

## BS 501 — Lecture Notes

### LECTURE 1

Go over Handout, concepts, outline. If you do not attend class, you almost certainly will get a lower grade than if you attend class conscientiously.

Chapters 52-55 of Boron and Boulpaep (2005) cover human reproduction reasonably well.

However, there are more than 4,000 species of mammals.

There is more species-to-species variation in reproduction than with other physiological systems such as cardiovascular, renal and nervous systems; gastrointestinal is intermediate.

Huge differences in placenta, litter size, gestation length, testis to body weight ratios, sperm shape, female reproductive cycles, maturity at birth, etc.

Impossible to cover all species. Information very limited except human, domestic animals and lab animals -- ~ dozen species. Most concepts apply across species. I will cover a few important exceptions.

Good general health and bodily function are essential for successful reproduction.

Suffers if illness, poor nutrition, environmental stress.

Nature follows the principle that reproduction can wait until better times.

For example, reproduction is delayed until puberty, inhibited during lactation and delayed until spring in seasonal breeding species. Parturition can even be delayed in stormy weather.

Sex differences in mammalian reproductive participation.

Essentially all healthy, breeding age females become pregnant during the breeding season.

Most males do not sire offspring in most species, but most males compete for mates.

In humans, older females do not reproduce directly, but have an indirect role in care of children and grandchildren. Reproductive life corresponds more closely to lifespan in most other mammals.

## Non-reproductive organs' role in reproduction.

Good feet and legs in bulls. Good nutrition essential.

Information processing in the brain, e.g. to identify sex of an individual.

The spinal cord is essential for ejaculation reflex, release of oxytocin at suckling, inducing ovulation in some species, etc.

The pineal gland at the base of the brain has an important role in reproduction in many species.

Cerebrum is involved in sexual behavior.

Hypothalamus is so involved in reproduction that it is a sex organ -- e.g. releasing hormones and source of oxytocin for the posterior pituitary.

## Anatomy of the reproductive system

Many parts can be removed surgically with only minor effects on other bodily functions.

Gonads -- ovaries and testes -- primary sex organs, source of the gametes (sperm and eggs) and steroid hormones.

## Secondary sex structures

### Internal

Tubular structures such as uterus and vas deferens

Accessory sex glands such as seminal vesicles

### External

Penis, clitoris, labia, scrotum

List of slides:

- 1) Human female reproductive tract, parts
- 2) Human oviduct, more accurate
- 3) Slide of ovary, follicles
- 4) Slide of 1-week-old calf reproductive tract with India ink
- 5) Human male reproductive tract, parts
- 6) Reproductive tract of the bull, plumbing
- 7) Histological slide of testis -- interstitial, seminiferous tubules, Sertoli and germ cells

## Secondary sex characteristics

Neutral (same for both sexes) in the late fetus and newborn.

Changes occur due to gonadal hormones, estrogens, progestins, and androgens binding to receptors in the tissues.

List of human male secondary sex characteristics:

- voice
- muscle and bone mass
- hair pattern, including baldness
- sexual and other behaviors

List of human female secondary sex characteristics:

- pattern of fat deposition, breasts and hips
- carrying patterns
- olfactory sensitivity changes with stage of menstrual cycle

## Embryology of the reproductive system

Slide:

8) list of parts of ~100-cell embryo

Gastrula stage (precursors to body are forming)

- ectoderm -- coverings and linings -- skin, central nervous system
- endoderm -- digestive system, some endocrine glands, liver
- mesoderm -- most of the body, for example muscle & reproductive system, except for the germ cells which originate in embryonic ectoderm

Slide:

9) Continuity of germ line — gametes → zygote/early embryo → inner cell mass → primordial germ cells → precursors of gametes → gametes

Slides:

10) day 40 human embryo

11) migration of primordial germ cells

All cells can be divided into somatic cells and germ cells or their precursors -- primordial germ cells are undifferentiated cells that form the ovum and the precursors to the sperm which, however, becomes very differentiated, during the final month before their release.

Primordial germ cells -- repositories of information on how to produce a copy of the organism. Divide as migrate.

DNA in many other cells also has sufficient information to reproduce the organism if reprogrammed -- for example the cloned sheep, Dolly. Irreversible changes occur in some cells, e.g. lymphocytes.

A chicken can be defined as an egg's way of making another egg.

The germ cells in the chicken connect the life cycle between the egg stages.

Embryos are bipotential with regard to sex.

They have the structures for both male and female development and both sexes are morphologically identical.

A few decision events determine if the indifferent structures become male or female -- it is easy to have errors in this process, which leads to intersexes -- intersexes are fairly common in some species, such as pigs

Some bipotential remains in adults -- for example nipples on males.

Slide:

12) hermaphrodite mare

Similar abnormalities in non-reproductive tissues usually lead to death before birth.

## **LECTURE 2**

### Sex chromosomes

Fundamental differences between male and female are genetic as reflected in sex chromosomes, XX female; XY male.

Functionally, the genetic information produced from two X-chromosomes is not very different from an X- plus a Y-chromosome.

Two principles make this so. First, there are only about 80 genes on the Y-chromosome (30 of which also are on the X chromosome), and second, one of the X-chromosomes is inactivated in most female mammalian tissues -- no mRNA transcribed from approximately 95% of the genes on the inactivated X-chromosome in differentiated cells. Note that X-chromosomes contain about 5% of all genes (about 30,000 total) in mammals.

Slides:

- 13) female cat demonstrating chimerism
- 14) Lyon hypothesis
- 15) consequences of Lyon hypothesis

Molecular Mechanism.

Females receive one X-chromosome from their mother and one from their father.

As cells differentiate, either the X- from the mother or the X- from the father is randomly inactivated. Inactivation is active expression of XIST gene (X-inactive specific transcripts); is non-translated RNA that binds DNA on inactive X. XIST expression is inhibited by T six expression by the active X. T six is also a regulatory RNA (not translated into a protein) produced by the anti-sense strand of the XIST gene.

Once a decision on inactivation is made, all progeny from that cell have the same X-inactivated for the rest of the life of the individual -- females are functional chimeras -- identical twin females are different from each other in this inactivation pattern. Define chimera.

Molecular mechanism – methylation of cytosines adjacent to a guanine; maintenance methylase whenever cells divide. Transcription inhibited from highly methylated DNA.

Slide:

- 16) methylated DNA

XXX females have 2 inactivated X chromosomes

Histological evidence for random X-inactivation:

- 1) Barr body seen in nuclei of spinal cord neurons
- 2) drumstick seen on polymorphonuclear leukocyte nuclei

Exception to these principles of X-inactivation:

- 1) both X-chromosomes are active in the ovum and early female embryo
- 2) the paternal X- is always inactivated in the placenta, so the pattern is not random
- 3) the X-chromosome from the father is inactivated in all cells of marsupials except for the ovum
- 4) about 5% of X is not inactivated, including gene steroid sulfatase – why women have smooth, silky skin (2 doses) and men do not (1 dose). If this gene mutated, women's skin like men's and men's is scaly – termed ichthyosis.

Slide:

- 17) karyogram

Y-chromosome.

Smallest chromosome in most species and has very few genes, probably fewer than 50 that are Y-specific.

Approximately 30,000 genes on all of the chromosomes in mammals, but depends on definition of a gene, e.g. alternate splicing.

Most Y-chromosome DNA is inert -- contains no genes -- therefore X,Y- is similar to X,X-inactivated.

Genes responsible for maleness are on Y-chromosome. XY, XYY, XXY, XXYY are all male; XX, XO, XXX, are all female; those underlined are sterile. OY are dead. Super males, super females, Turner's (XO) and Klinefelter's syndromes (XXY). X inactivation in male, Barr bodies, aneuploids. Nearly all autosomal aneuploids (extra = trisomy; missing = monosomy) are lethal during embryonic development; effects of sex chromosome aneuploidy are minor. Tricolor male cats are XXY.

Principle -- one gene on the Y-chromosome is a switch to turn on dozens of other genes on other chromosomes to make a testis and sequellae. Lack of the switch leads to development in the female direction.

Note that thousands of structural genes for male reproductive function; most are on the autosomes and X-chromosome, but around 20-30 are on the Y chromosome.

Detection of the first gene on the Y-chromosome -- discovered by skin grafting in inbred mice.

Male-to-male grafts accepted. Female-to-female grafts accepted. Female-to-male grafts accepted. Male-to-female grafts rejected.

Gene termed histocompatibility-Y or H-Y antigen.

Gene expressed on the surface of all male cells but not in females.

Mistakenly thought to be the sex-determining gene for many years.

Role of H-Y in fine-tuning development of parts of the testis.

Additional false starts in isolating the testis-determining gene.

This gene was eventually definitively identified and termed SRY (Sry in mice). It is a DNA-binding protein expressed in developing testes that bends DNA sharply where it binds.

Proven to be the testis-determining gene by transgenic (Transgenic = make genetic change in 1-cell embryo that is copied into all resulting cells) methods whereby female embryo develops into a male which, however, was sterile for reasons that will become clear later. Gene discovered in individuals that are sterile and had sex chromosome mutations -- XY females and XX males.

SRY AMH expression

Undifferentiated tissue → indifferent gonad → testis → male structures.

Summary: Y-chromosome makes the indifferent gonad to a testis. Lack of a Y-chromosome causes the indifferent gonad to develop into an ovary. This occurs at about 1½ months of gestation in humans.

Differentiation of the internal reproductive system during fetal life in the male or female direction.

Slide:

18) testis histology

Testis secretes two hormones in fetal life

- 1) testosterone (a steroid) from the interstitial cells
- 2) anti-Mullerian hormone, also known as Mullerian inhibiting factor (from the Sertoli cells)  
-- a protein hormone of the transforming growth factor- $\beta$  (TGF- $\beta$ ) family

Ovary secretes only very low levels of hormones at this stage.

Presence of testis leads to masculinization of the undifferentiated reproductive system.

Presence of an ovary or lack of a gonad results in development in the female direction.  
See figure 52.4 in Boron and Boulpaep.

Terminology -- Primitive kidney = mesonephric system = parts of male reproductive system (undifferentiated female also has all of these parts). Why reproductive tract so associated with urine.

- mesonephric (Wolffian duct)
- mesonephric tubules
- Mullerian duct

Draw indifferent reproductive system

Slides:

- 19) indifferent human reproductive tract at 7 weeks of gestation
- 20) male fetus at 2 months of differentiation
- 21) female fetus at 2 months of differentiation
- 22) summary – both sexes

The Mullerian duct develops into female reproductive tract structures and the mesonephric duct regresses in females.

The mesonephric (Wolffian) duct leads to male reproductive tract structures and the Mullerian duct regresses in males.

Mechanisms of deciding which duct develops:

Rule 1: Wolffian duct maintained in the presence of testosterone; if no testosterone, degenerates. Therefore if a testis is present, the Wolffian duct is maintained and if it is absent, it degenerates.

Rule 2: The Mullerian duct remains unless Mullerian-inhibiting hormone actively prevents its development. Mullerian-inhibiting hormone is produced by the Sertoli cells of the testis. It is a heterodimer of the transforming growth factor beta family.

Summary: In females, no androgen is present so the Wolffian duct degenerates. No Mullerian-inhibiting hormone is present so the Mullerian duct remains. In the male, androgen is present so the Wolffian duct is maintained. Mullerian-inhibiting hormone is also present, which regresses the Mullerian duct.

Experimental manipulations

- 1) Remove the gonad from either sex and development is in the female direction.
- 2) Give androgen to females and both ducts develop.
- 3) If a testis is grafted to a female, the system develops in the male direction.
- 4) If anti-androgen is given to a male, no ducts form at all.

Summary: SRY — indifferent gonad → testis → T + AMH → hormone action  
No SRY — indifferent gonad → ovary and little hormone production

Slides:

- 23) fusion of the female reproductive tracts
- 24) bovine freemartin
- 25) bovine freemartin
- 26-29) descent of the testis
- 30) temperature in different parts of the ram testis

### LECTURE 3

Mechanism of keeping testis and epididymis cool

Blood entering the testis is cooled by blood leaving the testis and blood leaving the testis is warmed by blood entering the testis. Occurs in pampiniform plexus.

Cooling of the testis is important in scrotal mammals. Sterility at body temperature. Some mammals do not have scrotal testes, termed inguinal.

Mechanism of testicular descent (Fig. 52.8 in Boron and Boulpaep)

- 1) Differential growth, gubernaculum
- 2) Maintaining an open inguinal ring (remains open, some species)

Cryptorchid - hidden gonad - sterile

Most common congenital defect in humans (congenital means present at birth).  
Insufficient testosterone at critical stage

Can be corrected hormonally with hCG or surgically, even age 7-8.

External genitalia also are bipotential.

Penis and clitoris are homologs, labia and scrotum are homologs.

Mechanism of differentiation of external genitalia.

Testosterone causes development in the male direction = penis, scrotum. Lack of testosterone causes development in the female direction = vagina, clitoris, labia.

E<sub>2</sub> is not involved in initial differentiation of female external genitalia.

Steroid pathway is cholesterol → progesterone → androgens → estradiol 17β  
(In adrenal, progesterone → androgen → cortisol) (see Fig. 53.5 in text)

Mutations adrenal enzymes result in adrenal androgen's masculinizing females and causing male babies to enter precocious puberty. Treatment usually cortisol.

Abnormality of differentiation in human babies in Central America, mutation in the enzyme 5α-reductase which causes testosterone to be made to dihydrotestosterone.

Dihydrotestosterone is the active androgen in most cells (is testosterone Wolffian duct).  
Cells that cannot convert testosterone to dihydrotestosterone will have very little androgenic response for most tissues. Describe how A ring of testosterone can be aromatized (with loss of hydrogens).

## Consequences of 5 $\alpha$ -reductase deficiency.

External genitalia in baby boys develop in the female direction because of not being able to mount an androgen response.

However, at the age of puberty, external genitalia become masculinized because of a great increase in testosterone production. This is a weak androgen compared to dihydrotestosterone, but the high concentration causes the little girls to turn into little boys and develop a penis at the age of 12.

## Masculinization of the brain, particularly the hypothalamus.

Numerous examples, will cover 2:

- 1) Sexually dimorphic nucleus: Nucleus is a collection of cell bodies of neurons. Sexually dimorphic nucleus is three times larger in males of rodent species than females. Testosterone prevents degeneration of neurons. Less anatomical dimorphism in human; homosexual implications.
- 2) Sexual differentiation of pre-optic area of hypothalamus. Preview of female reproductive endocrinology most species:  $E_2 \rightarrow$  GnRH release  $\rightarrow$  LH release  $\rightarrow$  ovulation of follicle(s) that secreted  $E_2$ . Give  $E_2$  to male and no GnRH release.

This system is very different in male than female rodents, much less human.

This differentiation occurs in late fetal or neonatal life. If testosterone is present during a critical window (~7 months of gestation in humans), male pattern results. Lack of testosterone is female pattern. Ovary results in the female pattern. Testis and testosterone result in male pattern (defeminization).

Important point: Female pattern is not due to estradiol-17 $\beta$  as the ovary does not make this hormone at this stage. In fact, estradiol-17 $\beta$  is about 1,000 X more potent than testosterone in causing this sexual differentiation in the male brain.

Explanation: Testosterone is converted to estradiol-17 $\beta$  in the male brain -- that is, aromatization (chemical change of A ring to benzene ring) of testosterone occurs to produce estradiol-17 $\beta$ .

Potential problem is masculinization of the brain of female fetuses from their mothers' estradiol-17 $\beta$  estrogen. To cancel this effect, sex steroid-binding globulin concentrations increase in maternal circulation during gestation. Also, in some species, the molecule alpha fetoprotein is made by placenta. These bind estradiol-17 $\beta$  so that one doesn't get large amounts of estrogen into the fetal circulation to masculinize the brain inappropriately. The area of the brain that is masculinized is the preoptic area.

Experiments in this area:

Give testosterone to males or females -- male pattern results.

Give estradiol to males or females -- male pattern results, even more so than with testosterone.

Castration -- results in female pattern unless there is replacement therapy.

Testosterone also affects the cerebrum in some species from a sexual differentiation standpoint setting up the potential for male sexual behavior in adult life. Lack of testosterone results in potential for female sexual behavior in the adult.

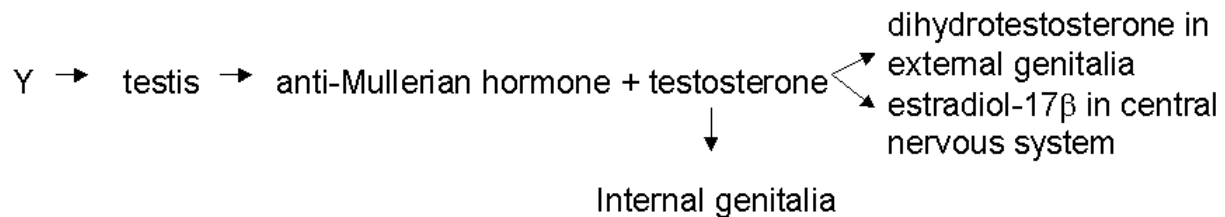
Summary: There are two roles for testosterone -- 1) an organizational role during fetal and neonatal development, and 2) an operational role that only occurs after puberty. Receptors are involved with both of these roles.

Also, estradiol-17 $\beta$  has a functional role in adult female, but not an organizational role in the fetal female.

Gender -- feminine/masculine.

Gender is how one perceives oneself sexually. It has hormonal and environmental components, as well as chance encounter components.

Summary:



This pathway affects the nature of the internal plumbing of the reproductive system, hypothalamic differentiation, and gender from the central nervous system. The process can be derailed by environmental contaminants with estrogenic or anti-androgenic properties.

Superimposed on the above are:

descent of the testis

secondary sex characteristics

post puberal function

## Illustrative genetic defect -- testicular feminization

For androgens to function, they require a receptor in the nucleus of the cell. The receptor binds testosterone or dihydrotestosterone. The gene for this protein (receptor) is on the X-chromosome.

Note: Males get their X-chromosome from their mother.

In some individuals, the gene for this receptor is defective, so the receptor is not made or is made incorrectly.

An X-Y fetus with the testicular feminization defect differentiates a testis. The testis makes testosterone, but there is no effect of the testosterone since receptors are not there to use it.

No internal plumbing in these individuals. Mullerian duct degenerates. Wolffian duct is not maintained. Testis does not descend; scrotum does not form.

External genitalia are female. Hypothalamus is female.

In these individuals, puberty does not occur because they have no ovaries. They look like females, but have no estrogen production (includes famous thin women, e.g. movie star Kim Novak). They produce huge amounts of testosterone, but it has no effect. Testis frequently becomes cancerous - removed surgically.

Condition found in many species -- example of XY female externally w/testes.

**CLASS 4** Recitation 1: Assisted Reproduction including artificial insemination, in vitro fertilization, embryo transfer, and cryopreservation will be discussed; Quiz 4

## LECTURE 4 (Class 5)

Male reproduction post-pubertal.

Functions of the male reproductive system:

- 1) produce spermatozoa
- 2) deliver the spermatozoa to the female reproductive tract.

Slide:

- 31) plumbing of male reproductive tract

Drawing of the male reproductive tract:

- testis
- seminiferous tubules
- rete testis
- efferent ducts
- epididymis
- vas deferens
- accessory sex glands
- Wolffian duct derivatives

Functions of the epididymis: maturation, transport, and storage of sperm. Sperm stored a week to months.

3 slides efferent ducts  
32-34)

Testicular histology.

Four types of cells:

- 1) germ cells
- 2) Sertoli cells } These in seminiferous tubules
- 3) Leydig or interstitial cells
- 4) infrastructure (e.g. blood vessels, nerves, connecting tissue, muscle)

Draw seminiferous tubule without germ cells

Slides of testicular histology:

- 35) standard histology
- 36) scanning EM slide
- 37,38) diagrams of ultrastructure including the blood-testis barrier, myoid cells, Sertoli-Sertoli cell junctions; basal and adluminal compartments
- 39) electron micrograph of interstitial tissue
- 40) diagram of interstitial tissue–seminiferous tubule interaction

Functions of the blood testis barrier: 1) keep toxins from the testis; 2) protect testis from immune system. Self-nonsel determined fetal life; sperm not made until after puberty. Different mechanisms from the blood-brain barrier which is tight junctions of capillary endothelial cells.

During spermatogenesis – X-chromosome is inactivated (functional aneuploid). Only one X-chromosome can be inactivated, which is why XXY is sterile. (Note rule in female is all but one X is inactivated.)

Characteristics of sperm.

Smallest nucleated cell in the body, and very specialized.

Haploid -- no DNA, RNA, or protein synthesis.

No ribosomes -- DNA is packaged tightly, and not available transcription, so no mRNA & no protein synthesis.

2 slides of sperm:

41)

42) diagram of a sperm:

- head including acrosome
- nucleus
- equatorial segment
- neck
- mid-piece
- mitochondria
- tail
- cell membrane

Production of sperm — takes about 2 months.

Many kinds of cells in the seminiferous tubule.

Germ cells are embedded in Sertoli cells which are somatic nurse cells.

Slides:

43) spermiogenesis and spermiation

List of germ cells in the testis:

spermatogonia – divide by mitosis

primary spermatocytes – undergo meiosis I division

secondary spermatocytes – undergo meiosis II division

No DNA synthesis

spermatids – differentiate into spermatozoa

} 2 cell divisions, but  
only 1 round of DNA  
synthesis

All of the above cells are spherical, except the spermatid which starts as spherical and develops into the sperm by the process spermiogenesis.

Spermiogenesis (results in smallest nucleated cell in the body).

Nucleus condenses

Histones change to protamines (rich in lysine and arginine) with lots of positive charge to neutralize negative charge on DNA.

3 slides:

- 44) acrosome formation, specialized lysosome developing from juxtannuclear Golgi
- 45) Manchette formation & Sertoli cell phagocytosis
- 46) sperm loses excess cytoplasm, phagocytosed by Sertoli cells

Slide:

- 47) spermiation -- release of sperm from Sertoli cells; protoplasmic droplet left

Sertoli cell -- nurse cell -- spermatid loses ability to care for self, e.g. inactive X chromosome. Also major regulating and remodeling functions.

Spermatogonia.

Seven or eight kinds in some species. We'll consider three for discussion purposes:

- 1) stem spermatogonia -- stem cell renewal system renews itself and divides into A spermatogonia (other examples of stem cell systems)
- 2) A spermatogonia -- divide into B spermatogonia
- 3) B spermatogonia -- divide into primary spermatocytes

Slides:

- 48) spermatogenesis -- intracellular bridges and degeneration
- 49) ultrastructure of intracellular bridges -- origin and functions of intracellular bridges (note: half of spermatids X and half Y)

## NOTES

**SPERMATOGENESIS** = the sum of the transformations which result in formation of spermatozoa. The entire spermatogenic process is initiated in early embryonic development and continues after birth and puberty as a consequence of continual renewal of stem cells

**SPERMATOCYTOGENESIS** = mitotic divisions of spermatogonia after birth to give rise to other aspermatogonia and preleptotene primary spermatocytes.

**MEIOSIS** = meiotic division of primary spermatocyte to form secondary spermatocytes and ultimately spermatids. Cells are called primary spermatocytes or secondary spermatocytes.

**SPERMIOGENESIS** = the differentiation of spermatids from round cells with considerable cytoplasm to cells with a highly condensed nucleus and scant cytoplasm with a flagellum. Cells are called spermatids. Based on changes in the spermatid acrosome, spermiogenesis can be considered as a continuum consisting of four phases: Golgi, cap, acrosome, and maturation. In addition to acrosomal evolution, condensation of the nuclear material and formation of the flagellum occur.

**SPERMATION** = release of spermatozoa from the germinal epithelium into the lumen of the seminiferous tubule. Prior to release, the germ cells are called spermatids and after spermiation they are called spermatozoa.

**GERMINAL EPITHELIUM** = the normal cellular components within the seminiferous tubule consisting of Sertoli cells and germ cells (spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids). Sertoli cells are somatic cells which are non-dividing in adult animals and probably are important for metabolic exchange between the germ cells in the luminal compartment and the exterior of the blood-testis barrier, coordination of spermatogenesis and have an endocrine function.

**BLOOD-TESTIS BARRIER** = a physiological barrier isolating the germinal epithelium from the interstitial tissue and dividing the germinal epithelium into a basal compartment and an adluminal compartment. Consists of the basement membrane (basal lamina plus fibers and a myoid cell layer) and occluding tight junctions between adjacent Sertoli cells. The Sertoli-Sertoli cell tight junctions form the principal component of the blood testis barrier.

**BASAL COMPARTMENT** – contains spermatogonia

**ADLUMINAL COMPARTMENT** – contains primary spermatocytes, secondary spermatocytes and spermatids

## Sperm production

One can measure sperm production rates by understanding how sperm are made. Healthy bulls,  $10^7$  sperm/gm testis/day. Sperm production rates are highly dependent on testis size, which in turn is dependent on the number of Sertoli cells and is related to follicle-stimulating hormone secretion at puberty in these animals and their relatives, including females. Whole process highly heritable.

Female relatives of males with large testes reach puberty earlier.

## Senescence.

In older mammals, on the average, fewer sperm are produced and numbers of morphological abnormalities increase.

Some 70 to 80 year old men can have reasonable fertility, but some younger men have very poor semen quality and are sub-fertile or sterile. Some problems due to endocrine “disruptors,” contaminant in environment due to plasticizers, synthetic hormones, etc.

Some problems due to mutations.

Freeze semen at a young age?

## X and Y sperm are very similar

Important consequence of the way sperm are made and connected with intracellular bridges -- X- and Y-bearing sperm are very similar. Recall that the X-chromosome is inactivated during spermatogenesis. -- draw diagram

During spermiogenesis, gene expression drops to very low levels making X- and Y-bearing sperm very similar; sperm depend on Sertoli cell for care.

The only difference seems to be that there is more DNA in X-bearing sperm than in Y-bearing sperm. This is the basis of methods for separating X- and Y-bearing sperm.

## LECTURE 5 (Class 6)

### Endocrinology of mammalian male reproduction

Regulating testosterone concentrations is key.

Effects occur only in tissues with androgen receptors.

### Functions of testosterone (T)

Required for normal Sertoli cell function and spermatogenesis, maintenance of accessory sex glands and secondary sex structures such as penis and epididymis. Accessory sex glands shrink to 1/3 size if no T.

Secondary sex characteristics, beards, voice, muscle mass.

Negative feedback to regulate gonadotropin secretion.

### Endocrinology.

In most tissues, the active androgen is a dihydrotestosterone, but it is testosterone for the Wolffian duct.

As covered earlier, the active androgen in the brain and Sertoli cell sometimes is estradiol-17 $\beta$  (e.g. stallion testis – rich source of estrogen!). Dihydrotestosterone, testosterone, and estradiol-17 $\beta$  work by classic steroid mechanisms of action covered earlier in the course, including binding to receptors in the nucleus resulting in regulation of RNA synthesis. (Note that there are some exceptions to this mechanism, in some cases steroids bind to cell surface receptors causing effects not directly related to regulating RNA synthesis.)

Cause and effect hormone pathways: GnRH  $\rightarrow$  LH  $\rightarrow$  testosterone (Leydig cells)  $\rightarrow$  (1) local effects on Sertoli cells, (2) systemic effects, and (3) feedback regulation. Receptors needed. Note different half-lives of hormones. (Draw curves on board.)

Testosterone is converted to estradiol-17 $\beta$  in Sertoli cells of most species.

Testosterone and estradiol-17 $\beta$  feedback on LH and GnRH-secreting cells.

Parallel -- GnRH  $\rightarrow$  FSH  $\rightarrow$  Sertoli cells (secrete extracellular androgen binding protein and inhibin). Feedback of inhibin on FSH secretion. Inhibin is member of TGF- $\beta$  family of proteins.

Slide:

50) male reproductive endocrinology

Explain diagram of endocrine pathways in male mammal.

Effects of exogenous GnRH, LH, and androgen to test causes of infertility (e.g. T normally suppresses LH).

Prolactin seems to have role in maintaining Leydig cells.

Testicular function.

Three methods of removing substances including hormones from the testes -- veins, lymphatics, rete testis (RT) fluid -- relative volumes are ~1,000:100:1.

Slide:

51) interstitial lymphatics

Concentrations of testosterone in various fluids and compartments, ABP & RT fluid.

How testosterone can function as a contraceptive.

Diagram of LH, GnRH, and testosterone secretions with time.

Effects of constant infusion of testosterone or release from an implant.

Testis starved for T, normally 10-100X peripheral concentration, now is 1X.

Anabolic steroids are androgens, suppress LH, cause small testes (see in some athletes).

Epididymis – Divided into head, body, tail (caput, corpus, cauda).

Functions -- storage, transport and maturation of sperm.

Takes 4-6 days for sperm to go from efferent duct to tail of the testis where they are stored. Rate of passage independent of mating frequency.

Epididymis requires cooler temperature than the body in the same way that the testis does. Perhaps storing sperm at a cooler temperature helps maintain their viability.

Sperm can be stored in the epididymal tail for some weeks or even months in a few species if they are not removed by ejaculation; in some species, sperm for dozens of ejaculates stored. Once ejaculated, sperm deteriorate (unless frozen).

Testes produce sperm at a constant rate. About 10 million sperm/gm/day in the bull. If no ejaculation is occurring, sperm overflow out of the epididymis and are expelled in the urine.

If 4 to 6 ejaculates are collected per week from typical mammals, only a few sperm are lost in the urine. Note that more frequent mating = fewer sperm/ejaculate.

Important concept: The number of sperm produced by the testis is not necessarily equal to the number of sperm that are collected with an artificial vagina because of losses in the urine and losses in the semen collection equipment. Description of artificial vagina.

Slide:

52) epididymis -- lumen epithelium infrastructure, stereocilia

Function of the head of the epididymis: absorbs 99% of rete testis fluid, secreted molecules cause maturation of sperm as they traverse the epididymis and facilitate concentrated storage in the tail.

Epididymal function depends on androgens, specifically dihydrotestosterone.

Androgen-binding protein is absorbed in the head of the epididymis from rete testis fluid and delivers testosterone directly to the epididymis.

Changes in sperm.

Infertile to fertile, non-motile to motile, whimpy motility to progressive motility.

- 1) sperm tail is stiffened by disulfide bond formation in the outer dense fibers; a non-stiff flagellum is like using a rope for a paddle; parenthetically, disulfide bonds also increase between protamines in the nucleus of the sperm
- 2) forward motility protein is secreted into epididymal fluid which, when combined with ATP, equals motility

Known functions of secreted proteins.

- 1) forward motility protein
- 2) acrosomal stabilizing factor -- functions to prevent premature acrosome reaction

Gross morphological changes.

Main one is migration of protoplasmic droplet from neck to partway down tail.

Male reproductive function.

Erection of penis -- engorgement of erectile tissue in penis with blood, more inflow than outflow.

Slide:

53) human penis

Prostaglandin E<sub>2</sub> - relaxes arterioles so more blood flow. Viagra.

Ejaculation -- reflex centered in spinal cord, traumatic neck injury induces ejaculation at that time. Input from cerebrum and tactile stimuli penis, both erection and ejaculation.

Ejaculate

seminal plasma -- nutrients and regulatory molecules for sperm, e.g. fructose, prostaglandins  
antibacterial properties  
gels and vaginal plugs -- human semen coagulates, liquefies

Site of semen deposition -- species specific, vaginal or uterine.

**LECTURE 6** (Class 7) (Starts female reproduction, 3rd part of course) (Dr. Winger will teach)

Microanatomy of the ovary.

Slides:

54) ovary -- point out germinal epithelium, parenchyma, how the mare differs from other species

55) SEM of follicle

56) ovary -- follicles, basement membrane, capillaries, theca interna, theca externa, corpus luteum, corpus hemorrhagicum, blood vessels -- follicle cell = granulosa cell

57) major hormones female reproductive system -- also relaxin, oxytocin, others

Theca interna is homologous with Leydig cells, has LH receptors, produces testosterone or other androgens. These transported across to granulosa cells.

Circulating levels of androgens in the female blood system are about one-tenth those of males and likely have a function in sexual behavior.

Granulosa cells are homologs of Sertoli cells. They have FSH receptors and an enzyme, aromatase, that converts testosterone to estradiol-17 $\beta$ . This is the same aromatase that is found in cells in the hypothalamus and in Sertoli cells.

Hypothalamo-pituitary-ovarian axis.

Quite homologous to the male, can exchange ovary for testis in male diagram, same embryological origins.

Estradiol-17 $\beta$  feeds back on the hypothalamus and the anterior pituitary under some circumstances, regulating GnRH and LH secretion.

This is a negative feedback system except for the preovulatory surge when it is a positive feedback system in females. Recall sexual differentiation of the hypothalamus.

Positive feedback systems unstable

Growing follicle produces estradiol-17 $\beta$  and has FSH receptors. As it grows, more FSH receptors are made and in final stages of follicular growth, LH receptors appear on granulosa cells (in addition to thecal cells). This sets up the system for the LH surge and ovulation -- major event, mittelschmerz (pain felt at ovulation in some women).

Large follicles produce the molecule inhibin from the granulosa cells. Another homology with the male reproductive system. This regulates litter size.

Many follicles grow simultaneously in the ovary.

Once a follicle becomes dominant and secretes inhibin, this turns off FSH secretion so other follicles will not grow, starved. Dominant follicle no longer needs large amounts of FSH

In the case of litter-bearing species, the inhibin from, for example, 10 follicles is required to shut off FSH secretion and, thereby, regulate litter size.

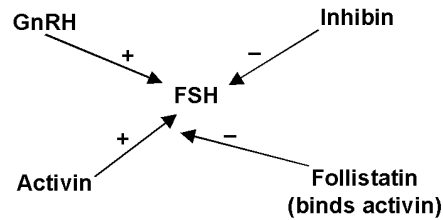
Slide:

58) activins and inhibins – TGF $\beta$  family, both from granulosa cells

Activins stimulate follicular growth by increasing FSH secretion by the anterior pituitary.

FSH secretion is regulated by GnRH positively. Activin positively, inhibin negatively.

Further complication -- follistatin is secreted by the follicle and is an activin-binding protein, thereby negating the effects of activin. Molecules secreted by granulosa cell, autoregulatory and autocrine function too.



2 slides:  
59-60) Superovulation

Superovulation is accomplished by administration of exogenous FSH. Even though inhibin concentrations become very high due to many follicles growing, the exogenous FSH continues to have an effect. Human IVF programs, cattle ET programs.

Luteinization.

The process of changing a follicle to a corpus luteum.

Steroid synthetic pathways: cholesterol → pregnenolone → progesterone → testosterone → estradiol-17β. Different sets of enzymes are involved. Are parallel pathways.

In the luteinized cell, the enzymatic pathways stop at progesterone.

Corpus luteum.

Three types of cells -- large luteal cells, small luteal cells and infrastructural cells.

Slide:  
61) CL

At ovulation and luteinization, blood vessels invade the follicle. This is known as a corpus hemorrhagicum.

Angiogenesis occurs.

Characteristics of large luteal cells:

- 1) 20-40 μ in diameter --  $\frac{4}{3} \pi R^3 \sim 14,000 \mu^3$
- 2) few LH receptors -- no LH regulation
- 3) baseline progesterone production
- 4) prostaglandin F<sub>2α</sub> receptors
- 5) oxytocin and relaxin granules

Small luteal cells:

- 1) 10-20  $\mu$  in diameter --  $\sim 1,700 \mu^3$
- 2) many LH receptors
- 3) LH regulation (essential to maintain CL)
- 4) regulated progesterone production
- 5) no prostaglandin  $F_{2\alpha}$  receptors
- 6) no protein peptide granules
- 7) large numbers of lipid droplets containing cholesterol esters (storage for rapid LH response)

Regression of the corpus luteum with prostaglandin  $F_{2\alpha}$  secretion from endometrium and ovary (from ovary only in primates).

- 1) Vasoconstriction
- 2) Influx of calcium into large luteal cells and sequellae resulting in apoptosis and demise of the entire corpus luteum.

Functions of progesterone (in cells with  $P_4$  receptors):

- 1) Synergistic with  $E_2$  for sexual behavior
- 2) Sets up cyclicity in the hypothalamus and ovary? after anestrus, whether prepuberal, lactational or seasonal; oral progestins fed to cattle for management
- 3) Stimulates the epithelium of the oviduct, uterus and cervix causing growth and secretions; particularly effective if  $E_2$  first
- 4) Maintenance of pregnancy by relaxing the myometrium, that is preventing contractions; most important function
- 5) Negative feedback inhibiting final follicular growth and therefore ovulation -- contraceptive aspects, estrus synchronization
- 6) Increases body temperature – pyrogenic (Basal body temperature shift in women)
- 7) Behavioral -- contributes to pre-menstrual syndrome?
- 8) Lactation -- mammary epithelium
- 9) Embryo transport from the oviduct to the uterus

Inhibition of progesterone receptor.

RU486 binds to progesterone receptor so that progesterone cannot have any effect.

Used as an orally active molecule to result in sloughing of the endometrium and early abortion if pregnant.

Orally active progestins -- a number of them used clinically and agriculturally, for example, melengesterol acetate (MGA) and oral contraceptives.

Functions of estradiol-17 $\beta$  -- cells with E<sub>2</sub> receptors. (Note hundreds of compounds have various degrees of estrogenic activity because they bind estrogen receptors, including some environmental toxins.)

Sexual behavior -- striking in most species -- 10 mg estradiol-17 $\beta$  in 500 kg cow.

Increase in physical activity. (Use pedometer in cattle.)

Stimulation of the epithelium of the oviduct, uterus, vulva, cervical mucus; further stimulation P<sub>4</sub>. Regression to 1/3 height with no hormones.

Causes cervical mucus to be less viscous.  
Mammary gland receptors.

Positive and negative feedback regulation of GnRH.

Fat cells in breasts, hips (at puberty in women).

Metabolic/anabolic function in long-bone growth, epiphyseal plate closure at puberty.

Androgens -- more anabolic, less plate closure effects in human, but there are androgen effects. For example, human castrates have the eunuch phenotype.

Note that there are several estrogen receptor types ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) and these vary from tissue to tissue.

Orally active estrogens. Are hundreds; potency varies; many occur in plants eaten regularly (e.g. soybeans). Some synthetic pesticides and/or their metabolites are estrogenic.

The most famous – diethylstilbesterol (DES) – used for anabolic properties agriculturally in the past and therapeutically in women.

An example of an anti-estrogen – clomiphene citrate, which may stimulate GnRH secretion in some species, e.g. human (and cause superovulation).

Functions and characteristics of relaxin (source larger luteal cells and placenta):

- 1) gene is derived from 2 insulin genes in tandem.
- 2) Other examples of insulin – gene family, insulin-like growth factors.
- 3) Relaxes the pubic ligaments at parturition.
- 4) Relaxes cervical infrastructure.
- 5) Possibly breakdown of collagen for widening cervix for parturition.
- 6) Possible effects on mammary system.
- 7) Source – large luteal cells and placenta – species differences
- 8) Receptor very different from that of insulin, IGF-1

Functions of oxytocin:

- 1) Stimulate myoepithelial cells in the mammary gland.
- 2) Stimulate the myometrial cells in the uterus for sperm transport and expulsion of fetus.
- 3) Stimulate endometrial cells to produce prostaglandin  $F_{2\alpha}$  in some species.

**CLASS 8** Recitation 2: Cloning mammals will be discussed; Quiz 5

**LECTURE 7** (Class 9)

Reproductive steroids and cancer.

Cancers of the reproductive system are fairly common – 10-15% of women; higher men but death due to other causes masks.

Can affect any reproductive tissues.

The most common human cancers are breast cancer in women and prostate cancer in males; most males benign prostate tumor: hypertrophy with age.

10-15% inherited – e.g. mutation in tumor suppressor genes; rest spontaneous mutations.

Estrogens and androgens drive cells to undergo mitosis. Billions

Most cancers of reproductive tract are steroid responsive but some are not. Many genes have steroid response elements in their regulatory regions.

If these regulatory elements mutated, drive cell division inappropriately, get cancer.

Solution: Get rid of  $E_2$  or testosterone. ↓ 95% (not 100%). Down regulate GnRH.

The anti-estrogen tamoxifen ↓ familial cancers.

Protective effects pregnancy, oral contraceptives.  $E_2$  less influence; takes years for cancers to develop. Being pregnant before age 20 decreases chances of breast cancer by half relative to never pregnant. Mechanism is regression of mammary tissue when lactation ceases; gets rid of pre-cancerous cells.

BRCA mutation – causes the inactive X chromosome to secrete a growth factor that can stimulate breast cancer cells.

## Female reproductive cycles.

Most species seasonal -- young born at the optimal time of the year, usually in the spring. Short season breeders become pregnant with decreasing daylight, for example sheep have a 5 month gestation so young are born in the spring.

Long season breeders have reproductive cycles with lengthening daylight and either have very long gestations (11 months in the horse) or quite short gestations (<1 month in rodents).

Non-seasonal breeders -- humans and domestic animals such as cattle and pigs -- these species have been selected for non-seasonality.

Note: Males also are seasonal, corresponding to female reproductive function. Regulated hypothalamus. In some species, testis regress during the non-breeding season and sperm are produced only during the breeding season.

Regulation of seasonality due to pineal gland regulating GnRH secretion, which regulates pituitary and ovarian function, testicular function in males.

## Pineal gland.

Anatomy -- small bulbous gland at the base of the brain, has nervous system input. Main cell type is pinealcyte.

Function of the pineal gland is a light to chemical transducer. Dark = melatonin secretion, light = no melatonin secretion -- input from optic tract (=eye). There is even a special pigment in the retina, melanopsin, that is in this signaling pathway.

Concentration changes in the blood are dramatic -- 3- to 4-fold increase in concentration in melatonin in dark over light -- happens to each of us within a few minutes in the dark.

Nomenclature -- melatonin regulates melanin granules in amphibia and reptiles causing the skin to be light or dark depending on the light/dark status.

Melatonin function -- in short day breeders results in GnRH pulses. Lack of melatonin results in limited GnRH pulses.

In long-day breeders, melatonin prevents GnRH pulses; GnRH pulses occur in absence of melatonin.

16 h light/8 h dark

ovine reproduction inhibited  
equine and rodent reproduction stimulated

8 h light/16 h dark

ovine reproduction stimulated  
equine and rodent reproduction inhibited

Chemical darkness -- melatonin can be taken orally, either by humans or fed to laboratory and farm animals and has same physiological effects as being in the dark -- feed to sheep at 4 pm equals short day.

Melatonin effects on non-seasonal breeders -- diurnal rhythms. For example, women have spontaneous LH surges for the most part between 5 and 7 am, men have more testosterone secretion in the late afternoon. Jet lag effects can be minimized by taking melatonin.

Chemistry of melatonin -- derivative of the amino acid tryptophan. Four enzymatic steps to melatonin that are regulated by light.

Reproductive cycles of females within the breeding season

Slide:

62) Regulation of reproductive hormones (Fig. 54-4 in text)

Due to changes in hormones, date by outward signs such as estrus, menstruation.

Cause & Effect

1. Follicle grows, secretes  $E_2$  in absence of high  $P_4$
2. GnRH/LH (FSH) surge
3. Ovulation/CL formation/fertilization?
4.  $P_4$  secretion
5. Demise of CL if non-pregnant; maintained if pregnant
6. Some species differences
7. Hormones secreted in pulses – not smooth curves

Can date cycles by epithelial cell changes, by biopsying vaginal tissue for example, or in the case of rodents, by lavage of the vaginal cavity and cytology.

Reproductive cycles can be divided up in several different ways. One is follicular or proliferative phase under estradiol- $17\beta$  domination vs the luteal or secretory phase under progesterone domination.

Another way of dividing up the cycle is into estrus, metestrus, diestrus, proestrus and back to estrus.

Estrus is defined as when the female permits the male to mate her. Diestrus is defined as the period during which progesterone is high. Proestrus and metestrus are between these two. Note estrous is adjectival form of estrus; same for proestrous, etc.

Anestrus is defined as periods when there is no reproductive cycle due to season, lactation, prepuberty, nutrition, stress, oral contraceptives (various species), and so on.

Concentrations of reproductive hormones are often drawn as smooth curves, when in reality nearly all reproductive hormones pulse to some extent. Illustrate with protein and steroid hormones.

	<b><u>Reproductive cycle (in season)</u></b>	<b><u>Species</u></b>	<b><u>Some features</u></b>
1.	Induced ovulators	rabbits, cats, skunks	Preovulatory LH surge from euroendocrine reflex
2.	Induced CL formers	rodents	2X daily prolactin surges set up by neuroendocrine reflex
3.	Spontaneous ovulators & CL formers, 3-6 day follicular phase	cattle, sheep, pigs, horses	Conceptus regulatory molecules cancel $PGF_2\alpha$ effects
4.	Spontaneous ovulators & CL formers, 10-14 day follicular phase	human, some other primates	Chorionic gonadotropin to maintain CL
5.	Sporadic cycles	dogs	2 month luteal phases
6.	Others	mole rats, marsupials	Very varied

Category 1: Induced ovulators (no cycles without mating).

Examples, rabbits, cats.

Mating causes ovulation of follicles and starts reproductive cycle via neuroendocrine reflex. Spines on penis to stimulate cervix.

Follicle to corpus hemorrhagicum to corpus luteum.

Diagram of  $E_2$ , GnRH, LH and progesterone.

GnRH secretion neuroendocrine reflex. High E<sub>2</sub> necessary but not sufficient; also need mating.

Main function of progesterone.

Maintain pregnancy by inhibiting uterine smooth muscles, myometrium.

If pregnant, progesterone stays high until just before birth. If pseudopregnant (mating is sterile) progesterone drops due to CL lysis two-thirds of the way through the normal pregnancy.

After pregnancy or pseudopregnancy, cycle starts over with follicles producing high concentrations of estradiol-17 $\beta$

Mating (and follicular growth, E<sub>2</sub>) is required for starting the cycle of events over.

Category 2: Induced corpus luteum formers (cycles are short without mating).

Examples are rats, mice and hamsters. Who cares about rodents? Models.

If mating occurs, corpus luteum forms due to prolactin secretion. In absence of mating, no corpus luteum forms and a short cycle ensues.

Diagram of E<sub>2</sub>, GnRH, LH and prolactin; neuroendocrine reflex.

Note: Designate when estrus occurs, prolactin luteotropic.

4- to 5-day cycles if no mating. Prolactin secreted anterior pituitary 2X/day; later from the placenta-choriosomatotrophin.

Regulation of prolactin secretion -- shut off PIF: dopamine.

If mating is sterile, pseudopregnancy occurs; ends two-thirds of the way through a normal gestation length.

Category 3: Spontaneous ovulators, spontaneous CL formers (no effect of sterile mating).

Short follicular phase. Examples horse, ewe, cow, pig.

Reproductive cycles are about 20 days in length, but species variation.

Draw diagram of progesterone, estradiol, GnRH, LH.

Follicular phase 3 to 6 days.

In non-pregnant animal, prostaglandin  $F_{2\alpha}$  is secreted by the uterus, causes progesterone to drop due to apoptosis of luteal cells in corpus luteum, positive feedback loop of oxytocin and prostaglandin  $F_{2\alpha}$ . Oxytocin initiated from CL or posterior pituitary.

The same happens at parturition.

Whether parturition or non-pregnant cycle, system starts cycling again with follicular growth.

Prevention of prostaglandin effects in pregnant animal.

Conceptus secretes molecules that prevent prostaglandin actions. In ruminants, interferon tau, secreted by conceptus, inhibits the actions of prostaglandin  $F_{2\alpha}$ . In pigs estradiol- $17\beta$ .

Slides:

- 63) utero-ovarian vasculature and anastomoses in ewe -- local transport  $PGF_{2\alpha}$ .
- 64) slide bovine tract with one uterine horn

## LECTURE 8 (Class 10)

Category 4: Spontaneous ovulators, spontaneous CL formers, long follicular phases.

Species are primates, including human.

Slide:

- 65) Blood levels of reproductive hormones (Fig. 54-3 in text)

Endocrinological changes same as in previous category with these exceptions:

- 1) prostaglandin  $F_{2\alpha}$  is not secreted by the endometrium to destroy the corpus luteum, rather the corpus luteum ceases function spontaneously (via ovarian  $PGF_{2\alpha}$ ) unless it is stimulated by human chorionic gonadotropin
- 2) follicular phase is about 10 to 14 days long, instead of 3 to 6 days, resulting in 25-30 day reproductive cycles

hCG secreted by the conceptus -- very LH-like,  $\alpha$  &  $\beta$  subunits.

Extremely long half-life due to heavy glycosylation -- 24 hours; secretes its own weight of hCG each day.

Conceptus maintains the CL to prolong its life by preventing expulsion from the uterus.

One other characteristic of this category is menstruation. Loss of lining of the uterus when progesterone concentration drops.

Slides:

66 & 67) human female reproductive tract and endometrium (same changes other species but less dramatic)

Menstrual cycles and estrous cycles are similar, but dated differently. Dated on overt signs -- blood in case of menstrual cycle; estrus behavior, that is standing for being mated, in estrous cycles.

In menstrual cycles, ovulation occurs in the middle, relative to dating. In estrous cycle, ovulation occurs toward the beginning, relative to dating.

Note: Not all blood discharge from reproductive tract is due to menstruation. For example in proestrus dogs, estrogen secretion causes discharge of blood and mucus. In cattle, withdrawal of estradiol-17 $\beta$  at metestrus causes bleeding.

#### **Discharge of Blood from Female Reproductive Tract**

<u>Species</u>	<u>Description</u>	<u>Cause</u>
Human	menstruation	decrease progesterone
Canine	proestrous discharge	increase estradiol
Bovine	metestrous bleeding	decreased estradiol

Equine conceptus also secretes chorionic gonadotropin -- LH gene but different glycosylation -- secreted days 40-100 of pregnancy and binds to FSH receptors-- accessory CLs.

Category 5: Dogs.

Cycle every 6 to 9 months. Have long periods of anestrus, but grow follicles and ovulate at irregular intervals.

Pregnancy lasts about 60 days in dogs and progesterone stays high for 60 days no matter if dogs are pregnant, mated and non-pregnant, or not mated.

Category 6: Others -- mole rats, marsupials, mink,

Continuum of spontaneous-induced ovulators.

Males speed up ovulation to some extent in most species including the human.

Under constant lighting conditions, rats become induced ovulators.

Women can be induced ovulators under certain stress conditions and intense sexual activity, for example when partner has been away for prolonged periods of time.

Factors affecting puberty.

Puberty occurs when follicles grow sufficiently to initiate an ovulation, and therefore ovum production.

In women, puberty is noted outwardly by menarche, the first menstrual bleeding. The age of menarche has been decreasing over the years -- in 1860 it was 17 in this country, in 1960 it averaged 13. This was probably due to much better health and nutrition in 1960 than in 1860.

Nutrition and age interactions occur in other species as well.

One question is how the hypothalamus knows it is time for puberty to occur. There seems to be a relationship with body fat content and reproductive cycling.

Evidence for that includes correlating body fat content with age at menarche, that thin women athletes reach puberty at a later age, or even become amenorrheic after puberty when they get below 20% body fat content.

Although women seem to require 25% body fat for normal menarche, it is likely that it is a correlated response to some other factors in the system, rather than just body fat.

A recent theory concerns the molecule leptin, derived from a gene expressed in adipose tissue. Leptin decreases appetite and, in laboratory animals, exogenous leptin decreases appetite. It is not clear that this occurs in the human, however.

Leptin may facilitate triggering of puberty, possibly via GnRH secretion.

Molecular mechanism puberty – ↓ receptors for E<sub>2</sub> in hypothalamus so E<sub>2</sub> less inhibitory to GnRH. Result is more E<sub>2</sub> in body.

## Oogenesis

Terminology of germ cells in mammalian ovary:

primordial germ cells -- diagram

oogonia

primary oocytes (in prophase of meiosis I from fetal life to LH surge, if LH receptors in follicle)

secondary oocytes (induced by the LH surge and release of a polar body)

ootid (induced by fertilization)

The ovary fills with primordial germ cells in fetal life, and they increase in number by mitosis and are termed oogonia. They have intercellular bridges like spermatogonia, and divide to form primary oocytes and start meiosis but arrest in prophase of meiosis I.

This occurs about the sixth fetal month in humans and is similar in other species -- that is, about two-thirds through the end of gestation, oocytes reach meiotic arrest.

In the case of women, no more primary oocytes are made after the seventh fetal month, so all women have all the oocytes they will ever have before they are born.

There are greater than a half million oocytes per ovary at that time and they are enclosed in follicles.

This results in a rather profound situation -- Women pregnant with a baby girl (draw it) actually also have within the baby girl the oocytes that will become the grandchildren of the pregnant woman. Therefore, the way one treats the fetus during pregnancy with nutrition, drugs, smoking, alcohol and so on affects not only the little girl that's born, but also the grandchildren, because the oocytes that will form the grandchildren are being formed.

Some years ago, one of my post-doctoral students proved that damaging drugs given to pregnant mice in fact affected the grand-mice.

Mammalian follicular nomenclature.

Slide:

68) ovarian follicles (Fig. 54-11 in text)

Primordial follicles

Primary follicles -- cells become cuboidal, increase in number

Secondary follicles -- zona pellucida, gelatin-like capsule around the oocytes formed and oocytes grow 3-4X diameter, follicle has multiple layers of granulosa cells but no antrum

Slides:

69 & 70) zona pellucida

Tertiary = Graafian follicles -- presence of antrum = fluid filled cavity

Ovulation

Not 1 cycle -- takes ~4 months

All follicle types contain primary oocytes.

Description of mature follicle -- antrum, stratum granulosum, cumulus oophorus, corona radiata, specialized follicle cells.

Three phases of follicular growth -- pre-antral, antral, large antral.

Pre-antral -- see above list -- cell numbers increase.

Waves of antral follicle growth.

LH (and other factors) needed to stimulate follicular growth after antrum forms.

FSH – inhibin system superimposed.

Occurs between the slow growth preantral phase and the pre-ovulatory follicles.

Slide:

71) follicular growth waves in cattle -- other species too

Intermediate waves of follicular growth occur on a recurring basis, averaging two or three waves per cycle. Not understood before ultrasonography.

Waves continue into pregnancy and also occur prepuberally.

Characterized by cohort of follicles starting to grow, regulated by FSH.

Repeats every 6 to 8 days – FSH driven.

One follicle becomes dominant and represses growth of other follicles.

In presence of high concentrations of progesterone, the dominant follicle regresses, loses its repressive effect and a new wave of follicles grows. Progesterone must decline for the last spurt of follicular growth, high E<sub>2</sub>, and frequent GnRH/LH pulses.

Dominant follicle makes inhibin, regulates litter size.

FSH concentrations increase when dominant follicle loses its dominance, starting new wave of follicular growth.

Estrus synchronization -- decrease P<sub>4</sub>

<u>Follicle</u>	<u>Timing</u>	<u>Granulosa Cell #</u>	<u>Gonadotropin</u>
pre-antral	3 months	few to 1,000's	None
antral	6-8 days	1,000's to 100,000's	LH & FSH
pre-ovulatory	3-6 or 10-14 days	millions	LH

## LECTURE 9 (Class 11)

Atresia = degeneration of follicle including oocyte.

99.9% of follicles degenerate. Only those that are at the right stage when P<sub>4</sub> falls go on to ovulate.

In the case of women, usually only one ovulation per reproductive cycle.

Considering pregnancy, contraception, etc., only several dozen to a few hundred follicles mature to ovulate in a normal lifetime. Millions degenerate. Atresia at any stage of follicular growth. Apoptosis.

50% of follicles degenerate by birth, 90% degenerate by puberty. Degeneration continues until, from a functional standpoint, too few follicles, resulting in menopause.

At menopause, ovary still has a few follicles, but not sufficient numbers for reproductive cycles, and the system is non-functional.

Menopause generally occurs in late 40s and early 50s although can be much earlier in some pathologies.

Reproductive function in women declines markedly before menopause. Fairly normal until age 35. At age 35, incidence of chromosomal abnormalities in oocyte and, therefore, children increases markedly and becomes especially high after age 40. Down's syndrome. Draw graph. Not surprising -- oocyte actually 40 years old.

Fertility declines clearly by age 35 and markedly by age 40. Assisted reproductive techniques. Mutations accumulate in sperm of older men.

Regulation of initiation of follicular growth. Mechanism unknown; GDF-9 gene has a role in primary follicle.

In older women, ovary runs out of primordial follicles, so fewer start developing each day.

All oocytes are primary oocytes until LH surge -- reemphasize point.

Menopause rare in non-human -- 2 year old mice, 15 year old cows, 25 year old mares.

In mice, oocytes/follicles may continue to be made into young adulthood, research controversial.

Slides:

- 72) Meiosis relative to ovulation, etc.
- 73) nuclear state of oocytes  
Nucleus in primary oocyte is termed germinal vesicle.

Regulation of meiotic maturation -- when LH surge occurs, in addition to ovulation, stimulates germinal vesicle (nucleus) breakdown (which must occur for any cell division), chromosome condensation and extrusion of the first polar body. This is an unequal cytokinesis -- there are no centrioles in oocytes. Therefore, a secondary oocyte is ovulated. Remains as a secondary oocyte until fertilization, when it becomes an ootid.

If not fertilized, oocyte dies as secondary oocyte.

Oocyte is prevented from maturing by inhibitors in follicular fluid. LH surge overcomes those inhibitors, via epidermal growth factor. – like molecules

FSH surge -- occurs the same time as the LH surge, causes hyaluronic acid secretion and cumulus cell expansion -- draw.

Slides:

- 74 & 75) Cumulus compaction and expansion

Ovulation.

Physical description -- The preovulatory follicle on the ovary extrudes from the surface like a blister -- diagram.

Small protrusion occurs on the follicle, termed the stigma. That region becomes avascular -- no blood flow. Like an inflammatory response.

Ovulation does not occur due to increasing pressure follicle, so it's not an explosive event, rather it is breakdown of the collagen in the follicle wall. -- graph

In some species, there is smooth muscular contraction that actually squeezes out the follicular contents, including follicular fluid, the ovum and the cumulus, or nurse cells around the ovum.

Some bleeding occurs with ovulation and the corpus hemorrhagicum becomes vascularized for corpus luteum formation.

In addition to ovum and nurse cells, follicular fluid is also released at ovulation and that may signal the oviduct to stimulate sperm transport.

Endocrinology of ovulation -- LH/FSH surge, cumulus expansion, cyclic AMP ↑ follicle cells, protein kinase A system, protein phosphorylation etc. Estrogen secreting system to

progesterone secreting system. Paracrine events, prostaglandin  $F_{2\alpha}$  increases hundreds of times in concentration of follicular fluid, prostaglandin  $E_2$  increases, there is an inflammatory response. Indomethacin or aspirin, which prevents prostaglandin synthesis, will delay ovulation.

Theca interna cells form small luteal cells of CL with receptors for LH.  
Granulosa cells form large luteal cells of CL – no LH receptor.

Pregnancy -- 4th and final major part of course.

Sperm and egg transport in the oviduct -- early stages under estrogen domination (list), sperm goes up the oviduct, oocytes go down the oviduct, meet and fertilize in the ampullary region of the oviduct; after 2-3 days the embryo goes to uterus when progesterone concentration increases.

Slides:

- 76) fimbria, ampulla, isthmus, uterotubal junction
- 77) human fallopian tube
- 78) inter circular and outer longitudinal musculature
- 79) cross-section of different regions of the oviduct
- 80 & 81) uterotubal junction
- 82) 2 cell types, cells secretions

Sperm movement up the oviduct -- two mechanisms: 1) the sperm's motility (minor relative to the distance needing to be covered), 2) contractions of the reproductive tract.

Barriers to sperm transport -- cervix and uterotubal junction. Cervix minor if uterine insemination.

Slides:

- 83) sperm in cervix -- important storage human
- 84) sperm numbers in tract

For egg transport -- two mechanisms: 1) ciliary movement, cilia beat very rapidly from the ovarian end of the oviduct toward the middle of the oviduct, 2) peristaltic contractions that move in that direction.

These mechanisms have some redundancy.

Kartegner's syndrome -- lack of functional cilia. Such women remain fertile.

Important point: Sperm are rapidly transported to the fimbria in mammals after they reach the uterus by peristaltic contractions. However, most of those sperm transported

within the first five minutes are dead and are not the sperm that fertilize the egg.  
Misleading.

## LECTURE 10 (Class 12)

Diagram of process of fertilization -- largest =  $150\ \mu\text{m}$  = 1/200 inch and smallest (nucleated) cells in body.

Slides:

- 85) sperm in zona (accessory sperm)
- 86) SEM sperm -- oocyte -- artificial -- zona removed artificially
- 87) compact cumulus
- 88) expanded cumulus
- 89) PV space

Events that occur in the sperm -- Capacitation, hyperactivated motility, penetration of cumulus, binding to zona pellucida, acrosome reaction, zona pellucida penetration, fusion of the sperm and oocyte.

Definition of capacitation - ability to fertilize an oocyte.

Evidence for capacitation -- Ejaculated sperm are infertile in in vitro fertilization systems without special incubation conditions. Sperm recovered from the female reproductive tract are fertile immediately.

Capacitation times vary according to species. One to two hours in the human and rodent, 10 to 12 hours in rabbits and cows, most species within this range.

Chemical mechanisms of capacitation:

- 1) Removal of acrosome stabilizing factor. This protein is added in the epididymis and accessory sex glands. Idea is to keep the acrosome intact until just at the time of fertilization when it needs to go from a very stable system to an unstable system to fuse with oocyte.
- 2) Deplete sperm cell membrane of cholesterol. Cholesterol is a rigid stiff molecule intercalated in cell membrane. Serum albumin binds cholesterol and remove it from the membrane in a sponge-like way. Result: more fluid membrane.
- 3) Increased cAMP in sperm, stimulates protein kinase A, phosphorylated proteins.

In vitro capacitation of sperm for in vitro fertilization -- remove seminal plasma, which is loaded with acrosome stabilizing factor (for example centrifuge through dense layer of albumin), add heparin or fusogenic phospholipids, and albumin, and/or time; 25 mM  $\text{HCO}_3^-$  important.

Hyperactivated motility -- only capacitated sperm.

Different from progressive motility (net forward motion) in that it is violent with very strong power strokes of the swimming sperm. Undesirable if premature.

Penetration of the cumulus oophorus. Need to break down hyaluronic acid. Hyaluronidase from the acrosome thought to be important in this respect. Leaks out before acrosome reaction? Recent finding that a sperm membrane-bound protein, PH-20, has a hyaluronidase functional domain; PH-20 found over the posterior part of the sperm head and inner acrosomal membrane. May function in penetration of the cumulus.

Acrosome reaction – only occurs capacitated sperm

Acrosome is specialized lysosome -- filled with enzymes -- 2 main ones are hyaluronidase and acrosin.

Physical description: Fusion of outer acrosome and sperm cell membranes, release of enzymes including hyaluronidase, breaks down hyaluronic acid.

Acrosin is a proteolytic enzyme of the trypsin family bound to inner acrosome membrane, will digest path through zona pellucida.

Acrosome reaction is induced when sperm binds to the zona pellucida --  $\uparrow \text{Ca}^{++}_i$ .

Slides:

- 90) LM and SEM zona pellucida
- 91) LM and SEM zona pellucida
- 92) sperm penetrating zona pellucida
- 93,94) acrosome reaction
- 95) zona pellucida structure and chemistry

Zona pellucida chemistry: three glycoproteins -- ZP1 is a structural protein that connects the other two, ZP2 = secondary sperm receptor, ZP3 = primary sperm receptor and inducer of the acrosome reaction.

Penetration of the zona pellucida. A molecule in the sperm plasma membrane binds to ZP3. Molecule on the sperm that binds initially is unclear. One possibility is, galactosyl transferase, an enzyme acting as a physical receptor. Thousands. Next, zonadhesin in the acrosomal matrix maintains binding to zona proteins.

Secondary binding to the zona pellucida. Needed because entire outer surface of sperm disintegrates. ZP2 binds to a binding domain of PH-20 as the sperm penetrates the zona pellucida by digesting zona proteins with acrosin. PH-20 found on posterior sperm head and inner acrosomal membrane (2 functions, see below).

Slide:

96) process of penetration

Sperm fusion with a secondary oocyte. The equatorial segment is very fusogenic on the sperm and microvilli of the oocyte are fusogenic. At the time of fusion, motility of the sperm stops.

Slides:

97) sperm-oocyte fusion

98) sperm-oocyte fusion

Molecules involved turn out to be fertilin on the sperm, which has a disintegrin domain that binds the integrin on oocyte microvilli. Snake venom also disintegrin.

Summary of molecular aspects of fertilization:

<u>Sperm</u>	<u>Site</u>	<u>Oocyte</u>
hyaluronidase	acrosomal matrix	hyaluronic acid in cumulus
PH-20	posterior sperm head	hyaluronic acid in cumulus
Galactosyl-transferase	sperm plasma membrane	ZP-3
zonadhesin	acrosomal matrix	zona pellucida proteins
acrosin	inner acrosome membrane	all zona pellucida proteins
PH-20	inner acrosomal membrane	ZP-2
Fertilin (disintegrin)	equatorial segment	integrin

Activation of the oocyte (second sperm function).

Slide:

99) secondary oocyte activation

Pre-fertilization situation with cortical granules. Homology with acrosome.

Steps of activation:

- 1) Sperm adds phospholipase C zeta (oscillin) – causes phosphatidyl inositol to split to diacyl glycerol and inositol triphosphate.
- 2) Electrical -- fertilization potential -- a depolarization that spreads across the oocyte.  $\uparrow \text{Ca}^{++}_i$ , caused by oscillin in sperm.
- 3) Phospholipase C zeta breaks down phosphatidylinositol into inositol triphosphate and diacyl glycerol which affect  $\text{Ca}^{++}$  concentrations and stimulate protein kinase C.
- 4) Cortical granule exocytosis by fusion with cell membrane, homologous to acrosome reaction. Cortical granules filled with proteolytic and hydrolytic enzymes.
- 5) Proteolytic inactivation of ZP3 prevents additional sperm from binding to zona pellucida (zona block) except in the rabbit.

- 6) Vitelline block to polyspermy, make sure character of oocyte membrane has changed so no longer fusogenic. Cortical granule membranes have been incorporated.
- 7) Enzyme activation due to increasing  $Ca^{++}_i$ . but evidence unclear.
- 8) Completion of meiosis II by polar body extrusion -- ootid stage.
- 9) Retraction of corona processes.
- 10) Continuing spikes of intracellular-free  $Ca^{++}$  due to oscillin from sperm every 30 min.
- 11) Contraction of the oocyte.

Third sperm function -- add centrosome (except rodents).

### **CLASS 13** Recitation 3 / Movies on fertilization and embryo technology / Quiz 6

#### **LECTURE 11** (Class 14)

Integrating themes.

Two blocks to polyspermy in most species: vitelline/zona pellucida.

Few sperm are at the site of fertilization at any given time.

Three fusion events: the acrosome reaction, sperm oocyte membranes, cortical granules.

Three distinct concepts:

- 1) Get the genome of the sperm into the oocyte.
- 2) Activate the oocyte -- otherwise dies.
- 3) In some species, add non-genetic components from the sperm, for example centrosome from sperm midpiece.

#### First cell cycle

Slide:

- 100) dehiscence -- breakdown of disulfide bonds of the sperm nucleus by enzymes in oocyte cytoplasm (opposite of what happens during spermiogenesis)

DNA repair.

Pronucleus formation.

S-phase synthesis of DNA for the first cell cycle.

Breakdown of nuclear (pronuclear) membranes -- no fusion in mammalian species.

There is no moment of fertilization, it is a process.

Syngamy -- fusion of pronuclei does not exist in mammals.

Two-cell embryo. Note: No centrioles for the first cell division, but is a centrosome.

Slides:

- 101) pronuclei
- 102) (non) syngamy
- 103) timing of fertilization
- 104) Mechanisms of Fertilization in Mammals

Maternal inheritance.

Slide:

- 105) mitochondria

About 100,000 mitochondria per oocyte. About 100 in the sperm. Both types specialized in different ways.

Sperm mitochondria degenerate. Even if they didn't, they would become diluted out as only a few cells of the early embryo develop into the fetus.

Mitochondria have their own DNA, about 16,000 base pairs. Genes for about 1/10th of the parts of proteins in mitochondria come from the mitochondrial genome in addition to tRNA, ribosomal RNA for mitochondrial function.

Slide:

- 106) mule/hinny

Cloning by nuclear transplantation -- consequences.

Preimplantation embryological development:

- 1) Cleavage -- when cells get smaller and smaller without an increase in mass of the embryo.

Slides:

- 107) terminology -- go over definitions
- 108) stages of development in rabbit embryos -- ICM
- 109) stages of development in cattle embryos
- 110) site in bovine reproductive tract
- 111) compaction
- 112) compaction
- 113) compaction

- 114) compaction
- 115) hatching embryo
- 116) ZP functions

Terminology, including morula, blastocyst, blastocoele, hatching, inner cell mass, trophoblast.

Slide:

- 117) Activation of the embryonic genome; prior to the 8-cell stage in human embryos, mRNA that was inherited via the cytoplasm of the oocyte drives most protein synthesis. New mRNA synthesis occurs at the 8-cell stage.

Infertility.

Many causes. Sperm and egg need to get together. Sperm need to be motile to pass barriers and zona pellucida.

Slide:

- 118) causes of fertilization failure

Examples of problems:

- 1) wrong timing
- 2) anatomical blocks
- 3) infections
- 4) antibodies to sperm or the zona pellucida

Human infertility frequently circumvented by IVF, GIFT (gamete intra fallopian-tube transfer) and ICSI (intracytoplasmic sperm injection). Pregnancy rates are on the order of 20% per embryo, usually 2 or 3 embryos are transferred. There is excess of twins (over 10% of pregnancies is >2 embryos transferred).

Can sort out abnormal embryos by biopsy and molecular techniques. Termed preimplantation genetic diagnosis (PGD).

Slide:

- 119) biotechnology involving fertilization -- unfertilize triploid when block to polyspermy fails

Biotechnology applications for embryos.

Freezing, sexing, transferring, culturing, splitting, adding genes.

Embryonic mortality.

Greater than 50% in women.

Can be studied by measuring hCG in sexually active women.

Embryonic mortality is 20-30% in most farm animals.

Slides:

- 120,121) shapes of advanced pre-implantation equine and bovine embryos
- 122) optimal conditions differ for natural vs assisted reproduction
- 123) mal-timing of ovulation leads to infertility

Implantation.

Slides:

- 124) endometrium of mare
- 125) apposition of human embryo to endometrium
- 126) nidation of human embryo

Definition depends on the species. In rabbits, rodents and the human, embryo nidates -- digests a path into the endometrium.

In many species, implantation implies the embryo's becoming fixed in place.

There are different degrees of attachment that depend on the species; will cover next time.

Timing of implantation -- day 5 to day 35 depending on the species. Rodents -- 5 to 6, human -- 7 to 8, cattle -- 20 to 25, horses -- about 35 days after fertilization.

## **LECTURE 12 (Class 15)**

If strong intermixing of tissues during implantation, then more damage to uterus at birth when the placenta is released. If attachment is more superficial, then less damage at birth.

For example, in the horse, there is relatively little damage when the placenta is released so the uterus is reasonably fertile a few days after birth. Conversely, takes a month to repair uterus of cow after birth.

Horses sometimes show estrus after giving birth -- known as foal heat. It can be fertile because there is little repair needed to the uterus.

One other term specific to rodents and humans -- decidualization. Uterine response to implantation by a thickening; increase in the cell number at implantation site.  
 The placenta -- is embryonic, not maternal tissue -- derived partly from inner cell mass, partly from trophoblast.

Very important species differences. Disaster occurs if there is inappropriate extrapolation from one species to the other.

Slide:

127) drawing of placental morphology illustrating amnion, allantois, chorion, yolk sac

Slide:

128) placental structure -- there are 6 potential barriers between fetal blood and maternal blood:

- 1) fetal capillary endothelium
- 2) fetal connective tissue
- 3) fetal = placental chorion epithelium
- 4) maternal endometrium -- epithelial lining of uterus
- 5) maternal connective tissue
- 6) maternal capillary endothelium

Slide:

129) Grosser classification -- 4 broad types of placenta:

	<u>Type</u>	<u>Maternal erosion</u>	<u>Form</u>	<u>Species</u>
1)	epithelial-chorial	none	diffuse	pig, mare
2)	syndesmo-chorial	endometrium	cotyledonary	ruminants
3)	endothelial-chorial	2 layers	zonary	carnivores
4)	hemo-chorial	all layers	discoid	primates, rodents

Ruminants -- mother's part of placentome is termed caruncle; fetal part is cotyledon.

No blood vessels human -- pools of blood in hemo-chorial.

Due to erosion of the human maternal tissues, there are relatively stagnant pools of blood, no capillaries are in the region, so the arterials release jets of fluid that mix the pools of blood so that nutrient and oxygen exchange can occur.

Slides:

- 130-132) cotyledonary placentas – anastomosis = freemartin  
 133-134) zonary placentas  
 135) discoid placenta

Transfer of passive immunity from mother to offspring. Real practical physiology.

Mother has made antibodies to most of the disease organisms in the environment. These pre-made antibodies are transferred to the fetus to protect it until its own immune system can make appropriate antibodies.

Antibodies are transferred three different ways depending on the amount of erosion of maternal tissue in the placenta.

In the mouse and human, much erosion, antibodies transferred directly to the fetal blood prior to giving birth.

Carnivores have intermediate erosion of maternal tissue, some antibodies are transferred via the blood but additional antibodies are transferred in the first milk colostrum.

The third category -- pigs, horses, ruminants -- little erosion, no antibody is transferred between the mother and the fetus via the blood because the tissue is relatively intact so all passive immunity must go to the offspring via the first milk -- colostrum.

Colostrum is extremely rich in IgM and IgG immunoglobulins; mother's blood to milk via mammary gland receptors and then taken from the stomach and small intestine directly into the circulation of the newborn. That is, the stomach and intestines do not digest the immunoglobulins; and they are taken up wholesale into the blood, probably receptor-mediated system.

Big problem -- mechanism only functions during the first 24 hours after birth and only functions well during the first 12 hours after birth because the gut epithelium closes up and immunoglobulins digested after that. Thus, extremely important to get colostrum to newborns to transfer the passive immunity. If this is not done, ruminants, horses, swine, death rates exceed 50%.

This mechanism is not operating in human babies because they receive the passive immunity directly from the mother before birth.

Exception to the above for humans -- IgA, a class of antibodies that act in the mouth and stomach -- are in the first milk as well and confer a passive immunity to the individual without having to enter the blood. This protects against microorganisms in the mouth, throat, esophagus, stomach and so on.

Thus, even though nursing babies do not obtain circulating immunoglobulins via the milk, have a slight health advantage because of the IgAs.

Practical point: Antibodies in mother reflect environment -- Sweden -- Iran study.

Placental function.

Slide:

136) functions as lung, liver, exchange of nutrients, removal of waste products, storage of energy in the form of glycogen, has many endocrine functions specific to human.

Slide:

137) embryonic signals

Oxygen exchange is quite efficient because fetal hemoglobin (different gene) carries more oxygen per molecule under given conditions than does adult hemoglobin. Also fetal blood has more hemoglobin molecules per unit volume than adult blood.

Circulating hormones in the blood during pregnancy.

Sources of progesterone depend on species -- primary corpus luteum, accessory corpora lutea, placenta.

Human placenta as an endocrine organ:

- 1) human chorionic gonadotropin
- 2) progesterone -- no need for the human ovary (or pituitary) after 6 weeks post-fertilization
- 3) choriosomatammotropin
- 4) estradiol-17 $\beta$

and numerous other minor hormones.

Slide:

138) graph of blood levels progesterone, hCG, human chorionic somatomammotropin, estradiol.

Slides:

139-140) endometrial cups of mare

Invasion of trophoblast cells into endometrium produces eCG - equine LH glycosylated differently, destroyed by immune system eventually

Functions of placental hormones:

- 1) maintain pregnancy
- 2) adjust homeostatic mechanisms of the mother
- 3) set-up the system for birth or parturition
- 4) set-up the mammary gland for lactation

## Functional changes at birth.

Changes in respiration (lungs function), circulation, excretory function (baby's kidney starts to function), nutrition (has to get nutrients via the mouth rather than the umbilical cord).

## Changes in fetal circulation at birth (Figs. 56-2 and 56-4 in text).

- 1) No more placental circulation -- the umbilical veins and arteries are closed.
- 2) Circulation to the lungs goes from about 10% of cardiac output to 100%, that is the circulatory system goes from pumping in parallel to pumping in series; change due to closing of foramen ovale and ductus arteriosus.
- 3) Circulation to the liver is greater after birth than before birth.

## Slide:

141) fetal circulation (simplified version of text Fig. 56.4A)

Ductus venosus – bypasses fetal liver.

## Why the fetus is not rejected by the mother.

Fetus is foreign to the mother.

Causes immune response.

Fetal tissue is rejected if grafted to the mother.

There are a number of mechanisms, some redundancy.

## Slide:

142) mechanisms

- 1) Progesterone is immunosuppressive, weakly but measurably. More infections occur during the luteal phase in female mammals, including women, than the follicular phase.
- 2) Immunosuppression by specific lymphocytes.
- 3) Antigens on the outer part of the fetal-placental unit are masked and internalized. For example, the zona pellucida is a physical barrier to cellular contact with the early embryo, and after that MHC (major histocompatibility antigens) are not present on the outside of the conceptus.
- 4) Acellular barriers of ground substance are secreted by the chorion to further mask antigens.

- 5) The immune system in the uterus is a local circulation. Lymphocytes do not seem to get back to the main circulation from uterine lymph but are destroyed.
- 6) Local immunosuppressive factors may be secreted by the fetal-placental unit that inhibits cell division of lymphocytes.
- 7) The immune system participates directly in fooling itself; HLA-G is expressed in placental tissue. Acts like a universal acceptor transplantation antigen of MHC. Immune system does not recognize as foreign. Placenta also secretes interleukin-10-anti-inflammatory balance of immune response.
- 8) Immune system cytokines stimulate the placenta.
- 9) Not all mechanisms function in all species.

### **LECTURE 13** (Class 16) Quiz at end of lecture

Maternal physiology of gestation.

Great species differences, will concentrate on the human.

Fetus is a parasite, relies on the mother for growth and maintenance. Receives nutrients, uses oxygen, mother removes waste products.

Homeostatic mechanisms of the mother are reset to deal with the extra load on the system -- traumatic disruption at the time of birth.

Resetting mechanisms is done by regulatory molecules, primarily placental hormones including progesterone, estradiol-17 $\beta$ , chorionic somatomammotropin. They also affect other hormonal loops in the mother.

Water and electrolyte balance.

Great increase in water during gestation -- 4 to 6 liters.

- 1) conceptus
- 2) extracellular space of the mother, especially lower extremities
- 3) blood volume

Concomitant increase in sodium and potassium. About 900 milli-equivalents in a typical gestation, but not enough compensation in sodium and potassium to maintain non-pregnant concentrations.

Therefore, blood is slightly more dilute during pregnancy.

Renin and aldosterone concentrations increase in attempt to correct this and they are partially successful in retaining sodium.

Normally at birth in the human, about 500 ml of blood is lost. All the extra blood and water volume in the mother acts as a safety factor in case more is lost. Placenta squeezes out the blood volume during birth back into the mother.

Much excess fluid right after birth in normal parturition -- the excess fluid is gotten rid of over the next month via adjusted kidney function.

Red blood cells and O<sub>2</sub> transport.

Pregnancy requires more oxygen transport, therefore more red blood cells are made for the blood.

The regulatory molecule, erythropoietin, stimulates extra red blood cell formation. However, this doesn't completely compensate for the need, especially because blood volume has increased 40%; result is known as a pregnancy anemia at about 30 weeks of gestation.

Red blood cell production decreases after birth.

Fetus makes fetal hemoglobin – different gene – fetal hemoglobin carries more oxygen than adult hemoglobin under most conditions.

Hemodynamics.

Cardiac output increases 40% during pregnancy from 5 to 7 liters per minute, leveling off by 20 weeks of gestation.

Upright posture problem.

Upright posture of the human poses special problems because of need to pump fluid out of the bottom of the body with the fetus restricting return. Draw picture.

Intra-abdominal pressure plus gravity keep extra blood fluids in the lower extremities.

In late pregnancy, even though there is increasing fluid volume in the mother, there is less venous return, so cardiac output plateaus.

Can result in hypotension, that is low blood pressure, because cardiac output is insufficient to keep blood pressure up. Heart can only pump the blood that is available.

Cardiac output (CO) made up of 2 factors, heart rate and stroke volume. Heart rate increases about 10% by 4 weeks of gestation, 25% by term. Stroke volume increases up to 30% above pre-pregnancy values by mid-pregnancy. Stroke volume less in later pregnancy.

During labor, cardiac output increases markedly, blood squeezed out of uterus so even more available for rest of body after birth.

At term, 17% CO to uterus; non-pregnant 2-3%.

Cardiac output increases markedly post-labor because fetus no longer occludes venous return from lower body.

Safety mechanism -- blood clots more easily during pregnancy to minimize blood loss during the birth process. However, this also results in greater tendency for blood clots to form in veins, particularly legs, which is exacerbated by the extra fluid in the legs. Rarely, clots lodge in the brain resulting in stroke.

Estradiol-17 $\beta$  and progesterone regulate increased blood clotting in complicated ways. Oral contraceptives mimic this to some extent.

When placenta is expelled, clotting mechanisms return to normal within weeks.

Excretory system.

Renal blood flow increases 40% by peak of pregnancy.

Kidneys work hard to retain sodium due to increased aldosterone to compensate for increased blood volume.

Respiration.

Both increase in the rate of breathing and the tidal volume in the lungs.

Carbon dioxide is lower and oxygen higher in the pregnant than non-pregnant woman to improve gas exchange at the placental level.

Metabolism.

Many nutrients used in larger quantities during pregnancy:

- 1) calcium
- 2) iron

- 3) vitamins
- 4) minerals
- 5) protein

Mechanisms are in place to enhance uptake of these molecules.

Will concentrate on energy.

Energy is required for fetal growth. Fetus uses huge quantities of glucose.

Energy is stored in the fetus and in the mother for lactation.

Energy costs of standard human pregnancy -- 80,000 kilocalories, about 280 calories per day for 280 days. Not constant over gestation.

Standard 56 kilogram woman, accretion of energy during pregnancy:

<u>Nutrient</u>	<u>Fetus</u>	<u>Mother</u>
925 gm protein (4 Cal/gm)	59%	41%
3825 gm fat (9 Cal/gm)	12%	88%
	7,300 calories	34,200 calories sum=41,500

Accretion of the above takes an additional 38,500 calories to move and synthesize these nutrients and maintain the tissues.

The fat accreted in the mother is needed for energy for lactation.

Weight increases: 5 kg water, 4 kg fat, 1 kg protein.

Oxygen consumption.

Increases 20% by term. Most to uterus, heart, lungs, kidney, mammary glands.

Actual basal metabolic rate decreases during the first two-thirds of pregnancies with some diets. One method of compensating for nutrient requirements.

Basal metabolic rate increases during last third of pregnancy.

Significant energy cost is carrying the excess weight of pregnancy. Behavioral adjustment to less physical activity the last one-third of gestation.

Where does the extra energy for pregnancy come from?

- 1) Increased food intake – about 20,000 calories normally.
- 2) Decreased metabolic rate – sparing effect 3% = 20,000 calories.
- 3) Use of maternal stores – depends on fat status of woman before conceiving. } **vary**
- 4) Decreased physical activity culturally determined. }
- 5) Decreased energy excretion due to increased efficiency of absorption – greater than 90% efficient without pregnancy so little room for improvement. However, only 3% increase in improvement = 20,000 calories per gestation.

In pregnant woman, there are functional and morphological changes in the small intestine. Food stays in the gut longer for more efficient nutrient extraction.

First two-thirds of gestation, nutrients are stored = anabolic phase. Fat accumulation, adipocytes in the hips and thighs particularly. Last third of gestation = opposite catabolic phase.

Glucose is shunted to the uterus, mother uses fat and lactate, spares glucose; insulin increases. Mother is insulin resistant, glucose stays in the blood for the fetus rather than being driven into cells, and insulin less effective in inhibiting gluconeogenesis.

Gluconeogenesis -- synthesis of glucose from non-glucose sources like fatty acids.

Diabetic mother -- too much glucose.  
Baby's pancreas ↑ insulin secretion -- little effect.  
Get macrosomic baby.  
Get hypoglycemic crisis in baby at birth.

## LECTURE 14 (Class 17)

Parturition.

The signal for parturition comes from the fetus, not from the mother.

There is a certain amount of genetic control.

There are considerable species differences, but also a lot of similarities.

Human parturition regulation is poorly understood. Sheep and goats are used as models for human parturition.

Common themes:

- 1) Need to open, ripen the cervix. Occurs from prostaglandin E<sub>2</sub>, relaxin and estrogen. Net result -- loss of collagen, elastin.
- 2) Myometrial contractions. These can be very powerful under some circumstances. Result in coordinated waves of peristaltic contractions. Regulating molecules include prostaglandin F<sub>2α</sub>, oxytocin; estradiol-17β facilitates contractions, progesterone inhibits the contractions.
- 3) Initiation of parturition -- a common denominator among species is decrease in progesterone and increase in estradiol, except human in which progesterone decreases gradually at the end of pregnancy rather than dramatically. Progesterone at the end of pregnancy can come from the corpus luteum, the corpus luteum and the placenta, or the placenta only, depending on the species. Therefore, mechanisms to decrease progesterone depend on the species.

Steps in endocrinology of parturition in the goat -- placenta makes no progesterone.

- 1) Sufficiently stressed fetus. Twins more stress & birth sooner.
- 2) Corticotropin releasing factor from the fetal hypothalamus (can't happen until sufficient maturity).
- 3) ACTH release from fetal anterior pituitary.
- 4) Cortisol production, fetal adrenal.
- 5) Increase in estradiol-17β production by the placenta.
- 6) Causes prostaglandin F<sub>2α</sub> to be secreted by the placenta.
- 7) This causes regression of the corpus luteum -- high E<sub>2</sub>/P<sub>4</sub> ratios.
- 8) PGF<sub>2α</sub> synthesized in the uterus. Oxytocin receptors synthesized in uterus due to E<sub>2</sub> (as occurs in non-pregnant reproductive cycles).
- 9) Contractions of the myometrium.
- 10) Reflex from the cervix -- fetus pushes against cervix causes signal to the posterior pituitary and results in release of oxytocin which causes uterine contractions and even more pushing against the cervix.
- 11) Stronger contractions.

Can short-circuit this process by giving exogenous ACTH, cortisol or an analog, prostaglandin F<sub>2α</sub>. These are used clinically. Oxytocin can be used as well, but it is dangerous because it can rupture the uterus if the cervix is not ready.

Oxytocin should be used as an aid, not as an initiator.

Parturition in the sheep -- Different from goat because placenta makes lots of progesterone.

In the sheep, placental progesterone is metabolized to estradiol-17β due to cortisol production and then similar things happen as in the goat.

In the human, instead of cortisol, dehydroepiandrosterone sulfate is secreted by the fetal adrenal. Otherwise pathways are similar.

Clinical problems -- Most species if no anterior pituitary in the fetus, birth is not initiated due to lack of CRF, resulting in a very prolonged gestation. This does not, however occur in the human.

In human, some question as to whether oxytocin participates in the normal parturition process. It is very useful clinically as a drug, however, and has role in expulsion of placenta.

Typical labor in women:

Stage I -- system is set up for parturition, one gets contractions of the uterus generally lasts about 10 hours but can be much longer or shorter.

Stage II -- expulsion of the fetus usually takes less than 1 hour.

Stage III -- expulsion of the placenta, less than 10 minutes.

During contractions, the uterus actually gets shorter. The smooth muscle cells, when they contract, do not regain their original length.

Pathology in some species: retain the placenta. This is rare in human.

Vaginal stretching induces maternal behavior. Very dramatically illustrated in the ewe by putting balloon in vagina of a ewe a week or 10 days before parturition. Ewe tries to steal lambs from other ewes.

Birth process in women may also induce maternal behavior.

Rh disease

+ mother, + father — OK

– mother, – father — OK

+ mother, – father — OK

– mother, + father — can be problem

No problem first pregnancy

Lactation.

Requires huge amount of nutrients including energy; much more than pregnancy.

During non-lactation, mammary system consists of nipple and rudimentary duct system.

Duct system morphology varies markedly among species.

Ducts grow under the influence of estradiol-17 $\beta$  and progesterone, especially with high concentrations during pregnancy.

Pregnancy stimulates further differentiation of the ducts to form alveoli at the end of the ducts. Alveoli consist of a globe of epithelial cells surrounded by myoepithelial cells and a rich capillary bed. Draw.

Slide:

143) mammary alveolus

Milk is secreted into the alveolus and is expelled by contraction of the myoepithelial cells by oxytocin.

Lactation could be thought of as occurring in three steps:

- 1) Developing the system during late pregnancy.
- 2) Milk secretion during lactation.
- 3) Milk expulsion by oxytocin.

Many hormones participate in setting up the mammary system and causing lactation to occur, including prolactin, placental somatomammotropin, estradiol-17 $\beta$ , progesterone, thyroid-stimulating hormone, adrenal corticotropic hormone, and others.

Important point in setting up lactogenesis for lactation – You want to set up the system but do not want it to make milk until birth.

Progesterone and estradiol-17 $\beta$  are needed to set up the system, but progesterone functions as a brake to actual lactation.

Because progesterone decreases at parturition, it is a perfect system for releasing the lactating tissue to make milk.

Key hormone of milk secretion is prolactin.

Prolactin is released primarily due to nipple stimulation. Neuroendocrine reflex to the hypothalamus decreasing dopamine (PIF), increasing thyroid-releasing hormone, both of which result in increased prolactin secretion by anterior pituitary lactotrophs.

There are prolactin receptors in the alveoli.

Prolactin also is released by stimulating nipples when there is no lactation. Even, for example, in jogging women.

Little consequence because there are no alveolar cells, no prolactin receptors, and little effects on the breasts.

May, however, result in increased growth hormone actions because prolactin binds growth hormone receptors to some extent.

Milk the perfect food for young of given species.

Milk is 50-90% water. Especially low in water in marine mammals.

Lactose 3-7%. Humans have quite high lactose – fortify cow's milk.

Fat 3-40%. Marine mammals have highest fat.

Protein is 3-6%.

Milk also is loaded with vitamins, amino acids, minerals, etc.;  $Ca^{++}$  – protein-bound.

It is especially draining on the lactating mother of calcium and energy.

Colostrum, the first milk, is higher than normal in protein, especially immunoglobulins. Also higher in fat. Loaded with growth factors, such as epidermal growth factor (EGF), PDGF, etc.

During lactation, great increase in blood flow to mammary glands to transport nutrients.

Glucose is precursor to lactose. Lipid precursors are transported for milk fat synthesis.

Mammary gland is a champion protein synthesizing system. Requires huge amounts of amino acids and energy to make the protein.

Milk ejection reflex via oxytocin. Acute neuroendocrine reflex; about 60 seconds after stimulating the teat, posterior pituitary releases oxytocin and 30 seconds later it is at the alveolus causing myoepithelial cells to squeeze milk into the milk duct and available for suckling.

Milk ejection evolves to a conditioned reflex. Women hear a baby cry, oxytocin is released before the baby even suckles.

In dairy cows, milk starts leaking from the teats when people come into the dairy barn in the morning. Can be conditioned to some extent by massaging the cow's udder and training the system so that the milking machine is placed on about 90 seconds after stimulating the teats.

This reflex is prone to inhibition. Stress, including epinephrine or norepinephrine inhibits oxytocin release in all species.

Four effects suckling on brain:

1. sensory
2. prolactin

3. oxytocin
4. inhibit GnRh

Stopping lactation – Stopping nursing stops lactation, but sometimes this is painful. One can give bromocriptine, a dopamine agonist, that shuts off prolactin secretion – is used clinically.

One can diminish lactation with progesterone, but generally just ceasing nursing stops the prolactin stimulus.

When lactation ceases, prolactin-secreting cells in the anterior pituitary are actively destroyed. Lysosomes destroy the prolactin-containing granules and the system shuts down in a few days.

One can initiate lactation in the absence of pregnancy – E<sub>2</sub>, P<sub>4</sub>, TRH, STH.

Nutrients → maintenance  
                  growth (when growing)  
                  lactation (when lactating)  
                  fat storage

Role of growth hormone in milk production as partitioning agent. Causes nutrients to be used for growth in young animals and lactation in older females; in the absence of growth hormone, excess nutrients go to fat deposition.