

# REPRO / FERTILIZATION- I

## Physiology of Reproduction - Gametes, Fertilization, Early Embryo Development

### Introduction

Thus far, we have maintained a gender separation in our consideration of the physiology of reproduction. In the second week of the reproductive medicine curriculum there will be an extensive investigation of human sexuality. One, almost incidental, aspect of that process is the deposition of semen in the vagina. It is the events that transpire after that process that we consider now. The fertilization of the oocyte, the maturation of the resulting cells, their implantation in the uterine lining and eventual development to birth represent a perilous time for new individuals. It is estimated that anywhere from 60 to 80% of all "fertile matings" fail to produce offspring. It is also believed that fertilization and implantation failures account for much of this outcome. While this may or may not be a good thing, these estimates provide ample reason to learn about initial events in the creation of a new individual.

### Objectives

**At the end of this unit the student will be able to:**

#### **I. Gamete Transport and Maturation in the Female Reproductive Tract**

- A. Describe egg transport from the ovulated follicle to the site of fertilization.
- B. Describe sperm transport from the vagina to the site of fertilization.
- C. State the functional changes in the sperm during this time.

#### **II. Fertilization, Implantation and Early Embryo Development**

- A. State the consequences of sperm binding to the oocyte
- B. Learn the principal events that control development of the zygote to the blastocyst stage.
- C. Draw the structure of a blastocyst and describe the two major cell types.
- D. Describe the early events of blastocyst "hatching" from the zona pellucida.

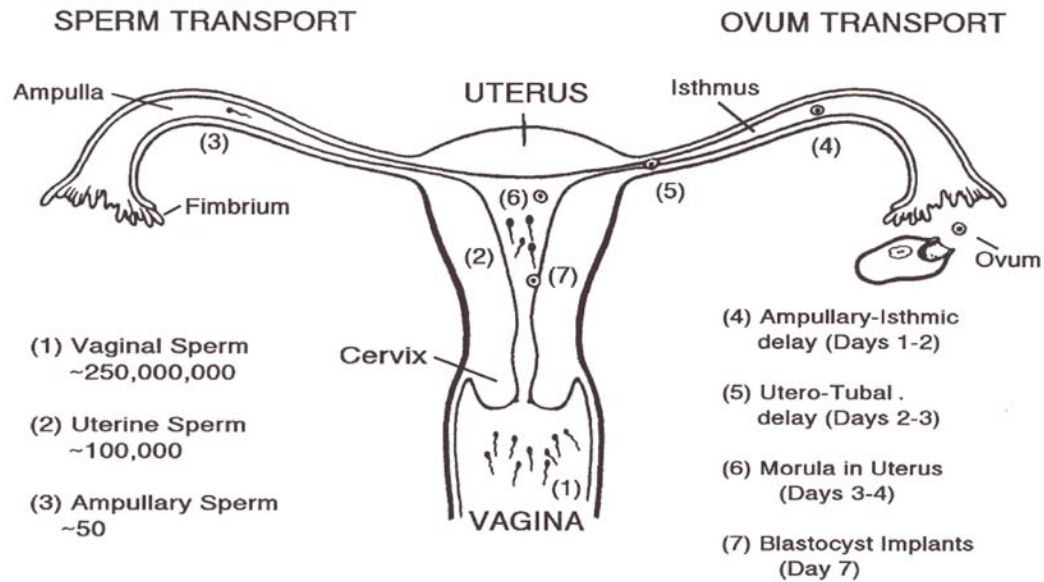
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### Physiology of Fertilization & Implantation: Achieving the Objectives

#### I. Gamete Transport and Maturation in the Female Reproductive Tract

- A. After ovulation the oocyte, surrounded by a mass of cumulus oophorous cells, is "picked up" by the fimbria of the fallopian tube. This action requires the motion of fimbrial cilia to remove the cumulus mass from the site of follicle rupture. These cilia also propel the mass along through the ostium and into the ampulla of the oviduct. The density of cilia in the fimbria is dependent on circulating estrogen concentration. Further movement of the cumulus mass is achieved by ciliary motion and smooth muscle contraction. Pharmacologic blockade of smooth muscle activity does not slow the progress of the oocyte, thus ciliary motion may be more important and muscle activity a "redundant" mechanism. Upon reaching the ampulla, the cumulus mass is held up for several hours. If sperm are present, fertilization will occur at this time and the early stages of development will occur.
- B. Of the 250,000,000 sperm in a typical ejaculate, only a very small number (estimates around 50 - 100) reach the vicinity of the cumulus/oocyte complex. Their transport involves several processes. Migration through the cervical mucus seems to occur by the sperm's own innate motility. The relatively low viscosity of this mucus at mid-cycle may aid sperm movement at this time. Sperm transport through the remainder of the female reproductive tract remains a bit of a mystery. Most authorities believe that uterine contraction is the major component because inert material moves through as quickly as live sperm. Upon reaching the isthmus the sperm are apparently "stored" for a period of time (a few hours). This is likely when the process of **capacitation** occurs. The signal for sperm release to finally migrate to the ampulla is not known, but this movement is nicely timed to coordinate with the arrival of freshly ovulated oocytes. Naturally, hormonal signals associated with ovulation are proposed.

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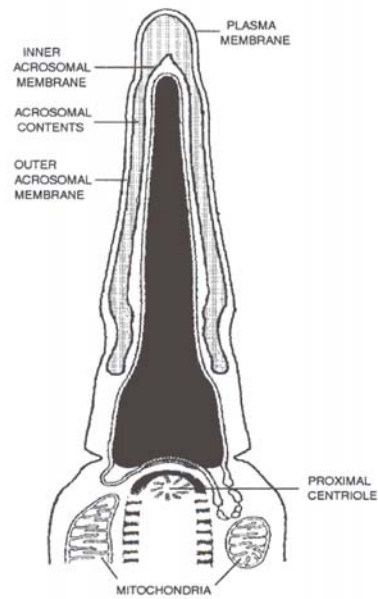
C. Freshly ejaculated sperm are incapable of fertilizing ova. Several changes must take place. These fall into three categories; **capacitation, binding to the zona pellucida and the acrosome reaction.**

**1. Capacitation** - Usually occurs in the female reproductive tract. The details are sketchy at best, however, the metabolic rate of sperm increases, changes in the protein components of the sperm head and changes in flagellar movement all occur. The changes in the sperm head membrane are important because they prepare the sperm for the subsequent processes of recognition and binding to the zona pellucida. The change in motility pattern is from progressive forward motility to "hyperactivated" with a "whiplash" type of flagellar movement. The latter seems essential to drive the sperm through the cumulus layer and the zona pellucida.

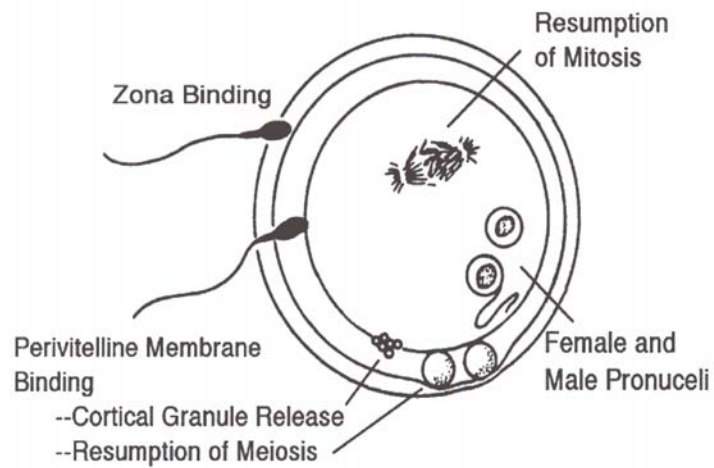
**2. The binding of the sperm head to the zona is species specific.** Because of the specificity, which is achieved by classic ligand-receptor interaction, this process is currently being manipulated in an attempt to develop new contraceptive strategies. The binding process may be the trigger for the next event - the acrosome reaction.

**3. The acrosome reaction** occurs at the outer surface of the zona. It involves distinct changes in the head of the sperm such that the enzymes in the acrosome are exposed to the extracellular space and are activated.

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D. Penetration of the zona is achieved by the enzymatic contents of the acrosome weakening the structure of the zona and flagellar movement propelling the sperm forward. Binding of the sperm head to the oocyte then occurs.



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## II. Fertilization and Early Embryo Development

**A. Fertilization** - This is initiated by the sperm binding to the surface of the oocyte. The sperm becomes passive and is then engulfed by the egg; this binding has two important consequences.

1. Cortical granules are released from the egg and alter zona structure prevent the binding of any additional sperm heads.
2. The binding induces the resumption of meiosis in the oocyte and this leads to extrusion of the second polar body.

The male pronucleus decondenses and combines with the analogous material from the egg.. This reconstitutes the diploid chromosome number and marks the end of the fertilization process.

### **B. Preimplantation Development**

The first week of development represents a perilous time for the early embryo as the majority of inseminated oocytes fail to successfully complete this developmental interval to implant to the uterine wall and establish a pregnancy. This interval called preimplantation development is uniquely mammalian and encompasses the free-living period of development during which the early conceptus traverses the oviduct and gains access to the uterine environment. This period culminates in the formation of an embryonic structure called the **blastocyst**, which is composed of the outer epithelial **trophectoderm**, a fluid-filled cavity and small group of cells called the **inner cell mass**, which are the progenitors of the embryo proper. The blastocyst is not simply the vessel that delivers its cargo of inner cell mass cells to the uterus as it contains the specialized cell type (the trophectoderm), which will mediate the maternal-fetal interactions required to support eutherian embryogenesis. The trophectoderm cells differentiate into the trophoblast to mediate either invasion or attachment to the uterus during implantation and as a prelude to their subsequent contribution to placental structures. Blastocyst formation is therefore essential for the establishment of pregnancy and is intimately coordinated by the events controlling trophectoderm differentiation.

Decades of research have elucidated many of the cell biological and molecular mechanisms that control this developmental interval. Although the mouse embryo has been the animal model of choice for most of the experimentation, the extension of these studies to the human and domestic agricultural species has stimulated vigorous research activity in these embryo systems leading to explosive growth in the fields of assisted reproductive technology and embryo transfer in humans and other species. Despite these advances, pregnancy rates following embryo transfer remain lower than expectations for all species, and further advances are required to optimize the general application of these technologies.

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Following fertilization, the embryo undergoes several cleavage divisions that divide the ooplasm into smaller compartments or blastomeres (embryonic cells). The first morphogenetic event, **compaction**, arises following the third cleavage division in the mouse, although the timing of this event varies greatly among the mammalian species arising, for example, at the 32-64 cell embryo stage in the cow embryo. Compaction represents the onset of cellular differentiation during mammalian development as the outer cells of the conceptus begin to polarize as a prelude to trophectoderm differentiation. **Blastocyst formation** is the morphogenetic event that follows compaction in this continuum of preimplantation development. Each succeeding phase of this developmental interval is dependent upon preceding events. It is therefore not possible to consider the mechanisms controlling blastocyst formation by only focusing on the blastocyst. We will begin by briefly examining the oogenetic to embryonic transition and compaction.

### Oogenetic to Embryonic Transition

Mammalian development is initially sustained by gene transcripts and polypeptides produced and stored in the oocyte during oogenesis. After one to several cleavage divisions (depending upon the species), genetic control of development comes predominantly under the embryo's control as oogenetic gene products decline, and embryonic transcription (RNA synthesis/gene expression) begins. The transition from oogenetic to embryonic control of development or **maternal zygotic transition** (MZT) varies in its timing between species. This is a critical event as blastocyst formation is dependent on expression of embryonic genes. However, it must also be kept in mind that specific oogenetic products can still influence development to at least the blastocyst stage and potentially later in development.

### Compaction

Compaction is a common feature of preimplantation development within all mammalian embryos, although the timing of this event varies greatly arising later in the agricultural domestic species. Compacting embryos undergo an increase in inter-blastomeric contact that obscures the distinct individual cell boundaries and continues until the embryo ultimately appears as a uniform cell mass called a **morula**. The cellular events associated with compaction include: (i) the development of  $\text{Ca}^{2+}$  dependent cell adhesion; (ii) the establishment of gap junction mediated interblastomeric cell communication; (iii) the initiation of cell contact-induced cell polarization, and (iv) the appearance of tight junctions which eventually divide the plasma membrane of the outer blastomeres into distinct membrane domains. These events, particularly the development of asymmetrical cell contact and polarity within the outer blastomeres are essential and directly contribute to the formation of the blastocyst. By the late morula stage, the embryo acquires the remaining proteins that are necessary for blastocyst formation to occur.

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### Blastocyst Formation (Cavitation)

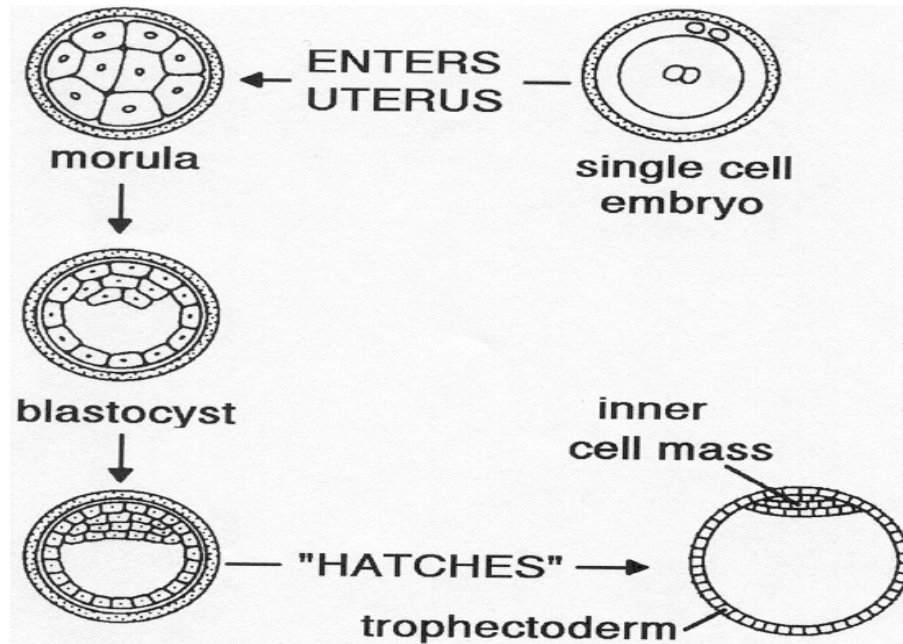
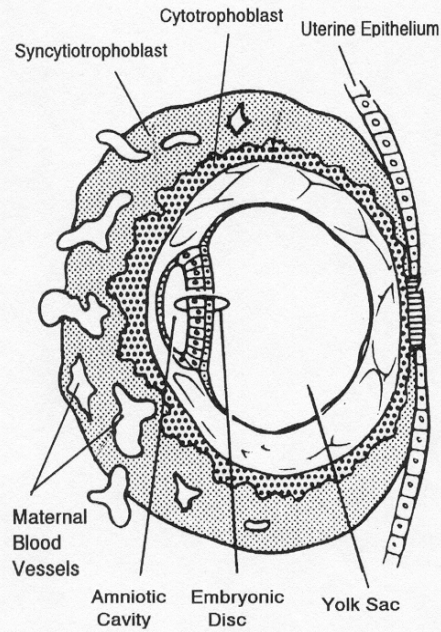
The trophoctoderm acquires the capacity to initiate and regulate blastocyst formation by expressing proteins that facilitate the transport and retention of the blastocoelic fluid as it accumulates in the nascent blastocyst cavity. Therefore, blastocyst formation is dependent upon the development of a polarized epithelium. The blastocoelic fluid is predominantly water, thus the mechanism of its production and accumulation relies on the ion transport properties of the trophoctoderm. In many ways, the trophoctoderm is comparable to an early kidney tubule epithelium as many ion and water transport characteristics are shared between these epithelia. For these reasons blastocyst formation is tightly coupled to the development of trophoctoderm ion transport systems, the epithelial junctional complex, and the primary role played by establishment of cell-to-cell adhesion between blastomeres during compaction.

The vectorial transport of  $\text{Na}^+$  and  $\text{Cl}^-$  (but not  $\text{K}^+$ ) from the medium is essential for the onset and progression of blastocyst formation. The  $\text{Na}^+$  ions likely enter the trophoctoderm cells through apically localized  $\text{Na}^+$  channels and/or via various  $\text{Na}^+$  co-transporters such as the  $\text{Na}^+/\text{H}^+$  exchanger or  $\text{Na}^+/\text{glucose}$  co-transport system.  $\text{Cl}^-$  transport is also mediated across the epithelium. It has been proposed that the trans-trophoctoderm  $\text{Na}^+$  gradient is completed by the active transport of  $\text{Na}^+$  out of the cell into the blastocoelic cavity by a basolaterally localized  $\text{Na}/\text{K}$ -ATPase. The presence of such a gradient would result in the movement of water down the gradient across the epithelium and into the blastocyst cavity. A great deal of evidence collected in the past twenty years supports this hypothesis as it has been determined that the  $\text{Na}/\text{K}$ -ATPase is present in the basolateral plasma membrane domain of the trophoctoderm for several species including the mouse, rabbit, pig, and cow.

Blastocyst formation is thus a complex cellular process that requires the precise coordination of several cellular events.  $\text{Na}/\text{K}$ -ATPase distribution is influenced by the stability of the trophoctoderm tight junctional seal; the membrane cytoskeleton and by cell to cell adhesion. All of these events must occur properly to enable the embryo to develop to the stage where implantation may be attempted.

**C. Growth of the blastocyst** leads to "zona hatching" and at day six post-conception it attaches to the endometrial epithelium. The embryo then erodes the uterine wall and literally sinks into the endometrium and the site of penetration becomes covered with a fibrin plug.

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# REPRO/IMPLANTATION and EARLY PREGNANCY-1

## Physiology of Reproduction- Implantation and Maternal Recognition of Pregnancy

### Introduction:

It is beyond the scope of this section to consider the complete physiology of pregnancy. However we will focus on implantation and maternal recognition of pregnancy as these are critical events that determine whether a pregnancy will be established or not. Implantation is regulated by communication between the embryo and the uterus; the corpus luteum and the uterus; and also by the embryo and the corpus luteum. If an embryo is not present in the reproductive tract the corpus luteum is not maintained, progesterone levels fall and menstruation occurs. We are studying these events because they represent a critical check point in the pregnancy cascade. The majority of early embryos do not reach the blastocyst stage and do not successfully implant. Pregnancy cannot be established without successful implantation occurring and maintenance of progesterone production by the corpus luteum.

### Objectives:

#### At the end of this unit the student will be able to:

#### I. Implantation

- A. Become familiar with the different types of implantation
- B. Learn the three stages of implantation
- C. Present a model for embryo attachment to the uterine epithelium
- D. Know the three possible routes for embryo transgression of the uterine epithelium
- E. Have an understanding of the general mechanisms that regulate trophoblast invasion

#### II. Hormonal Control

- A. Understand the concept of “Uterine receptivity” and the “Implantation window”.
- B. Understand the role played by progesterone and estrogen in initiating pregnancy
- C. Understand the changes that occur to the corpus luteum if pregnancy does not occur.
- D. Be familiar with the structure of human chorionic gonadotrophin and the role it plays in “rescuing” the corpus luteum.

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### I. Overview of Key Features of Implantation

- A. The cells of the trophoctoderm, which are also called trophoblast cells, are the source of human chorionic gonadotropin (hCG) a hormone with LH activity which acts to maintain the corpus luteum formed at ovulation, thus hCG is an essential signal generated by the embryo to signal its presence to the mother.
- B. After adhering to the uterine wall, the trophoblast cells "burrow" between the cells of the uterine epithelium and reach the basement membrane of this layer. Presumably through the action of locally produced proteases, the blastocyst can then breach this membrane and come to rest among the endometrial cells.
- C. The trophoblast cells are derived from the fetus which is immunologically distinct from the mother. These cells, although in contact with maternal tissues do not normally elicit a maternal immune response.
- D. The human endometrium stroma cells undergo differentiation into cells called decidual cells, which rapidly proliferate under both the influences of ovarian steroid hormones encountered in the luteal phase of the menstrual cycle as well as by poorly characterized messages from the implanting blastocyst itself. Increases in permeability of the vasculature of the endometrium is an early event after blastocyst contact with the endometrium. The change in permeability is apparently mediated by prostaglandins formed in the uterine wall. It is believed that the decidual cells provide an initial source of nutrients for the embryo until the vascular connections between fetus and the mother, which will be manifested in a placenta, are established. The decidual cells give rise to the maternal components of the placenta.

### A.Modes of Implantation

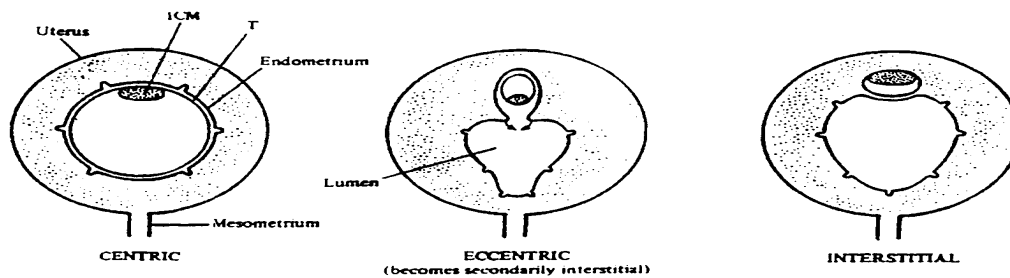
The purpose of implantation is obviously to establish a metabolic connection between the developing embryo and the mother. The free-living blastocyst supports its own metabolism by drawing oxygen and metabolic substrates from the oviductal and uterine fluids. There is however a limit to the size that the "free-living" embryo can reach before these mechanisms become inadequate. To maintain its development the embryo must establish a connection to the maternal vascular system, however it cannot directly invade the maternal circulation and thus a specialized organ called the placenta is required to serve the metabolic needs of the developing fetus. Implantation is the route the embryo takes to establish this important metabolic connection. The outer cells of the blastocyst (the trophoctoderm) play a critical role in mediating

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implantation and also in eventually contributing to the fetal components of the placenta. Across mammalian species there are two general types of implantation: The first is “non-invasive” implantation and the second is “invasive” implantation. The invasive implanters are further divided into either interstitial or eccentric implantation. The following table breaks down the species distribution for these implantation types. You can see that humans fall into the interstitial invasive implantation group.

**Table 9.3.** Classification of implantation and placental forms in several species.

Species	Depth of invasion	Extent and shape of attachment (see Fig. 9.5)	Maternal tissue in contact with conceptus (see Fig. 9.6)	No. of layers of chorionic trophoblast (see Fig. 9.6)	Histological classification (see Fig. 9.6)
<i>Invasive</i>					
Man	Interstitial	Discoid	Blood	1	Haemomonochorial
Rabbit	Eccentric	Discoid	Blood	2	Haemodichorial
Rat/mouse	Eccentric	Discoid	Blood	3	Haemotrichorial
Rhesus monkey	Eccentric	Bi-discoid	Blood	1	Haemomonochorial
Dog	Eccentric	Zonary	Capillary endothelium	1	Endotheliochorial
Cat	Eccentric	Zonary	Capillary endothelium	1	Endotheliochorial
<i>Non-invasive</i>					
Sheep	Central	Cotyledonary	Epithelium	1	Epitheliochorial
Pig	Central	Diffuse	Epithelium	1	Epitheliochorial
Cow	Central	Cotyledonary	Epithelium	1	Epitheliochorial
Horse	Central	Diffuse	Epithelium	1	Epitheliochorial

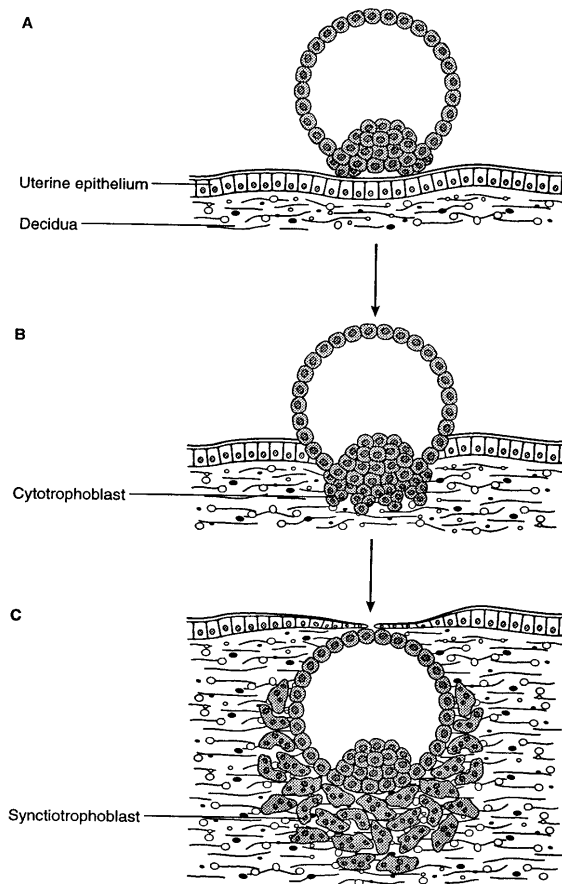


Implantatio

n in invasive species such as the human can be divided into several phases that include:

- A) attachment to the uterine epithelium
- B) transgression through the uterine epithelium
- C) Invasion into the uterine stromal tissue

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**FIG. 16-1.** Human implantation. The embryo attaches to the surface epithelium at the embryonic pole (A), penetrates the epithelium by intrusion led by cytotrophoblast (B), and establishes a trophoblast bed in the decidua with fused syncytiotrophoblast (C).

### A) Attachment:

Attachment of the embryo to the uterine epithelium is a unique process in many ways. It involves the establishment of epithelial (trophectoderm): epithelial (uterine epithelium) interactions which seldom occurs. This is possible only because of the expression of specialized cell adhesion molecules on the surfaces of these two cell types. The most likely candidates for mediating these interactions are **integrins**. This is a very large family of molecules with at least 21 different members. They function to anchor cells to specific locations and can also act as signaling molecules as well. The functional integrin is composed of a  $\alpha$  and a  $\beta$  subunit, thus the functional integrin is composed of a  $\alpha/\beta$  combination such as  $\alpha 1\beta 1$ . These molecules can interact with themselves, other integrins, growth factors, or extracellular matrix proteins such as fibronectin or laminin. Both trophoctoderm and uterine epithelium express integrin molecules.

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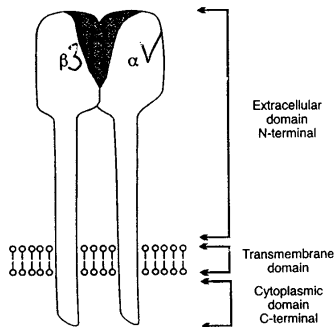
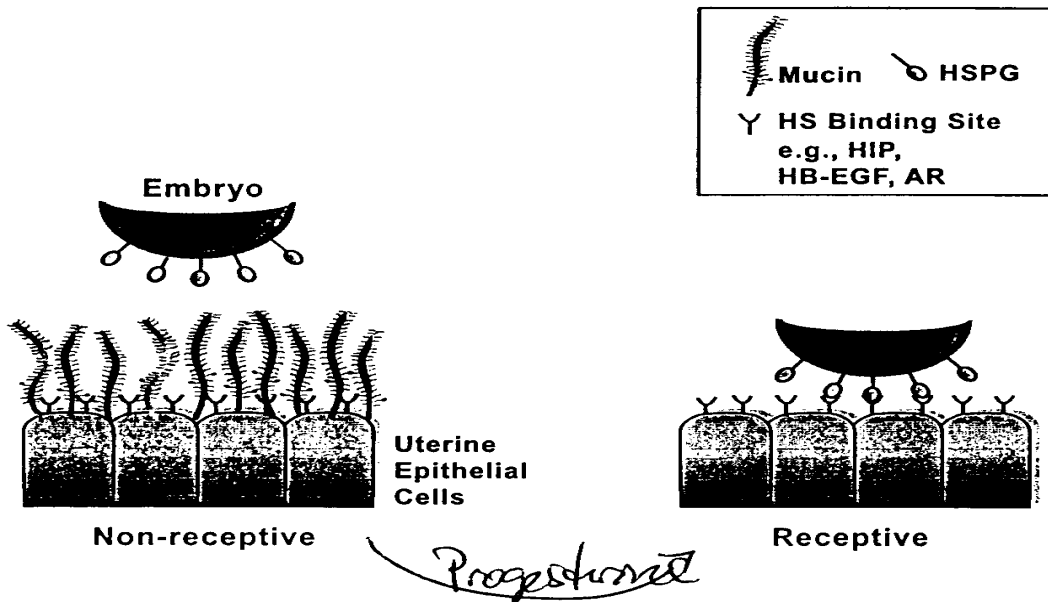


Fig. 1. Structural features of integrin cell surface adhesion receptors. The molecule has two chains ( $\alpha$  and  $\beta$ ) and three domains: extracellular, transmembrane and cytoplasmic. The shaded area, at the junction of the two chains, represents the ligand-binding region.

**TABLE 16-4. Integrins and possible functions during reproduction**

Integrin		Site	Effect
$\beta_1$	$\alpha_4$	Endometrium	Cyclic expression
$\beta_3$	$\alpha_5$	Endometrium	Cyclic expression
$\beta_3$	—	Endometrium	Infertility?
$\beta_1$	—	Embryo	Inner cell mass involution
$\beta_1$	$\alpha_1$	Trophoblast	Limits invasion
$\beta_1$	$\beta_5$	Trophoblast	Accelerates invasion

Interactions between trophoblast  $\alpha 1\beta 1$ ,  $\alpha 1\beta 5$  integrins and uterine  $\alpha v\beta 3$  may mediate attachment. Support for this view comes from studies that show the absence of the  $\beta 3$  integrin has been linked to infertility in some patients. Attachment in the mouse embryo is mediated by changes in the presence of mucin glycoproteins such as MUC-1 on the uterine epithelium. MUC-1 is removed from the receptive uterus in response to progesterone and this allows heparan sulfate proteoglycan (HSPG) on the trophoblast to bind to receptors on the uterine epithelium. It is uncertain whether this mechanism occurs in the human but what is certain is that attachment is a critical first step to the implantation process.



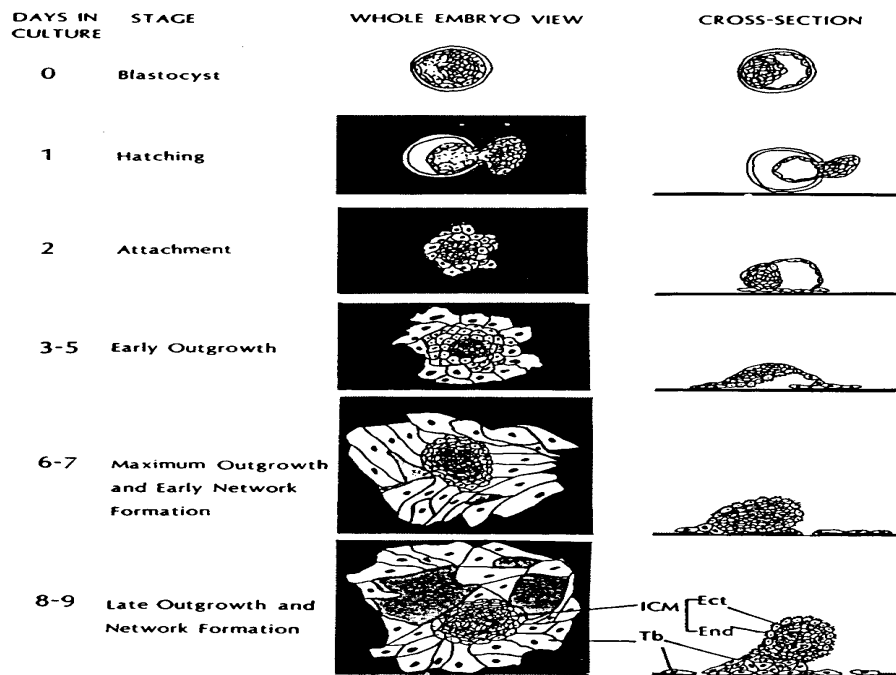
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### B) transgression through the uterine epithelium

As you can appreciate it is very difficult to investigate the mechanisms underlying the movement of the attached blastocyst across the uterine epithelium as it gains access to the uterine stroma. In vivo experiments are pretty near out of the question and while in vitro models of blastocyst invasion exist each has some limitations that affect the interpretation of experimental outcomes. There are at least three proposed mechanisms though for this migration. The trophoblast may penetrate between the uterine epithelial cells (intrusive penetration); directly replace the epithelial cells (displacement penetration) or fuse directly with the endometrial cells (fusion penetration). It is unknown what mode is operational in the human but since rhesus monkeys employ an intrusive mechanism it is thought that humans may as well.

### C) Invasion into the uterine stromal tissue

Although it may not be clear how the blastocyst traverses the uterine epithelium it is clear that its target is the uterine stroma. The ability of the blastocyst to invade the stroma is tightly related to its intrinsic invasiveness. In other words the blastocyst has the ability to migrate and penetrate into tissues. We know this from experiments where mouse blastocysts were transplanted into foreign tissue sites such as the kidney capsule or the cornea. In these situations however the invasion is not as controlled as is observed in the uterus and the embryo can disrupt the structural integrity of the entire organ. A form of in vitro invasion can be observed by culturing zona hatched blastocysts in plastic dishes using serum supplemented media as demonstrated below.



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These embryos cannot obviously penetrate or invade into the plastic bottom but they do spread out over the bottom of the dish and display this migratory nature of the invading trophoblast. This in vitro blastocyst outgrowth model has been used to investigate which molecules may mediate trophoblast invasion into the endometrium. The most likely candidates are a family of proteinases called the matrix metalloproteinases (MMPs). This is a large gene family as demonstrated in the following table.

**Table 1.** Classification of matrix metalloproteinases

Sub-family	MMP	Other names	MW	Substrates
Gelatinases	MMP-2	Gelatinase A 72 kDa gelatinase	73 882	Col IV, V, VII, X, gelatine Fibronectin, elastine
	MMP-9	Gelatinase B 92 kDa gelatinase	79 427	Col IV, V, gelatine
Collagenases	MMP-1	Interstitial collagenase Fibroblast collagenase	54 007	Col I, II, III, VII, X, MMP-5, entactin
	MMP-8	Neutrophil collagenase, PMNL collagenase	53 412	Col I, III
Stromelysins	MMP-13	Collagenase-3	53 819	Col I
	MMP-3	Stromelysin-1 Transin-1	53 977	Col III, IV, IX, X, gelatine, laminin Fibronectin, elastine, casein
	MMP-7	PUMP-1, matrilysin	29 677	Casein, fibronectin, gelatine
	MMP-10	Stromelysin-2 Transin-2	54 151	Col II, IV, V, fibronectin, gelatine
	MMP-11	Stromelysin-3	54 595	Col IV
	MMP-12	Metalloelastase	54 000	Elastine, fibronectin
	Membrane bound	MMP-14	MT1-MMP, MMP-X1	65 883
MMP-15		MT2-MMP	75 807	MMP-2
MMP-16		MT3-MMP	69 158	MMP-2
MMP-17		MT4-MMP		

Col: collagen; PMNL: polymorphonuclear leukocytes; PUMP-1: putative metalloprotease-1.

These molecules digest extracellular matrix or the “cellular glue” that holds the stroma cells together. As you might expect the trophoblast expresses several members of this gene family and it is the release of these molecules that paves the way for the invasion into the uterine stroma. The uterus also expresses these molecules thus facilitating the invasion as well.

What controls the invasion? How is the invasion regulated unlike what was observed during blastocyst transfer to kidney? The answer lies in the production of specific inhibitors of these MMPs. The so called tissue inhibitors of metalloproteinases or TIMPs. There are four such inhibitors and the uterine stroma is an active producer of these inhibitors. So invasion is tightly controlled in the uterus. The embryo advances by the degradation of extracellular matrix but this invasion is controlled and eventually halted by the production of uterine inhibitors. Thus invasion is a tightly regulated process.

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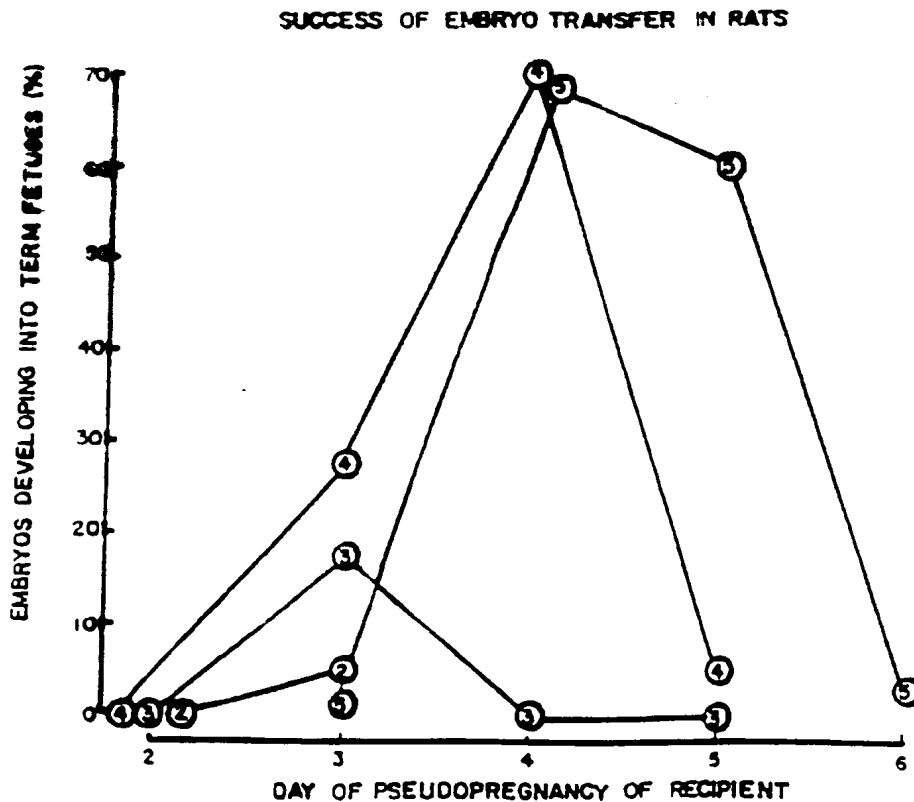
### II. Overview of Hormonal Control

- A. The first two steroid hormones to be considered are ones already encountered in the study of ovarian physiology. They are progesterone and estrogen. Progesterone is essential for successful implantation and the initial development of the fetus. Progesterone stimulates the glands of the endometrium to secrete a variety of nutrients which sustain the early zygote and is required to maintain the decidual cells of the uterus.
- B. As with progesterone, estrogens are initially produced by the corpus luteum. Eventually the placenta will assume this role, and once again an interaction among the components of the fetoplacental unit are required.
- C. Human chorionic gonadotrophin (hCG) is a protein hormone secreted by the trophoblast cells that appears in maternal plasma and urine less than 9 days after fertilization. Its action at that time is to maintain the secretory activity of the corpus luteum, namely its capacity to form progesterone. Thus hCG is the signal provided by the conceptus to the mother which makes her ovaries aware that a developing blastocyst is present. Circulating levels of hCG decline later on in pregnancy when the placenta itself becomes capable of forming the steroid hormones necessary to maintain pregnancy.

#### A. Understand the concept of Uterine receptivity and the “implantation window”.

The concept of uterine receptivity and the “implantation window” varies dramatically between species. It is an important concept though because it demonstrates that the uterus must be properly primed to receive the embryo and it demonstrates that the uterus is not always receptive and in fact can be quite hostile to the embryo. For rodent species this relationship is quite strong as demonstrated in the following figure. As you can see to achieve a successful pregnancy in the rat it is vital that embryo development be synchronized to uterine development. This is critical for embryo transfer as a day 4 embryo must be transferred optimally to a day 4 uterus. In other species such as the cow or sheep it is possible to transfer an embryo into a uterus that is delayed by up to one day and still achieve an acceptable pregnancy rate however the embryo cannot be transferred into an advanced uterus (ie day 5 embryo into a day 6 uterus). For humans this concept does not hold fully as in vitro fertilization procedures often result in day 2 or 3 embryos being transferred into the uterus. Something that would not be effective in animal species.

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From Howe and Dickman (1960) J.R.F. 1:186

### B. Understanding the role played by progesterone in initiating pregnancy

- 1) For all species a progesterone dominance is required to support initiation of pregnancy.
- 2) Progesterone prepares the endometrium for pregnancy; maintains the endometrium during pregnancy (prevents sloughing); maintains the myometrium during pregnancy (prevents uterine contractions); increases mucous viscosity in the cervix (creates a physical barrier between the uterus and the external environment); promotes breast development; down regulates estrogen receptors and thus reduces estrogenic effects; and restricts secretion of LH/ blocks GnRH surges and reduces the amount of LH released in response to GnRH.

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The Corpus Luteum is the Primary source of progesterone. The preovulatory surge of LH results in luteinization of the granulosa and theca cells and alters their steroidogenic pathways to dominate in progesterone production.

Two types of luteal cells: 1) large luteal cells (from the granulosa) and 2) small luteal cells (theca)

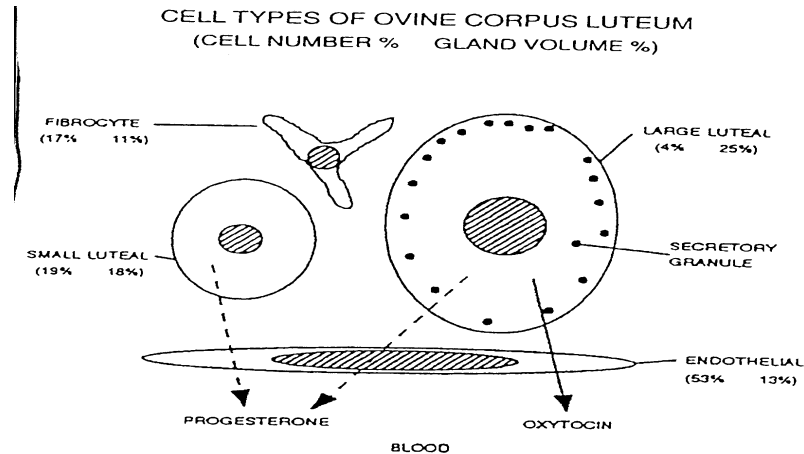
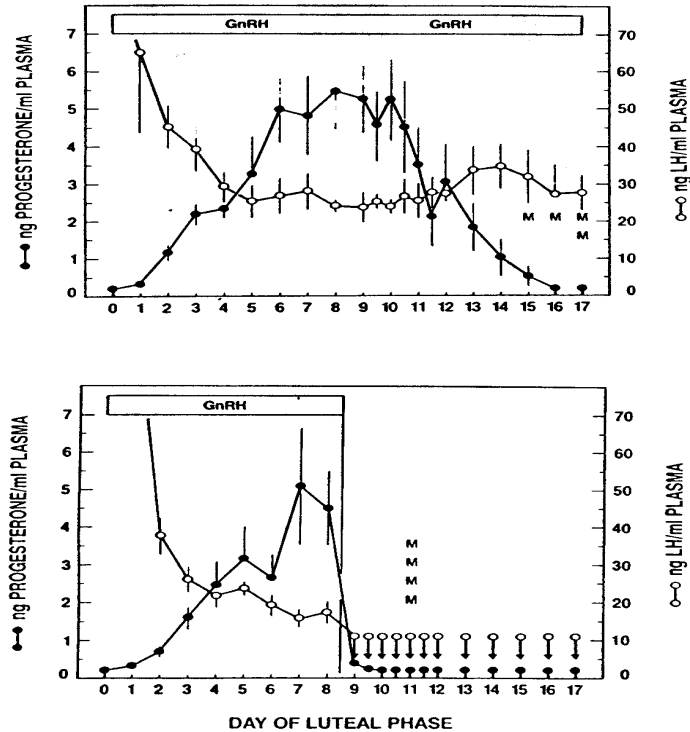


FIG. 3. Major cell types in ovine corpus luteum. Relative number of cells (%) and relative cell volume (%) of each cell type in corpus luteum are shown diagrammatically. Remaining number of cells (7%) are composed of plasma cells, lymphocytes, and granular leukocytes. Residual tissue volume (33%) is occupied by vascular lumina and intercellular spaces. Granulosa-derived large luteal cell constitutes 4% by number but 25% by volume of gland. [Based on data from Rodgers et al. (586) and Farin et al. (195).]

In the human progesterone production by the CL is required only for the first 40 days of pregnancy as shown in the following table. After that time the placenta becomes the principal source of progesterone production for the maintenance of pregnancy.

Species	Duration of non-pregnant luteal phase (Days)	Day of pregnancy when ovariectomy without effect	Duration of pregnancy (days)
Man	12-14	40	260-270
Sheep	16-18	55	147-150
Cow	18-20	Term	280-290
Pig	16-18	Term	115
Rat	**no luteal phase pseudopregnancy 12 days	12	22

## REPRO/IMPLANTATION and EARLY PREGNANCY-11



**FIG. 7.** Serum LH and progesterone concentrations in MBH-lesioned rhesus monkeys whose menstrual cycles were restored by a pulsatile infusion of synthetic GnRH. The **top panel** illustrates serum LH and progesterone concentrations of animals that received GnRH at a frequency of one pulse per hour throughout the menstrual cycle. Note that these animals have luteal phases of normal duration. The **bottom panel** illustrates data from animals in which the infusions of GnRH were terminated on day 8 of the luteal phase. Serum LH and progesterone concentrations fell rapidly on the cessation of GnRH treatment, and premature menses were observed in all animals. (Reproduced from ref. 173.)

Luteinizing Hormone (LH) is the principal luteotrophic hormone in primates and humans as can be observed in this figure. As long as GnRH pulses continue, LH is released from the pituitary and progesterone production continues. As soon as the GnRH is removed from the picture both LH and progesterone levels fall. Once the CL reaches its maximum size it persists for a few days if pregnancy does not occur and the CL regresses and menses occurs.

## REPRO/IMPLANTATION and EARLY PREGNANCY-12

### C. Understand the changes that occur to the corpus luteum if pregnancy does not occur

The process that results in the demise of the corpus luteum is called luteolysis. The CL first loses its capacity to produce progesterone and then the luteal cells begin to undergo apoptosis and remodeling resulting eventually in the formation of a corpus albicans.

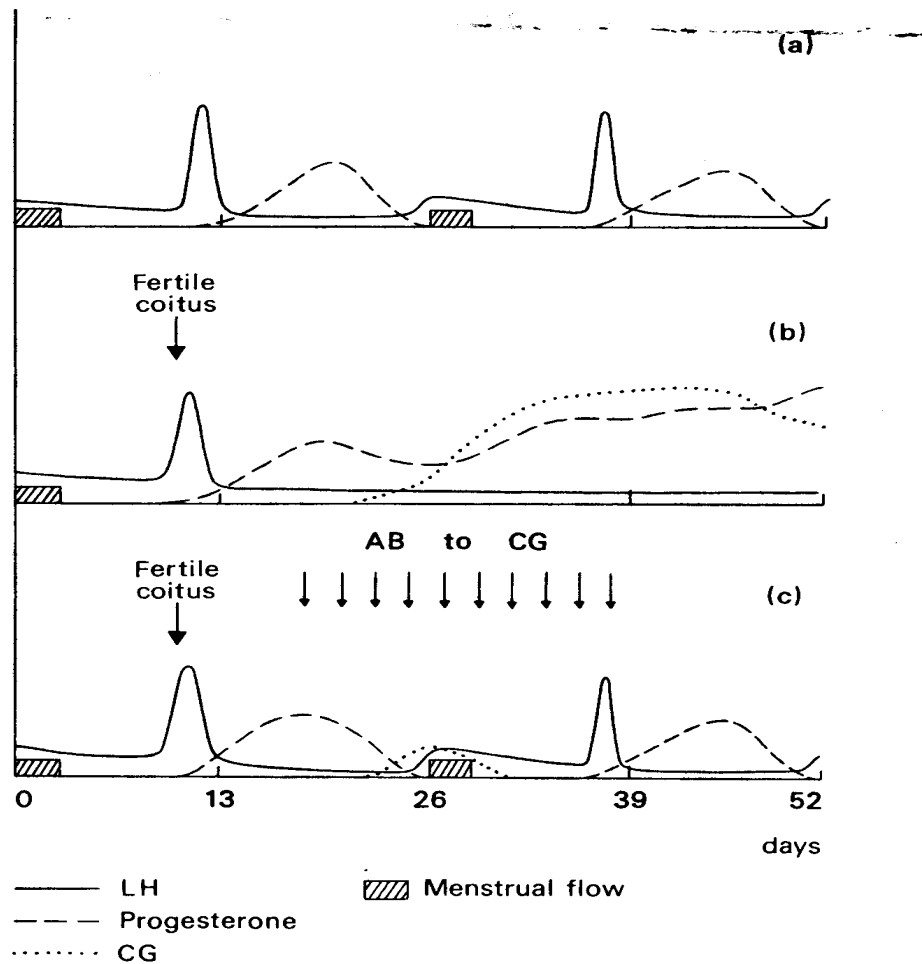
In most species luteolysis is dependent upon the presence of a uterus. In other words there is a uterine factor that promotes the destruction of the CL. If one performs a hysterectomy on a cow or sheep at the appropriate time you can delay luteolysis significantly. The likely mediator of these events is **prostaglandin F<sub>2</sub> $\alpha$**  which is subject to control by oxytocin in these species.

The picture is very different in the primate or human as removal of the uterus has no effect on the lifetime of the corpus luteum. So control of luteolysis in the human does not involve uterine prostaglandin. **What happens in the human?** The true answer is that the details are still being worked out but two principal hypothesis are being investigated. The first suggests that over time LH secretion simply declines and reaches a level so low that progesterone cannot be produced any longer. The second hypothesis suggests that there is a source of intraovarian prostaglandin F<sub>2</sub> $\alpha$  and that this molecule will actively promote the regression of the CL. Stay tuned for future updates.

## REPRO/IMPLANTATION and EARLY PREGNANCY-13

D. Be familiar with the structure of human chorionic gonadotrophin and the role it plays in “rescuing” the corpus luteum.

How is the CL maintained in the human?



**Fig. 10.1.** Levels of hormones in the blood during two cycles of a higher primate. (a) Non-pregnant cycles; (b) cycles in which fertile mating occurs; (c) similar to (b) but passive administration of a highly specific anti-CG antibody is given (arrows), depression of CG and loss of pregnancy occurs but there is no effect on LH levels and cyclicity.

## **REPRO/IMPLANTATION and EARLY PREGNANCY-14**

In humans there is the production of a luteotrophic factor by the embryo which replaces LH as the primary luteotrophin. To signal pregnancy and maintain the CL progesterone production the embryo (trophoblast) releases human chorionic gonadotrophin or hCG. This hormone is similar to LH but is composed of a beta chain which is 30 amino acids longer than the one found in LH. They both interact with the same receptor but the half-life of hCG is 10X longer than that for LH. Human chorionic gonadotrophin directly stimulates CL progesterone production and therefore overcomes any luteolytic tendencies stemming from low LH levels. It may also be luteoprotective as it may antagonize the effects of ovarian prostaglandin F<sub>2</sub> $\alpha$  in promoting luteolysis.

### **Hormonal Events Summary**

- 1) The CL and progesterone production are essential for pregnancy maintenance in all species.
- 2) progesterone production and CL formation are stimulated by mid-cycle LH surge in all species.
- 3) The ovarian (CL) source of progesterone shifts to placental production after 40 days gestation.
- 4) LH is an important luteotrophic factor.
- 5) Luteolysis may be induced by intraovarian prostaglandin F<sub>2</sub> $\alpha$  in the human or simply a normal decline in LH levels
- 6) Pregnancy is signaled by repressing CL regression or luteolysis
- 7) In primates and human hCG produced by the embryo replaces LH and rescues the CL from its demise