**REPRODUCTION REVIEW**

**SEXUAL DIFFERENTIATION – KEY CONCEPTS**

1. Gonadal sex (ovary or testis) is determined by chromosome (XX or XY).
2. Internal and external genitalia is determined by hormonal factors.
3. Testes produce AMH (anti-Mullerian hormone) – causes regression of Mullerian duct.
4. Female pattern is generally considered the “default” – lack of testis will result in female pattern of development.

**REVIEW OUTLINE**

**Selected Slides from lectures last year – NO NEW SLIDES except Key Concept slides**

**Key Concepts noted before each section topic**

1. Sexual Differentiation
2. Male HPG axis and Important Hormones
3. Male Genitalia and Spermatogenesis
4. Female HPG axis and Hormone Cycles
5. Fertilization and Embryo Implantation
6. Placental Development
7. Endocrinology of Pregnancy
8. Parturition and Lactation
HIERARCHY

1. chromosome
2. gonads
3. genitalia
4. gender identity

GENETIC
(hormones not involved)

HORMONES REQUIRED
INTERNAL STRUCTURES
EXTERNAL STRUCTURES
SOCIAL BEHAVIOR

EARLY DEVELOPMENT

Primordial germ cells (PGC) migrate to gonadal ridge (5-6 weeks gestation)

Primordial (indifferent) gonad bipotential for testis or ovary

Germ cells direct the specific development of the gonad

Specific gene expression patterns lead to gonad differentiation

Male (Y): Sry (encodes for testis determining factor) + Sox9
Female: Rspo1 + Wnt4 (required for gonad development in both sexes, but ovaries upregulate to repress testes development)

SEXUAL DETERMINATION

Gonadal sex: determined by chromosome (or SRY)
MALE INTERNAL GENITALIA

TESTES -
- Sertoli cells make AMH and Inhibin B – AMH induces regression of the Mullerian duct
- Leydig cells make androgens - testosterone stimulates formation of internal genitalia from Wolffian duct:
  - Testosterone: top = epididymis, middle = ductus deferens, base = seminal vesicle, ejaculatory duct
  - DHT: prostate, external genitalia

FEMALE INTERNAL GENITALIA

Absence of Testes (not presence of ovary) induces regression of Wolffian ducts
- No AMH, No Androgens

Mullerian Ducts differentiate:
- Top – fallopian tubes (oviducts)
- Middle – fuses to become uterus
- Bottom – cervix and upper 1/3 of vagina

MALE HPG AXIS – KEY CONCEPTS

- HYPOTHALAMUS >> GnRH is obligatory for reproduction! GnRH pulsatility determines LH/FSH ratio
- PITUITARY >> 2 main hormones "gonadotropins": LH (steroidogenesis) and FSH (spermatogenesis).
- TESTES >> major hormone is testosterone (converts to DHT and/or Estradiol in select tissues)
HPG AXIS:
Major hypothalamic hormone:
Gonadotropin-releasing hormone (GnRH/LHRH)
Absolutely required
Must be released in a pulsatile manner

Major pituitary hormones =
Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH).
LH: stimulates steroidogenesis in ovaries and testes.
FSH: stimulates gametogenesis (egg and sperm maturation) in ovaries and testes.

INHIBIN B – specific inhibitor for FSH
Expressed in gonads
Inhibits FSH beta subunit synthesis in gonadotropes

ACTIVIN –
Expressed in pituitary and gonads
Stimulates FSH beta, LH beta, and GnRH receptor synthesis in pituitary

MALE REPRODUCTION - FSH
Sertoli cells – have high affinity FSH receptors
Form the blood-testes barrier

FSH –
Stimulates spermatogenesis
Increases sperm motility
Stimulates growth of seminiferous tubules – primary determinant of testes size
Stimulates androgen binding protein (ABP) – maintains high local T
Stimulates aromatase
Stimulates inhibin
Stimulates growth factors
Leydig cells – have high affinity LH receptors

LH – Stimulates steroidogenesis from cholesterol
Androgens = 19 carbon steroids
Stimulates STAR protein – rate limiting
Stimulates Leydig cell growth

Testosterone precursors made in extragonadal tissues: brain, adrenal, skin, adipose tissue

Testes – primary source of circulating T
Circulation – bound to SHBG
Intracellular – converted to estrogens or DHT
Metabolized – diols, triols
T and DHT both bind androgen receptor (AR)
Nuclear steroid receptor
DHT higher affinity than T for AR

Masculinization of the brain requires T to E conversion

Male pattern baldness
**COMPARISON OF TESTOSTERONE AND DIHYDROTESTOSTERONE (DHT) ACTIONS**

<table>
<thead>
<tr>
<th>Fetal Development</th>
<th>Testosterone</th>
<th>Dihydrotestosterone</th>
</tr>
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<tbody>
<tr>
<td>Epididymis</td>
<td>Penes, Penile urethra</td>
<td>Seminal Vesicles</td>
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<tr>
<td>Vas Deferens</td>
<td>Seminal Vesicles</td>
<td>Prostate</td>
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<tr>
<td>Seminal Vesicles</td>
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<tr>
<td>Penis, Penile urethra</td>
<td>Prostate</td>
<td>Prostate</td>
</tr>
<tr>
<td>Skeleton</td>
<td>Skelton</td>
<td>Penis, Penile urethra</td>
</tr>
<tr>
<td>Pubic Hair</td>
<td>Spermatogenesis</td>
<td>Penis, Penile urethra</td>
</tr>
<tr>
<td>Sebaceous glands</td>
<td>Spermatogenesis</td>
<td>Penis, Penile urethra</td>
</tr>
</tbody>
</table>

**MALE ANATOMY AND SPERMATOGENESIS**

**KEY CONCEPTS**

- Spermatogenesis begins in the seminiferous tubules of the testes.
- High concentrations of intratesticular testosterone are required for spermatogenesis.
- Sertoli cells provide nutrients for developing sperm and make important hormones/enzymes.
- Leydig cells produce testosterone.
- Sperm mature in the epididymis.
- Accessory glands (prostate, seminal vesicles) provide nutrients and fluid to keep sperm alive in the female reproductive tract.
- Erection is under parasympathetic control and ejaculation is under sympathetic control.

**MALE REPRODUCTION - ANATOMY**

**PENIS**
- Glandular and muscular tissue
- Penile urethra

**ACCESSORY GLANDS**
- Seminal vesicles
- Prostate gland
- Bulbourethral gland (Cowper’s gland)

**TESTES**
- Seminiferous tubules
- Scrotum
TESTES

Each adult testis weighs ~ 40 grams with 80% germinal tissue and 20% supportive connective tissue with Leydig cells.

Seminiferous tubules are about 200 meters of coiled mass.

100 – 200 x 10^6 sperm per day (oligospermia is <20x10^6/ml)

**LEYDIG – SERTOLI CELL INTERACTIONS**

High T concentrations in lumen support sperm development

**SPERM MATURATION**

Progressive increases in motility

Formation of acrosome: sperm "cap" containing hydrolyzing enzymes used to penetrate ovum.

Acquisition of receptors necessary for binding to ovum.

Decreased cytoplasm and cell volume.

*maturation not fully complete until sperm enter vagina (capacitation)
ACCESSORY GLANDS

SEMEN =
10% sperm (150-600 million)
70% fluid from seminal vesicles
10% fluid from epididymis, prostate, bulbourethral glands

PENIS – FUNCTIONAL ANATOMY


ERECITION
Relaxation of vascular smooth muscle (corpora cavernosa and corpus spongiosum) leads to increased blood flow in cavernous tissue. Engorgement compresses outflow pathway and creates tumescence (swelling).

Erection is Primarily Under Parasympathetic Control
Parasympathetic postganglionic nerves release Ach and NO. 
Ach can bind muscarinic receptors and activate PLC (via Gαq). This increases calcium and activates NO synthase. NO activates guanylyl cyclase.
**FEMALE HPG AXIS AND HORMONE CYCLES**

**KEY CONCEPTS**

- HPG major hormones are the same in males and females - same general functions.
- Changes in HPG hormone ratios regulate the female monthly menstrual cycle.
- Menstrual cycle consists of parallel changes in ovary and uterus.
  - Ovarian cycle describes the maturation of a follicle and oocyte, ovulation, and luteolysis (follicle degeneration).
  - Uterine cycle describes the preparation of uterine endothelium for oocyte implantation and degeneration (menses).

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**FEMALE REPRODUCTION - ANATOMY**

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**FEMALE HPG AXIS**

HPG axis feedback mechanisms generate a cyclical monthly pattern of hormone secretion called the "Menstrual Rhythm".

The menstrual cycle consists of physiological changes in 2 organs: ovary and uterus.

- Ovary = ovarian cycle
- Uterus = endometrial cycle
MENSTRUAL CYCLE - OVERVIEW

DAY 1 = FIRST DAY OF MENSES

OVARIAN CYCLE – 3 PHASES (OVARY)
1. Follicular phase = growth of dominant follicle
2. Ovulatory phase = follicle rupture and release of oocyte
3. Luteal phase = formation of corpus luteum

ENDOMETRIAL CYCLE – 3 PHASES (UTERUS)
1. Menstrual phase
2. Proliferative phase
3. Secretory phase

HPG axis hormones drive coordinated physiological changes during the menstrual cycle. Physiological changes signal hypothalamus.

OVARIAN CYCLE - OVERVIEW

FOLLICULAR PHASE – variable length (10-14 days)

OVULATORY PHASE
1-3 days

LUTEAL PHASE – 14 days

Graafian follicle

OVARIAN CYCLE SUMMARY
TWO COMPARTMENT THEORY OF E2 SYNTHESIS

Thecal cells have LH receptors -> synthesize androgens.
Granulosa cells have LH and FSH receptors:
FSH stimulates LH receptor expression — required for response to “LH surge”
FSH increases aromatase expression -> converts androgens to E2

3 LAYERS:
1. Endometrium — mucosal layer (innermost)
2. Myometrium — thick muscular layer
3. Perimetrium — outer connective tissue and serosa (also called serosal layer)

CERVIX — distinct from endometrium

UTERINE ANATOMY —
organ dedicated entirely to pregnancy

ENDOMETRIAL CYCLE
**FUNCTIONAL ZONE** – shed during menstruation

Uterine glands – secrete substances for embryo survival

Spiral arteries – reduced blood flow to spiral arteries results in ischemia >> necrosis of endometrial layer

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**ESTROGENS**

E1 = estrone - produced in higher amounts after menopause; lower binding affinity for estrogen receptors

E2 – 17β-estradiol – primary circulating estrogen during reproductive years

E3 – estriol (weak) – produced by the placenta. Also converted from estrone in the liver

**TRANSPORT IN BLOOD:**

38% bound to SHBG

60% bound to albumin

2-3% free

High conversion in target tissues by aromatase >> high local concentrations

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**ACTIONS OF ESTRADIOL - E2: SUMMARY**

Inhibit growth of cohort follicles

Prime Graafian follicle

Produce luteinizing hormone surge

Prime cervical lining for sperm transport

Prepare uterine endometrium for progesterone to evoke secretory response

Affect fallopian tube to favor transport of ovum and sperm
PROGESTERONE (P4)

Progesterone receptors A and B – E2 upregulates expression of these receptors. Nuclear steroid hormone receptor family

Transport: bound mostly to albumin; low affinity for SHBG

Actions:
• Prepares endometrium for implantation of embryo (proliferation, synthesis of enzymes that lyse zona pellucida)
• Inhibits myometrial contractions – maintains pregnancy
• Stimulates mammary gland development – preparation for lactation
• Antagonizes actions of estrogen – important consideration for hormone replacement therapy

FERTILIZATION AND IMPLANTATION

**KEY CONCEPTS**

• 3 major events must occur for ONE spermatozoa to successfully penetrate oocyte: capacitation, acrosomal reaction, and cortical reaction.
• Meiosis II does not complete in oocytes until fertilization.
• Implantation into endometrium is invasive: requires balance of factors that defend against too deep of invasion (decidual cells of uterus) and factors that promote implantation from embryo trophoblast cells.
• Embryo is completely enveloped into superficial layer of endometrium – pathological if embryo goes beyond endometrium into myometrium.

FERTILIZATION

**Step #1 - GAMETE TRANSPORT**

**SPERM:**
150 million – 600 million sperm deposited into vagina – 50-100 reach ampulla of oviduct (site of fertilization)
Reach ampulla within 5 minutes – retained for 24-48 hours

**Helpers:**
- Vaginal secretions become more alkaline
- Uterine and cervical contractions propel sperm forward
- Prostaglandin in seminal plasma induce muscle contractility
- Seminal “plug” – semen coagulates upon ejaculation
- Vaginal mucus less viscous
FERTILIZATION

Step #2 – SPERM PENETRATION OF OOCYTE

1. Sperm capacitation
2. Acrosomal Reaction
3. Cortical Reaction

MEIOSIS COMPLETES

Decondensation of sperm DNA
Oocyte is released from metaphase II arrest and completes meiosis II
Second polar body is extruded – oocyte has haploid, unduplicated chromosomes

EMBRYO IMPLANTATION

"Hatching" of embryo – dissolution of zona pellucida by trophoblast cells
Blastocyst at implantation - trophoblast differentiates into cytotrophoblast and syncytiotrophoblast
Cytotrophoblast – (initially) feeder for continually dividing cells
Syncytiotrophoblast – 3 functions: adhesion, invasion, and endocrine
PLACENTAL FORMATION

KEY CONCEPTS

• Trophoblast cells of embryo differentiate into 2 types to form placenta: cytotrophoblasts and syncytiotrophoblasts.

• Cytotrophoblasts form chorionic villi – essentially “stem” cells of syncytiotrophoblasts. Invasion of cytotrophoblasts into uterine spiral arteries is necessary for arterial remodeling.

• Syncytiotrophoblasts are epithelial covering of villi – direct contact with maternal blood and facilitate nutrient exchange between mother/fetus.

• Placenta is the major endocrine organ during pregnancy.

PLACENTAL FORMATION

Lacuna = fluid filled spaces in the syncytiotum make contact with the maternal blood vessels

Cytotrophoblasts proliferate and invade the syncytiotrophoblast – form the chorionic villus

Mature villus = fetal tissue protruding into maternal blood “brush border” of syncytiotrophoblast faces maternal blood
PLACENTAL FORMATION

Vascular remodeling at site of implantation is critical for fetal life: conversion of high resistance, low capacity >> low resistance, high volume vessels

Spiral arteries increase in diameter; muscular and elastic components are lost – increases perfusion of the placenta

Failure to develop vasculature results in relative placental ischemia.

ENDOCRINOLOGY OF PREGNANCY

KEY CONCEPTS

• Two unique hormones made by placenta: hCG and hPL
  • hCG is required to keep corpus luteum viable for first few weeks – binds to LH receptors. Corpus luteum makes lots of progesterone.
  • hPL allows more glucose delivery to fetus and promotes mammary gland development.

• Pregnancy is a physiological stressor: increased glucocorticoids to mobilize glucose.

• Pregnancy is insulin-resistant state: to increase glucose availability to fetus.

• Progesterone and Estradiol high! Progesterone – keeps uterus quiescent; Estradiol important for mammary gland remodeling

Syncytiotrophoblasts:

ENDOCRINE FUNCTION

First hormone secreted is hCG – human chorionic gonadotropin.

hCG binds to LH receptors on corpus luteum and keeps it viable

hCG is what pregnancy tests detect in urine

Rapid rise in hCG is responsible for nausea “morning sickness”

hCG also stimulates fetal Leydig cells and fetal adrenal cortex

Negative feedback actions on maternal HPG axis
Syncytiotrophoblasts: ENDOCRINE FUNCTION

Also produce human placental lactogen (hPL) (also called hCS – human chorionic somatomamadropin)

Similar to GH and prolactin

**GH-like:**
Counters-regulatory to insulin (anabolic and lipolytic)
Mobilizes glucose for fetal use
Stimulates fetal IGF-I

**Prolactin-like:**
Stimulates mammary gland development

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PREGNANCY IS AN INSULIN-RESISTANT STATE

Estrogen and progesterone reduce insulin sensitivity in mother

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ENDOCRINE PLACENTA – STEROID BIOSYNTHESIS OVERVIEW

Placenta does not express CYP17
ENDOCRINE PLACENTA - GLUCOCORTICOIDs

ENDOCRINE PLACENTA – ANDROGENs/ESTROGENs

PARTURITION AND LACTATION

KEY CONCEPTS

• Release from inhibitory effects of progesterone is key factor that signals parturition.
• High cortisol at the end of pregnancy is important for final stages of fetal maturation.
• Decreased progesterone and estradiol initiate lactation.
• Prolactin stimulates milk production.
• Oxytocin stimulates milk secretion.
PARTURITION

Stages of Labor:

**Stage 1 – activation of the uterus**
- Release from inhibitory actions of progesterone
- Uterine stretch from mature fetus stimulates oxytocin (Ferguson reflex)
- Fetal HPA axis is activated – CRH levels peak

**Stage 2 – Positive feedback phase**
- Widening (dilation) and thinning (effacement) of cervix

**Stage 3 – Evacuation of uterus**
- Expulsion of fetus from uterine compartment and release of placenta

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LACTATION

Initiated after delivery by decreased progesterone and E2
- Repeated transient hyperprolactinemia sustains milk production
- Suckling stimulates prolactin release
- Prolactin – suppresses reproductive function (inhibits GnRH)
- Prolactin – stimulates maternal behavior during pregnancy and after parturition
Oxytocin – released in response to neural input to NTS (mother’s response to infant crying) and via circulation to breast.

Increased contraction of myoepithelial cells, alveoli and smooth muscle of duct walls causes “milk letdown” for infant.

Prolactin as a lactogenic hormone:
mammogenic effects, galactogenic effects

Breast development  Milk production