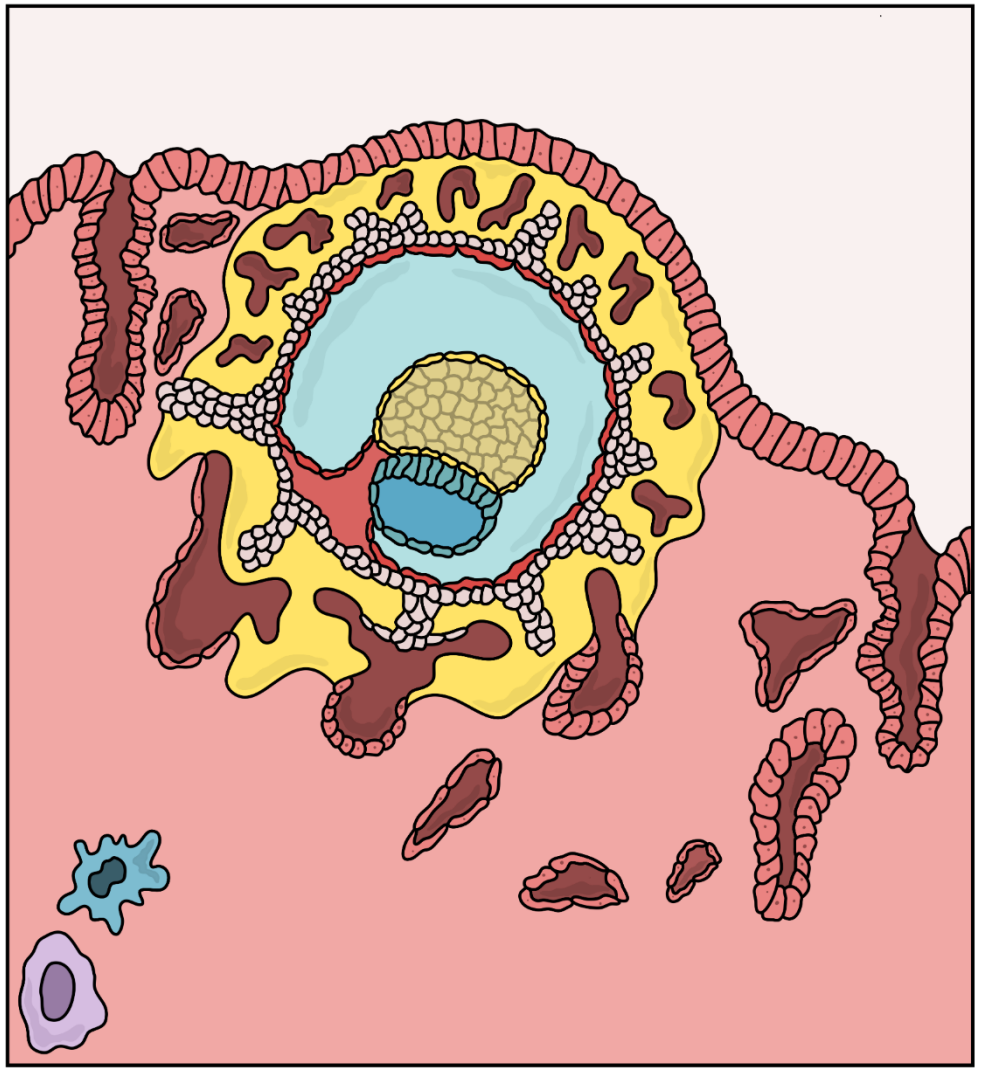
Placental production of other substances

- Placental production of cytokines and growth factors involved in prenatal growth and development
  - Regulates transfer of nutrients across placenta to fetus
  - Help with differentiation of cytotrophoblast cells to syncytiotrophoblasts

The Process of Implantation

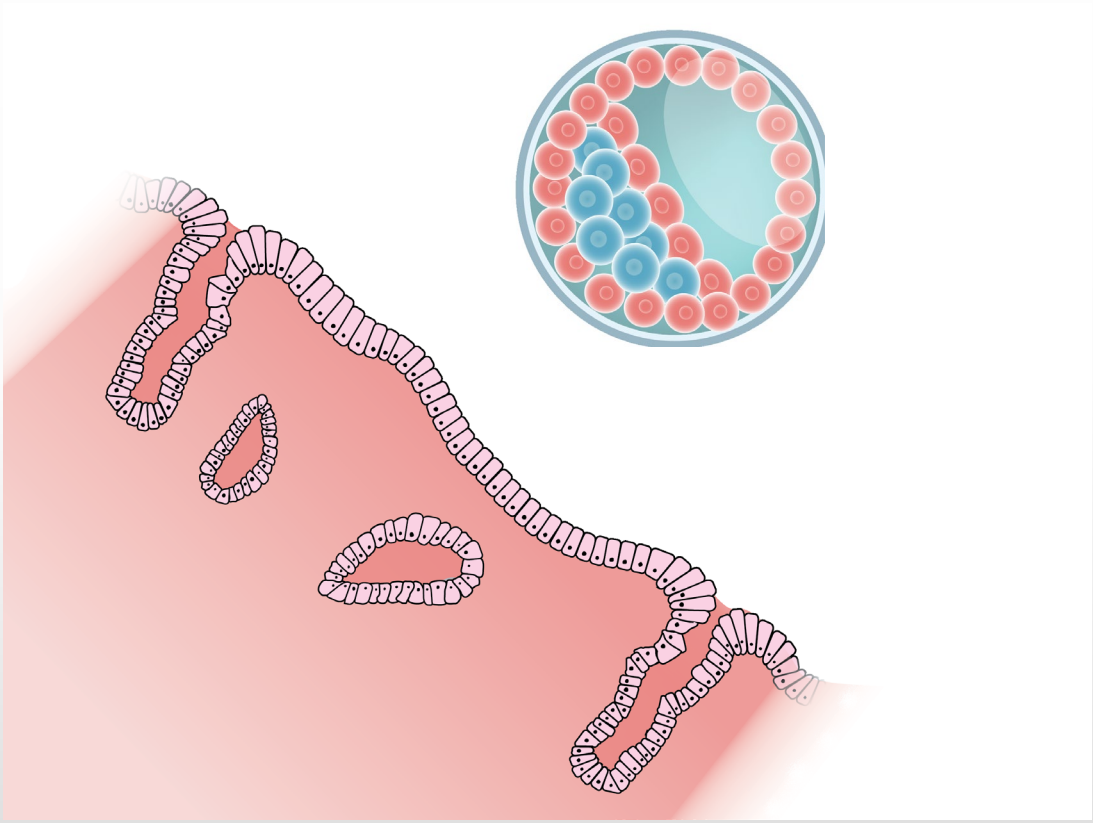
Process of Implantation

- An inflammatory process
- Prostaglandins important for troph invasion
- Starts with having the necessary lining of uterus
- Then, a timely and ordered regulation of cellular and genetic changes in the endometrial tissue surrounding the implanting embryo is critical.



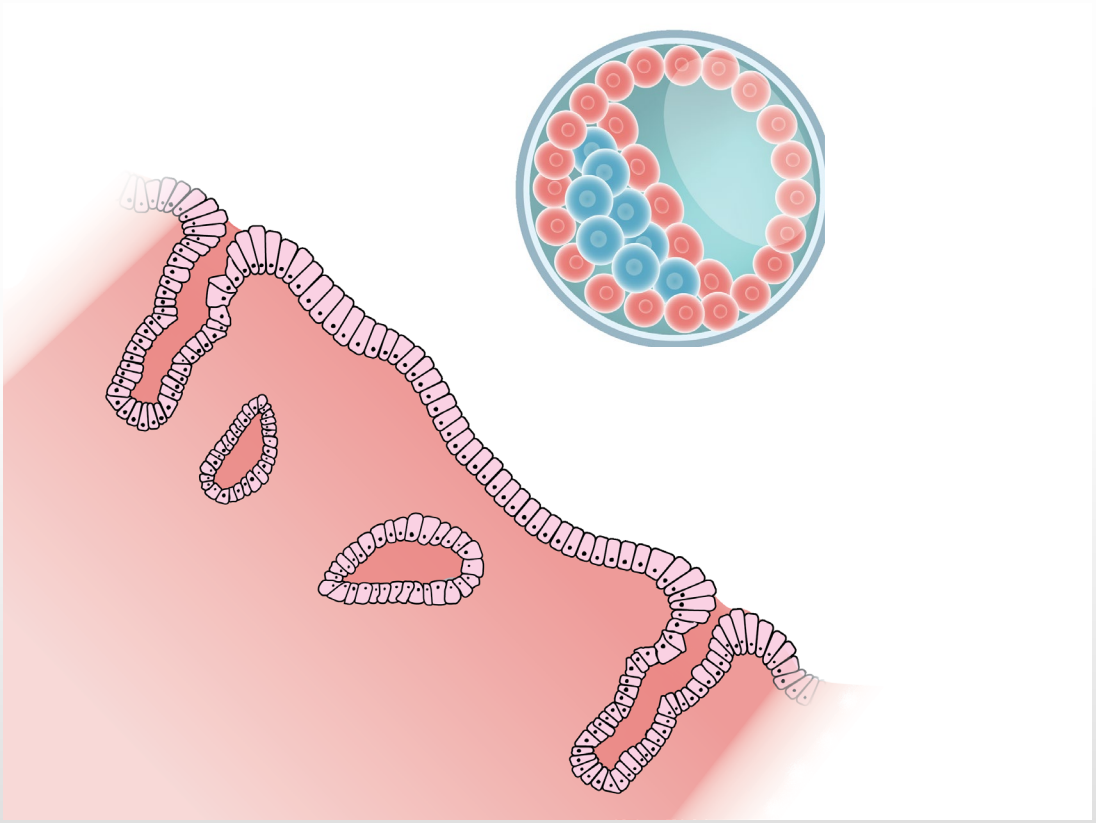
Apposition-Embryo effects

- Trophoblast cells need to come into contact with endometrium
  - Approximately 6-7 days post-fertilization (2-4 days after morula enters uterine cavity)
- The blast differentiates into an ICM and trophectoderm during this process
- Inner cell mass aligns itself closest to the decidua



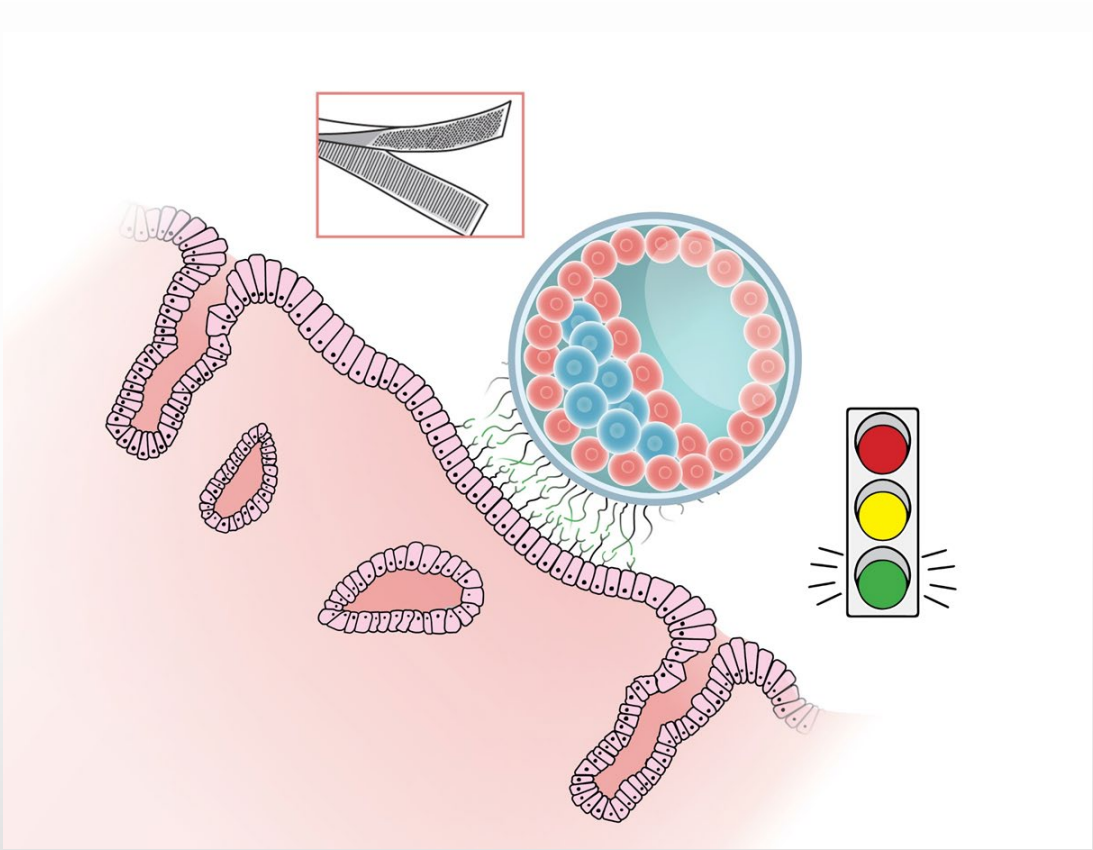
Apposition-Uterine effects

- Stromal cells differentiate into a special cell type called decidual cells.
  - Supports embryo growth and maintains early pregnancy
  - Function mostly replaced by placenta
- The substance/molecules necessary for apposition to take place are unknown



Pinopodes

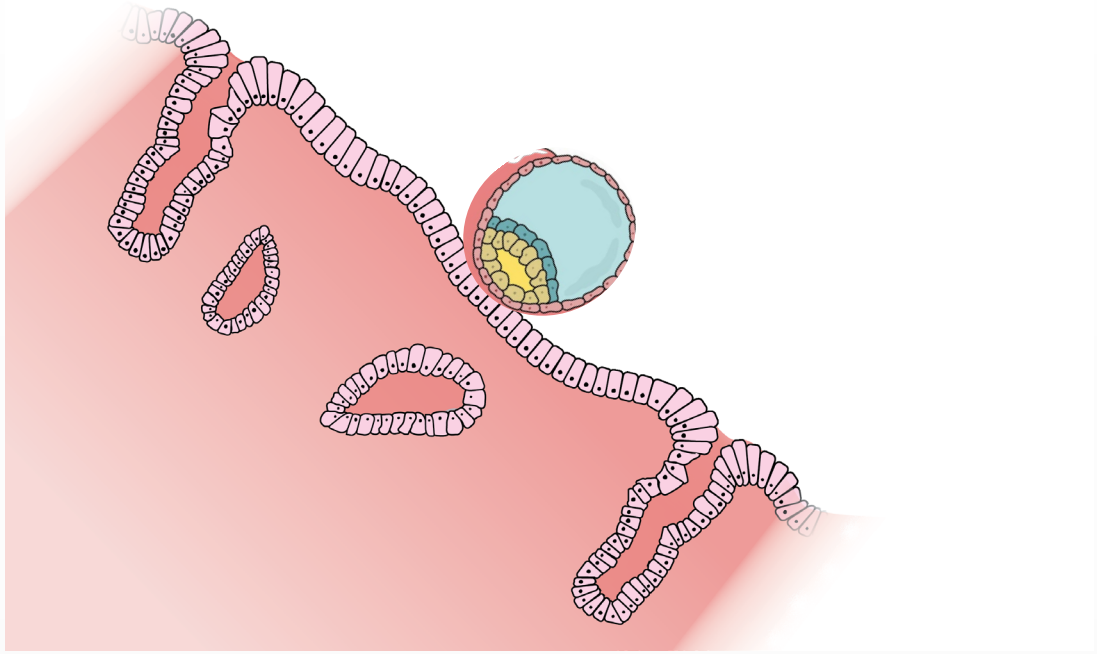
- Small protrusions from the endometrium
  - Microvilli come into contact with pinopodes (Velcro effect)
- Only last for 1 to 3 days (around the window of implantation)
- Not sure of their exact role
  - Probably have role in implantation and endometrial receptivity
  - They might decrease uterine fluid and the molecules found in it (might approximate uterine walls so that floating blast can adhere)



Mirkin et al, 2004; Nikas et al, 1999.

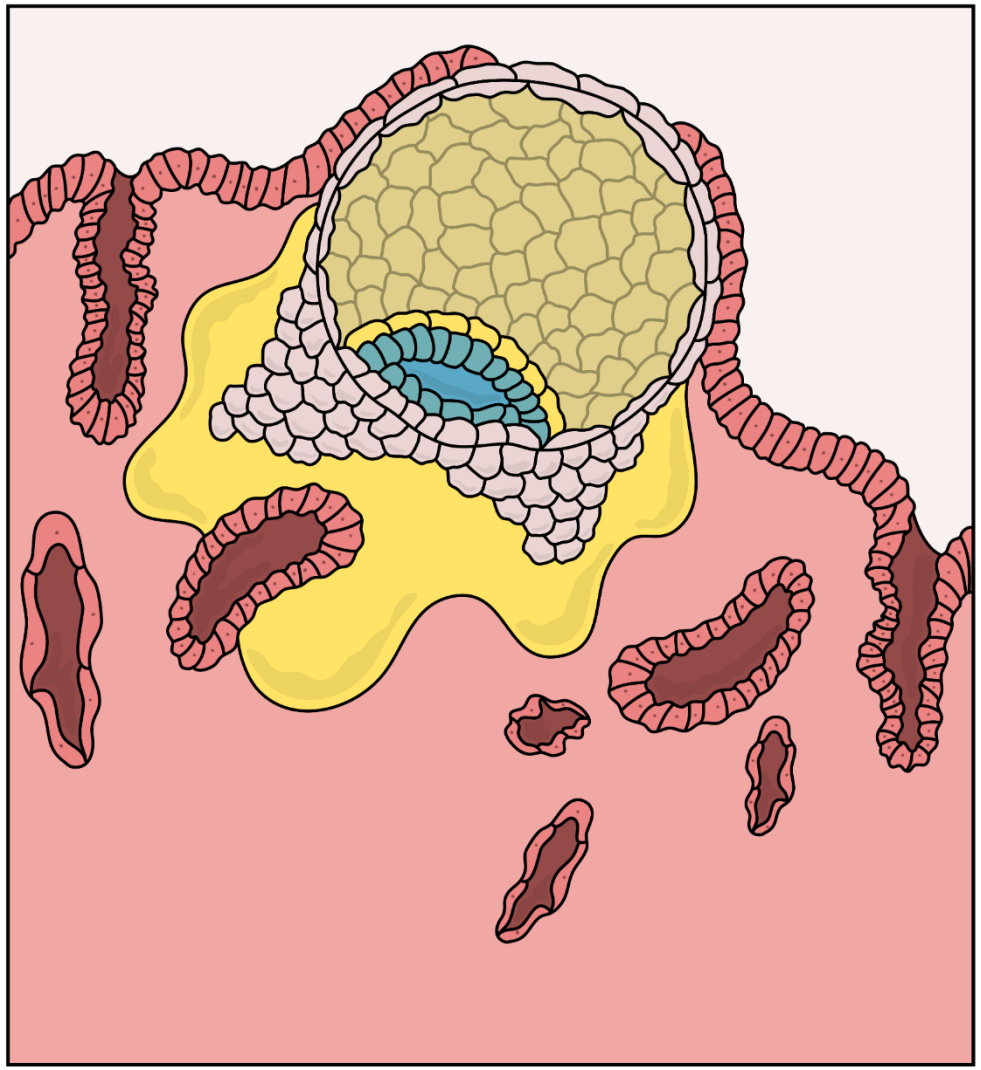
Adhesion

- Next step
- Stronger attachment
- Trophoblasts penetrate the endometrium
- Communication between endometrium and blastocyst is critical at this stage.
  - Blast “alerts” the decidual cells that it’s attaching so they “allow” the invasion.



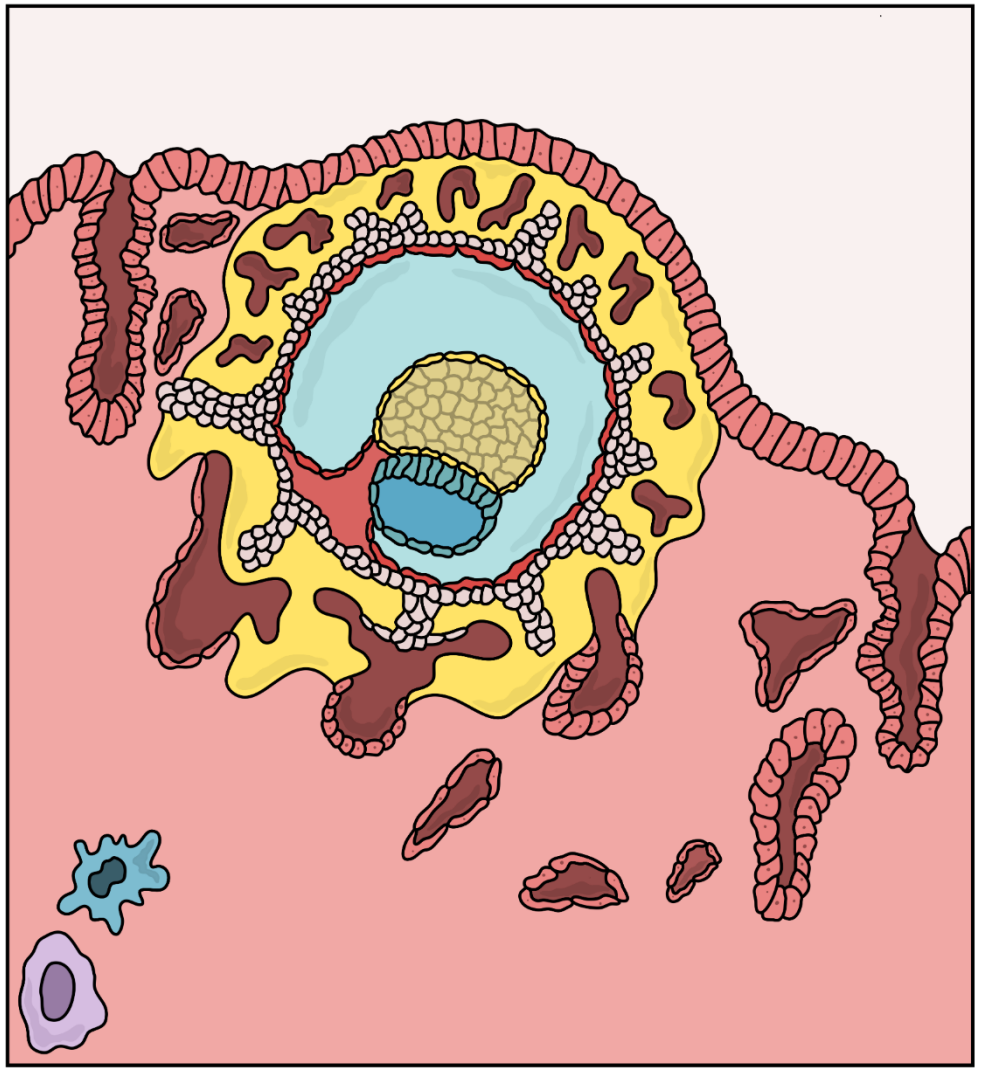
Adhesion/Invasion

- Trophoblast cells continue to penetrate and proliferate into the endometrium
- They further differentiate to become syncytiotrophoblast cells which are the functional cell of the placenta, the major site of hormone and protein production.
    - These continue to invade and reach the basement membrane, then the whole embryos is embedded in endometrium.
    - These form chorionic villi

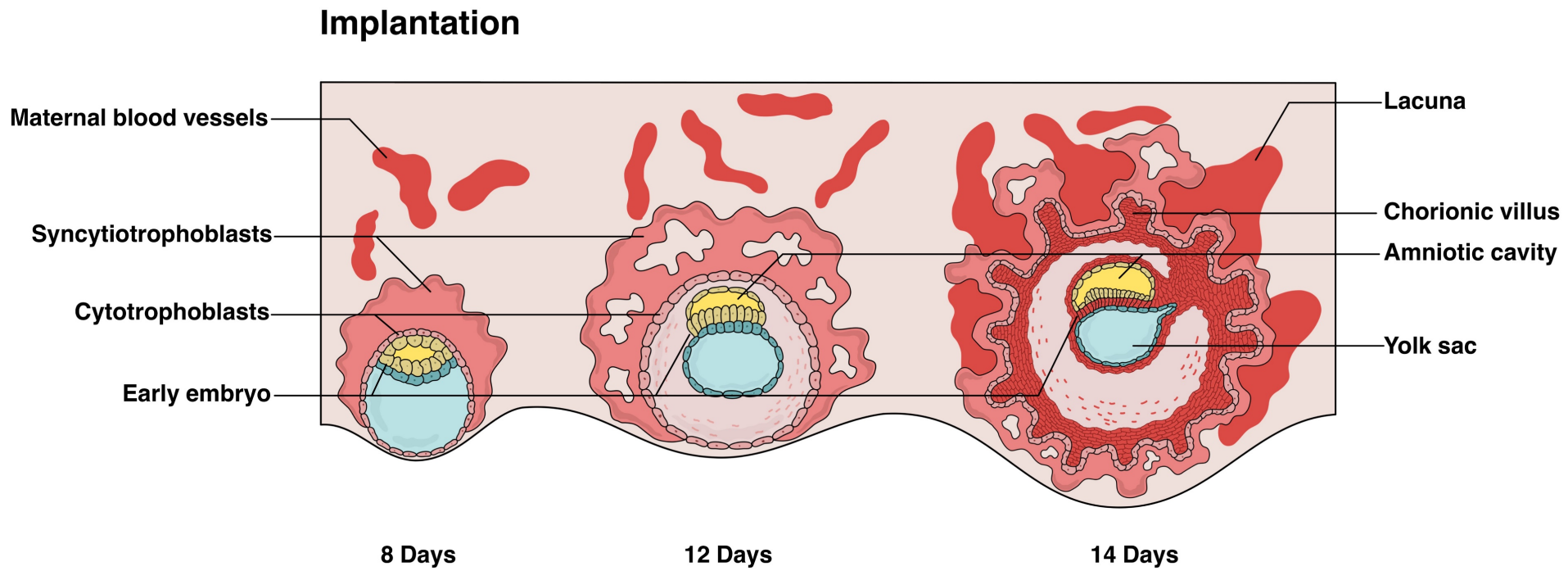


Invasion

- At this stage, blastocyst secretes autocrine factors (like hCG) which loosen decidual cells, prevent the embryo from being rejected by the mother and prevent menstruation from occurring.
- Embryo also secretes immunosuppressive agents (such as platelet activating factor, prostaglandin, estrogen) which keep the embryo from being rejected by the mother.



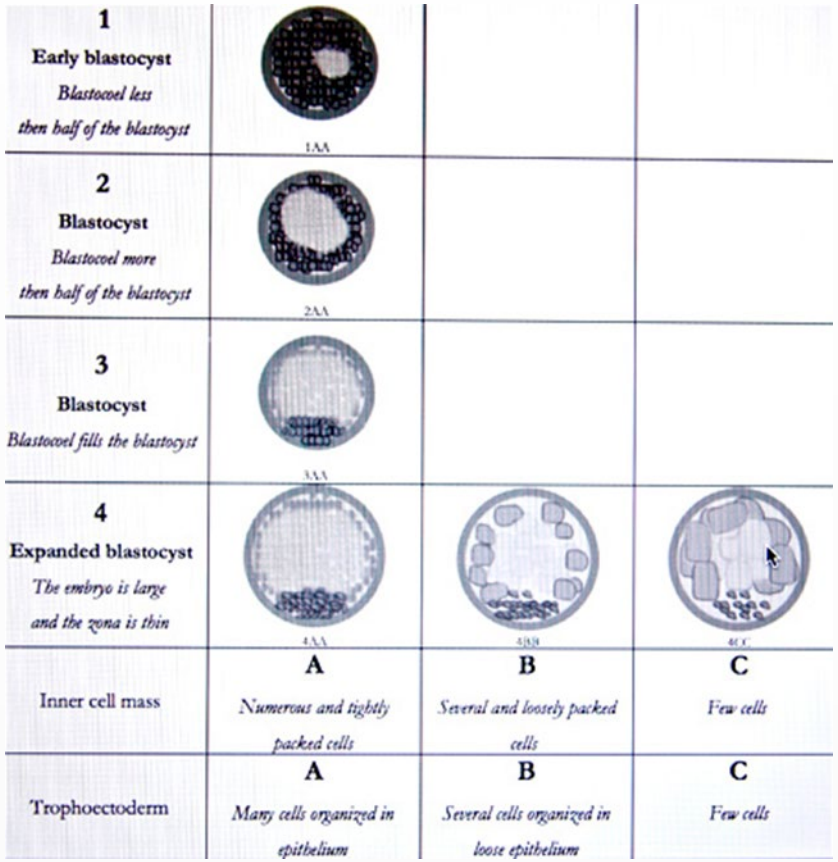
Implantation



The effect of COS on Endometrial Receptivity

Blastocysts grow at different paces

- Normal variation in blast growth rate, some women make expanded blasts on day 5, some on day 6



1 **Early blastocyst** *Blastocoel less then half of the blastocyst*	1AA		
2 **Blastocyst** *Blastocoel more then half of the blastocyst*	2AA		
3 **Blastocyst** *Blastocoel fills the blastocyst*	3AA		
4 **Expanded blastocyst** *The embryo is large and the zona is thin*	4AA	4AB	4CC
Inner cell mass	**A** *Numerous and tightly packed cells*	**B** *Several and loosely packed cells*	**C** *Few cells*
Trophoectoderm	**A** *Many cells organized in epithelium*	**B** *Several cells organized in loose epithelium*	**C** *Few cells*

IVF Phoenix. www.acfs2000.com

COS can generate uterine/ovarian dysynchrony

- In IVF, gonadotropins stimulate ovary to produce supra-physiologic levels of E and P which can advance endometrium
  - Premature progesterone elevation detrimental to implantation
- Ovaries (and therefore follicular development) may be out of synch with uterus.
- Mature pinopodes (structures on endometrium that appear at time if implantation) appear 1-2 days earlier in cycles with COS and are less numerous

Nikas, et al, 1999; Kolibianakis, et al, 2002; Shapiro et al, 2010

“Following COS, the endometrium is histologically advanced, biochemically different, and genomically dysregulated.”

Horcajadas et al, 2007.

Early Pregnancy

Early Pregnancy Basics-Terminology

- Gestational timing begins with day 1 of the last menstrual cycle (LMP)
  - So day 1 of first missed period is considered day 1 of “gestational week 4”.
  - If undergoing a treatment cycle, based timing on day of ovulation or embryo transfer since follicular phase may vary.
- Due date (EDC) is 280 days from the LMP
  - Difficult to detect solely based on timing.
  - Early vaginal ultrasound (before 12 weeks) helps with timing
- Terminology
  - Clinical Pregnancy-a pregnancy is confirmed by ultrasound
  - IUP-Intrauterine pregnancy
  - Ongoing Pregnancy-Current, viable pregnancy confirmed by ultrasound

Early Pregnancy-Monitoring

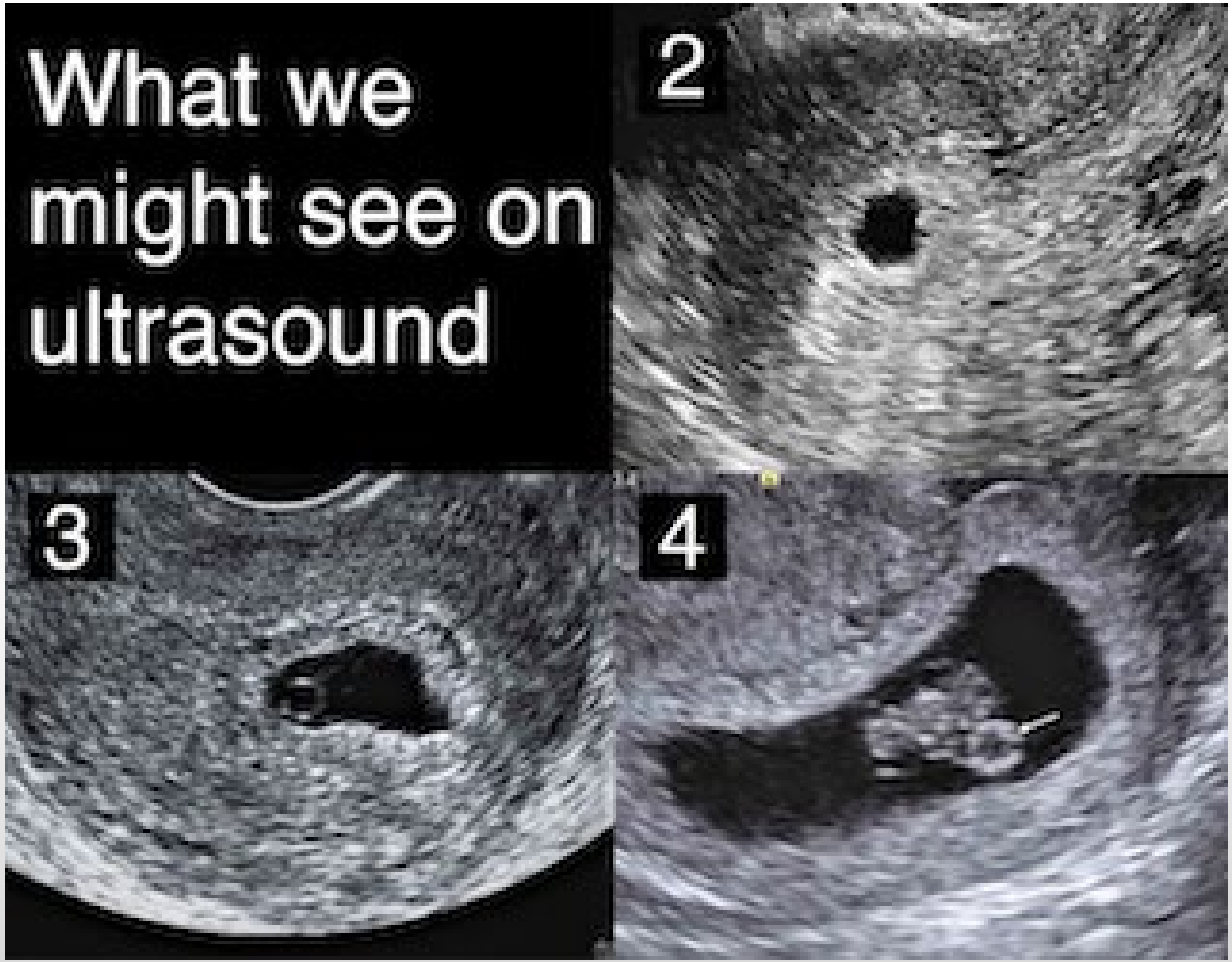
- bHCG levels important. Initial level differs depending on lab assay (75-100 IU/L) and number of embryos that implant
- Levels peak around 8-10 weeks gestation-the range of normal levels at this point make the serum level alone less reliable
- Early pregnancy characterized by rising levels of hCG (at least 60% every 2 days)
  - Trophoblastic disease-very high levels of hCG
  - Ectopic pregnancy-level increases at different (lower) rate-when hCG levels reach 1500 to 3000 IU/L, a gestational sac should be detected by vaginal u/s.

Early Pregnancy-Ultrasound Findings

Gestational Age	bHCG level	US Finding
4 weeks	1,000-2,000 IU/L	Gestational Sac
5 weeks	7000+ IU/L	Yolk Sac
5-6 weeks		Fetal Pole
6 weeks	10,000+ IU/L	Fetal Pole with cardiac activity*
7 weeks		Fetal Movement
8 weeks		Head, limb buds
12 weeks		Hands, fingers

*early cardiac activity may be detected at the end of 5 weeks (5 5/7-5 6/7) but should be detected by the time the embryo is 5 mm in diameter

Adapted from ASRM course: Early Pregnancy



https://utswmed.org/medblog/patience-key-understanding-timing-early-ultrasounds/

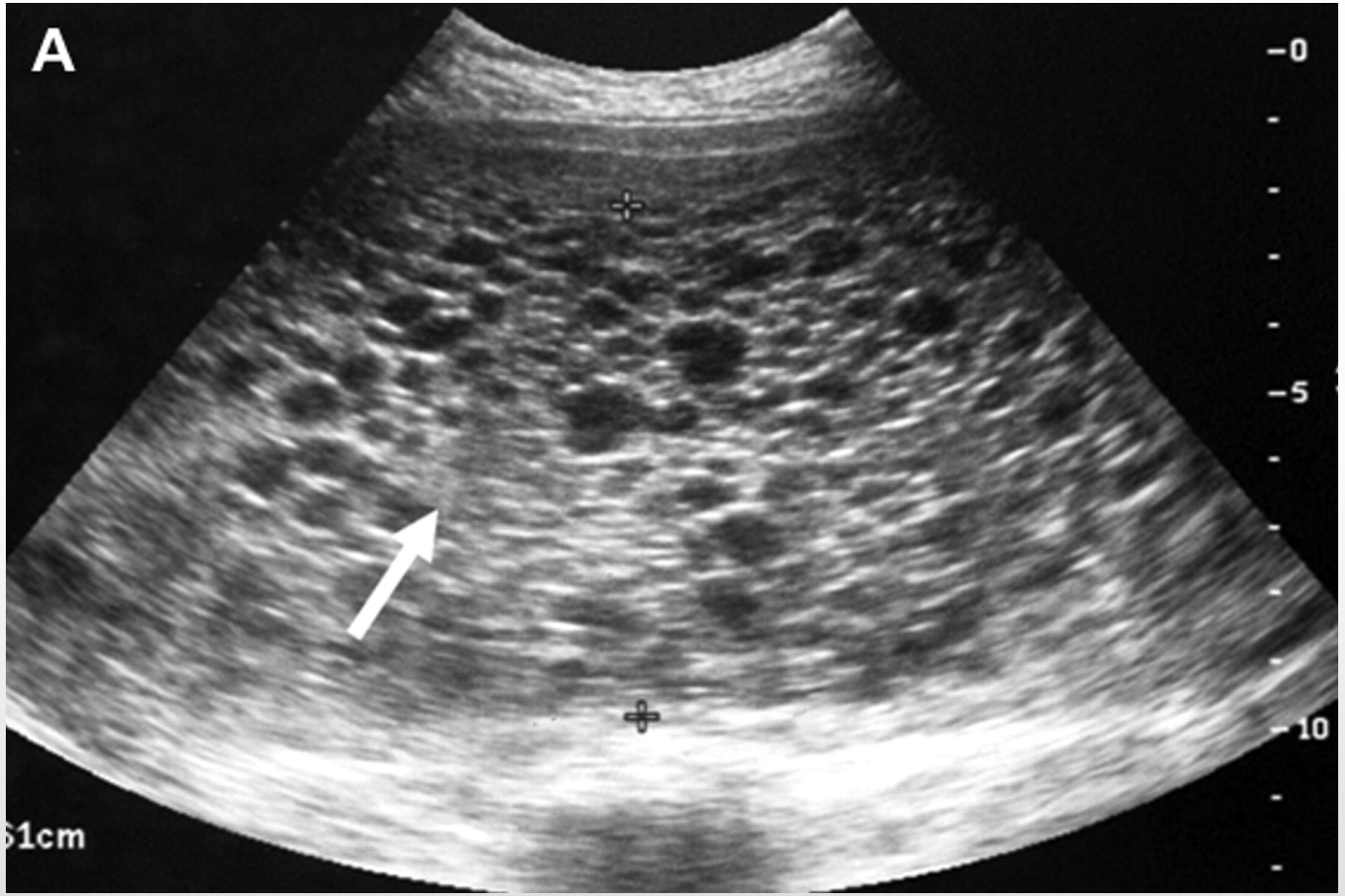
When the pregnancy isn’t viable

When the pregnancy isn't viable-Terminology

- Pregnancy of unknown location
  - Evidence of pregnancy due to elevated bHCG levels, but unable to visualize structures by ultrasound in the uterine cavity
  - hCG levels may be affected
    - Low levels at beginning (less than 75 IU/L)
    - Levels do not increase appropriately, plateau or decrease
- Blighted ovum
  - Gestational sac without fetal structures
- Spontaneous abortion (Sab)-rate 16%
  - "Miscarriage"
  - Might have spotting/cramping; bHCG decline; absence of FHB >6-7 weeks gestation
  - May or may not have bleeding
- Missed abortion (Mab)-Pregnancy does not continue to grow or loses fetal heartbeat, but remains in uterus
- Chemical/biochemical pregnancy-bHCG levels present (usually low), sometimes rise or rise slowly, then plateau. No IUP ever identified-R/O ectopic!

Molar Pregnancy

- Caused by abnormally fertilized egg, but instead of embryo growing normally, placenta develops into an abnormal mass of cysts.
- Complete or partial
  - Complete-no embryo or normal placental tissue
  - Partial-might contain abnormal embryo or some normal placental tissue
- After molar pregnancy removed, some tissue might remain and penetrate into uterine wall-persistent gest trophoblast disease (GTD)
- Rarely cancerous form of GTD develops



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Early Pregnancy Loss

- Check initial bHCG. Follow your center's parameters for a good first level (75-100)?
- The next level should be check in 2-3 days and preferably double (or at least increase by 60%). If levels plateau or drop, consider stopping luteal meds if applicable.
- If spontaneous loss is documented by u/s (no fetal growth, lack or loss of FHB), medical or surgical treatment vs expectant management

Misoprostol for medical management of early pregnancy failure

- Misoprostol is a synthetic prostaglandin E1 which was developed and approved originally for the prevention of gastric ulcers.
  - It is not FDA approved for uterine evacuation in pregnant women, but used off-label in the practice of OB/GYN
- Prostaglandin E1 causes myometrial contractions-causes cervix to soften and uterus to contract
- Usual regimen is vaginal administration of 800 mcg.
  - 75-85% success rate
  - Allow 7-14 days for completion of tissue passing and might need a second dose
  - Ultrasound to confirm complete abortion

Misoprostol

- Contraindications:
  - Pelvic infection or sepsis
  - Hemodynamic instability or shock
  - Known bleeding disorder
  - Confirmed or suspected ectopic or molar pregnancy
- Treatment effects:
  - Heavy bleeding and cramping
  - Nausea/vomiting/diarrhea
  - Fever and chills

Ectopic Pregnancy

- Extrauterine pregnancy
- Account for about 2% of pregnancies in US. Incidence has risen 6 fold over the past 20 years due to the use of ART.
- Early diagnosis based on clinical suspicion, u/s findings and hormone levels
  - hCG levels-abnormal trend
  - Ultrasound-detection threshold (1500-2000 IU/L)
  - May be asymptomatic
- Two treatments: Medical or Surgical
  - Medical usually preferred in non-emergent situation. Avoids anesthesia and surgery risks, cost-effective, can preserve fallopian tube(s).
- The success of medical treatment is approximately 90%.

Methotrexate

- Chemo agent that disrupts cell multiplication (folic acid antagonist)
  - Dose 50 mg/m2
- Toxic to replicating trophoblastic cells
  - Used in treating molar pregnancies
- Administered in single or multiple IM injections
- Best results when:
  - Size of gestation doesn’t exceed 4 cm (or 3.5 cm with FHB)
  - No evidence of tubal rupture
  - Pt must be reliable to return for f/u care
  - bHCG <5000 mIU/ml

Single dose Treatment Regimen

- Day 1-Give MTX 50 mg/m2 IM
- Day 4- Measure Quant bHCG (common to see a rise from day 1)
- Day 7- Measure Quant bHCG. If there has been a decline of > or =15% from day 4 level, follow serum levels weekly until <5mIU/ml
  - If there has NOT been a decline a second dose may be given to the patient (that would be there new day 1).

Multi-dose MTX

Considered in the following situations:

- Cervical/corneal/ C-section ectopic
- Tubal pregnancy with cardiac activity
- Bhcg > 5000
- Leucovorine is folinic acid and allows a higher dose of MTX to be used by mitigating some of its side effects.

Multi-dose MTX Regimen

Treatment day	Laboratory Evaluation	Intervention
1	bHCG (baseline)	MTX 1.0 mg/kg IM
2		Leucovorin 0.1 mg/kg IM
3	bHCG	MTX 1.0 mg/kg IM if bHCG<15% decline from day 1 to day 3. If bHCG >15%, stop treatment and start surveillance’
4		Leucovorin 0.1 mg/kg IM
5	bHCG	MTX 1.0 mg/kg IM if bHCG,15% decline day 3-day 5. If bHCG>15% stop tx and start surveillance.
6		Leucovorin 0.1 mg/kg IM
7	bHCG	MTX 1.0 mg/kg IM if bHCG<15% decline day 5-day 7. If bHCG>15% stop tx and start surveillance.
8		Leucovorin 0.1 mg/kg IM

Patient Instructions

- Avoid intercourse
- Refrain from taking food and multivitamins containing folic acid
- Review adverse affects (see next slide)
- Review S & S of rupture
  - Hemodynamic instability, increasing abdominal pain (regardless of hCG values), syncope, shoulder pain

MTX

- Contraindications
  - Liver disease
  - Blood disorders/thrombocytopenia/anemia
  - Renal, hepatic or pulmonary dysfunction
- Adverse effects
  - Nausea/vomiting
  - Diarrhea
  - Dizziness
  - Transient elevation in LFTs
  - Increase in abdominal pain (separation of pregnancy from implantation site?)
  - Increase in bHCG levels in first 1-3 days of tx
  - Vaginal bleeding or spotting

Conclusions

- Early pregnancy regulated by embryo/endometrial communication and signaling.
- Ovarian and uterine events must “match”
- COS can have a negative effect on the implantation window
- The complexity of implantation precludes RE centers from having 100% pregnancy rates.