IN SUMMARY HST 071
MALE SYSTEM

MALE SYSTEM ANATOMY

TESTIS

- Testis descends from a retroperitoneal position through the inguinal canal to take to scrotum during the eighth fetal month.
- Lower temperature required for spermatogenesis
- Countercurrent vascular heat exchange system
- Seminiferous tubules comprise 95% of testicular volume,
- 500 tubules per testis.
- Surrounded by the tough tunica albuginea.
- Seminiferous tubules -> rete testis -> epididymis -> vas deferens ->prostate ->ejaculatory duct ->urethra
- Seminal vesicle secretions join sperm in prostate
- Gonad undifferentiated at 8 weeks
- Influence of Y chromosome
- Wollfian duct -> male
- MIS -> regression of Mullerian ducts (uterus and fallopian tube in female)
- Testosterone to DHT by 5 alpha reductase causes masculinization of external genitalia

SERTOLI CELLS

- Sertoli cells and germ cells make up the seminiferous tubules
- Tight junctions between Sertoli cells (blood-testis barrier)
- Sertoli cells separate germinal epithelium into basal and adluminal compartments.
- Single Sertoli cell envelopes up to 20 germ cells
- Sertoli cells: support and nutrition of germ cells, release of mature germ cells, secrete androgen binding protein, transferring, inhibin, cell-cell communication (gap junctions)

GERM CELLS

- Spermatogonia – line basement layer of seminiferous tubules – small, round, mitotically active
- Develop into type B spermatogonia -> primary spermatocytes during first meiotic division (tetraploid)
- Pre-leptotene, leptotene, zygotene, pachytene, diplotene
- Secondary spermatocytes result from first reduction division (diploid)
- Second meiotic division lasts a day and result in spermatids (haploid)
- Cells in any cluster are synchronized

SPERMATOZOA

- Morphologically mature – highly polarized – 60 uM
- Condensed nucleus (acrosome) and membrane
- Tail – has neck, mid piece (sheath of mitochondria), principle piece, end piece
- 9+2 axoneme extends from neck to end piece
- Entire tail covered by plasma membrane

LEYDIG CELLS

- Between seminiferous tubules
- Production of testosterone – local and distant purposes
- In testes, testosterone bound to androgen binding protein secreted by Sertoli cells
- In plasma, testosterone is bound to circulating testosterone binding globulin

SEMEN

- Suspension of sperm in seminal plasma
- Seminal plasma: seminal vesicles, prostate and also bulbourethral gland (Cowper's), urethral glands (Littre), rete testis, epididymis, vas deferens, ampullae
- Seminiferous tubules drain to mediastinum testes and then into rete testis where 90% of fluid is produced
- 8-12 efferent ducts from rete testis to epididymis

EPIDIDYMUS
- 20 meter long duct, pseudostratified columnar epithelium, cilia
- Sperm conduit, fluid resorption, sperm reservoir (cauda), sperm maturation (fertilizing ability and motility

VAS DEFERENS
- Continuation of epididymis
- Straight duct, pseudostratified columnar epithelium, cilia, 25-45 cm long
- Transport of sperm through scrotum, inguinal canal, behind base of bladder (ampullae)
- Thick muscular wall for rapid transport of sperm during ejaculation

PROSTATE
- 20 grams

EJACULATE
- 2-5 cc with 150-200 million sperm

HORMONAL CONTROL OF SPERMEOGENEISIS
- Testosterone (for germinal epithelium), FSH (stimulates Sertoli cells to make protein kinases and protein synthesis)
- LH stimulates Leydig cells to make testosterone
- GnRH made in hypothalamus – pulses every 70-90 minutes – half life 2-5 minutes
- Testosterone inhibits LH release from pituitary and GnRH release from hypothalamus
- Estradiol derived from aromatase conversion of testosterone
- Estradiol more potent inhibitor of LH and FSH
- FSH secretion down regulated by inhibin (made by Sertoli cells)

SPERM PHYSIOLOGY
- Traverse female genital tract, bind to ova, penetrate zona pellucida, fuses with plasma membrane of ovum
- Fresh ejaculate is gel (made in seminal vesicles), liquefies in 5-20 minutes (by prostate protease)
- Fructose (seminal vesicles) is energy substrate for sperm
- Capacitation -> acrosome reaction, release of lysosomal enzymes, alter plasma membrane of sperm head, affects sperms stickiness to egg, cause hypermotility, change in tail beat frequency

MALE INFERTILITY EVALUATION
- History
- Prior history of infertility, timing of puberty, genital abnormalities, medications, prior surgery, chronic illness
- Physical exam
- Evidence of endocrine abnormalities->gynecomastia, hair pattern, phallus, urethral meatus, size and consistency of testes (4.5 cm diameter), vas deference, varicocele

SEMEN ANALYSIS
- Volume 2-5 cc
- Sperm density >20 million/cc (avg. 50-60)
- Motility >60%
- Morphology Absence of WBC's, RBC's, bacteria, agglutination
- >60% normal forms

VARICOCELE
- Dilated pampiniform plexus, left side, 10-15% of all men, 50% of infertility patients

- Abnormal thermoregulation
- Testes usually 20 degrees C cooler than core temperature
- Ligation of gonadal vein, improves sperm quality in 70% of men

EJACULATION – ERECTION

- Emission (deposition of sperm into posterior urethra) and expulsion from urethra
- Under control of sympathetic nervous system
- Emission – contractions of vas, seminal vesicles and prostate
- Ejaculation – closure of bladder neck and contraction of periurethral muscles, bulbocavernosus
- Point and shoot

ERECTION

- Neurologic initiation, reflex or centrally mediated
- Arterial filling of erectile bodies – corpora cavernosa and corpus spongiosum
- Venous occlusion of erectile bodies
- Flaccid state – resting sympathetic tone, small caliber cavernosal arteries and contracted muscle
- Signal for erection – relaxation of arterioles, increased caliber, relaxation of cervernosal sinusoids, venules are compressed by inflow of blood and increased intracorporal pressure causing reduced outflow
- When sympathetic tone returns the arteries constrict and the veins allow outflow

VIAGRA AND SIMILAR DRUGS

- Sildenafil and many others
- NO (nitric oxide) released in corpus cavernosum during sexual stimulation
- NO->guanylate cyclase->cGMP->smooth muscle relaxation->blood flow
- Sildenafil enhances effect of NO by inhibiting type 5 phosphodiesterase (degrades cGMP)
- Has no effect in absence of sexual stimulation
- Most potent effect on PDE5 (4000 times that for PDE3)
- Also affects PDE6 found in retina (color vision problems with high dose)
- Hepatic metabolism (cytochrome P450) – active metabolite
- Caution if using other P450 inhibitors (itraconazole, ketoconazole, erythromycin, ritonavir, cimetidine)
- Rapidly absorbed, maximum plasma concentration within 30-40 minutes
- Decrease in supine BP

FUNDAMENTAL QUESTIONS

1. Where are sperm produced?
2. What is a Sertoli cell? A Leydig cell?
3. In what cell in the testes is testosterone synthesized?
4. On what cell does FSH act? LH?
5. What is the function of the epididymis?
6. What is the function of the seminal vessicles?
7. What causes the undifferentiated gonad to differentiate into the males testes?
8. What is semen made of?
9. Describe the structure of a sperm? What is the acrosome? The midpiece? How does the tail move?
10. What do Cowper's glands do?
11. When a man has a vasectomy what comes out when he ejaculates? What test would one do on a woman who states she was raped in order to prove ejaculation occurred in her vagina?
12. Describe the contribution of the sympathetic and parasympathetic nervous systems to ejaculation.
13. What is the role of the prostate gland? If a man has had a prostatectomy can he make a woman pregnant?
14. How does Sildenafil (VIAGRA) work?
15. What are some of the significant side effects and contraindications of VIAGRA?
16. What is the range of normal sperm count for a male?
17. What is the volume of an average ejaculate?

TEMPERATURE AND THE TESTIS

Historical

- Greece, -heat was related to vitality
- Large penis was considered less desirable
- Thought to allow the semen to cool during ejaculation
- Recognized for centuries that cryptorchidism - results in sterility
- Core body temperature is actually lethal for the male germ cells
- Humans & many other mammals - extracorporeal pouch (scrotum)
- Not universal among mammals
- 1/3 mammals maintain testes internally (elephant, rhinoceros, aquatic mammals..)
- Does not exist at all among birds, reptiles, amphibians, or fish
- Unique among the body's organs – vulnerability

Evolutionary benefit

- Cool scrotal environment - greater sperm storage within the epididymis
- Greatest benefit to the seasonal breeder (not humans !!!)

Anatomy and Physiology

Five main anatomic features that allow the testes to remain cool

1) Dartos muscle
2) Cremaster muscle
3) Countercurrent heat exchange system,
4) Absent fatty skin layer
5) Abundant sweat glands

Clinical Aspects

Causes of male infertility related to heat

- varicocele,
- cryptorchidism
- fever

Early correction of cryptorchidism results in improved fertility

Unilateral cryptorchidism can cause significant subfertility

Varicoceles

- Dilated veins in the scrotum
- Become fuller with standing position
- First noted by Celsus(born in 25 AD)
- Intratesticular and scrotal temperature -higher in men with varicoceles,
- Pampiniform plexus of veins
- Carries blood from the testis through the spermatic cord
- Coalescing into the testicular vein(s)
- Travel upwards in the retroperitoneum
- Finally drain into the renal vein on the left
- Inferior vena cava on the right

Incompetent Valves

- Gravity pulls blood down into the scrotum
- Veins become dilated.
- Blood from retroperitoneum is at core temperature
- Reflux of this relatively warm blood
- Contributes to the scrotal warming

Varicoceles can also cause a reduction of serum testosterone level

Basic science

- When stem spermatogonia have been lost - no capability for
- spermatogenesis to recover
- First cellular manifestations of heat exposure - condensation of nuclear components (pyknosis),
- P53 highly expressed in testes
- Apoptosis form of cell death in biologic systems - loss of germ cells in response to heat
- Longest stage (pachytene) – crossover and DNA exchange, ability to repair breaks
- Time-temperature threshold

Heat and Male Contraception

Insulating the underwear of male volunteers

- Significant drop in sperm concentration and motility
- Change in scrotal temperature of only 1°C
- Unreliable form of contraception

Controversy

No temperature differences were noted between men wearing tight or loose underwear in one study while sperm count twice as high in another

FUNDAMENTAL QUESTIONS

1. Discuss the adaptive need for extracorporeal testes. Do all mammals have these?
2. What happens to cells exposed to increased temperature?
3. What is a varicocele? Discuss the aberration in blood flow that occurs.
4. Does surgical correction of a varicocele help fertility in males?
5. Do boxer shorts help fertility? What is the evidence for this?

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IN SUMMARY
FEMALE REPRODUCTIVE SYSTEM

FEMALE PELVIC ANATOMY

UTERUS

- Composed of smooth muscle, collagen connective tissue, vessels, nerves, lymphatics
- Corpus – body (mainly smooth muscle)
- Cervix (neck) – mainly collagen
- Supported by utero-sacral ligaments, cardinal ligaments
- Round ligaments – no supportive value
- Broad ligament – not a ligament but rather two layers of peritoneum containing vessels, lymphatics
- Space between leaves of broad ligament are extra peritoneal
- Lymphatic of cervix drain to base of broad ligament
- Lymphatic of upper uterus drain to ovarian plexus
- Ovaries are BEHIND uterus and usually lie in cul-de-sac
- Blood supply: uterine arteries (ascending and descending branches, ovarian vessels
- Ureter travels UNDER the uterine vessels ("water runs under bridge")
- Ureter is about 1.5 cm from cervix (easily injured during hysterectomy)
- Bladder and urethra above uterus – may prolapse into vagina (urethrocele, cystocele)
- May cause urinary stress incontinence (loss of urine with cough or sneeze)
- Short female urethra may result in urinary tract infections
- Perineum innervated by S2,3,4 by branches of pudendal nerve
- Genitofemoral nerve innervates the upper vulva

HISTOLOGY

- Portio of cervix – stratified squamous epithelium, originally was columnar epithelium that underwent squamous metaplasia
- May contain "Nabothian cysts" – glands that became obstructed through overlying squamous metaplasia – normal finding of no significance
- Cervical canal lined with ciliated columnar epithelium with crypts that extend into the collagenous lamina propria
- Endometrium consists of columnar epithelium that undergoes hormonal change each month and ultimately sheds (known as "decidua")

ABNORMAL BLEEDING

- Menorrhagia - excessive at regular intervals
- Menometrorrhagia – excessive at regular intervals
- Intermenstrual Bldg – between normal cycles
- Polymenorrhea - <21 day intervals
- Oligomenorrhea - >35 day intervals
- Hypomenorrhea – regular but small amounts
- Postmenopausal – unanticipated after one year of amenorrhea
- Pregnancy related, Infection, Neoplasm, Hormonal, Iatrogenic, Systemic

ENDOMETRIOSIS

- Attachment of endometrial cells to the peritoneal surface
- Invasion of these cells into the mesothelium
- Recruitment of inflammatory cells
- Angiogenesis around the nascent implant
- Endometrial cellular proliferation

- Monkeys are only animal (cyclic)
- No cases reported prior to puberty
- Often seen in teenage years
- Short cycle and longer flows have twice the risk of endometriosis
- Early menarche
- Delayed childbearing
- Menstrual outflow obstruction

- Implantation (Sampson's theory)
- Viable endometrial tissue is refluxed through the fallopian tubes during menstruation
- Implants on peritoneal surface or pelvic organs

Retrograde menstruation
- Occurs through the fallopian tubes
- Refluxed endometrial cells are ***viable***
- Refluxed endometrial cells are able to ***adhere*** to peritoneum

Coelomic Metaplasia
- Ovary & Mullerian ducts derive from coelomic mesothelium
- Germinal epithelium attempts to recapitulate endometrium
- Only explains ovarian endometriosis
- Peritoneal mesothelium is **totipotential**
- Develops from metaplasia of cells that line the pelvic peritoneum
- Infectious, hormonal, or other inductive stimuli may result in metaplasia

Dissemination
- Disseminated tissue can cause metaplasia
- Injection into ear vein of rabbit causes endometriosis of lungs
- Laparotomy scar
- Episiotomies
- Cesarean sections
- Transplantation confirmed in animal experiments

Embryonic rest theory

- Cell rests of Mullerian origin
- Lymphatic and hematogenous dissemination of endometrial cells
- Evidence suggests that endometrial cells can metastasize
- Pleura, umbilicus, retroperitoneal space, lower extremity, vagina, and cervix - are anatomically possible
- Endometrial tissue in uterine veins in women with adenomyosis
- Induced pulmonary endometriosis by injecting endometrial tissue intravenously in rabbits
- Lymph node endometriosis was found to be present in 6.7% autopsies

Found In

- Bone
- Muscle
- Brain
- Nerve
- Lung parenchyma
- Vertebral space
- Extremities

Genetics

- Much more common in patients with a FH
- Maternal inheritance pattern
- 7% in first degree relatives
- More severe in women with a + first degree relative
- 6/8 monozygotic twins had endometriosis
- 3.8% of non-monozygous sisters
- Polygenic/multifactorial
- No HLA system seems involved
- Perhaps different diseases

Adherence

- Endometrial fragments obtained in either phase of the cycle – adhere to the epithelial side of the amnion but only at locations where the amniotic epithelium was damaged or absent
- Cultured peritoneal explants adhered to peritoneal explants only at locations where the mesothelium was absent or damaged and the basement membrane was exposed
- Intact mesothelium constitutes a defense barrier
- Occasionally there is attachment to intact mesothelium

Integrins

- Intracellular adhesion molecule-1
- Vascular cell adhesion molecule-1
- Integrin-blocking antibodies do not interfere with endometrial stromal or epithelial cell adherence to mesothelium

Hyaluronic Acid

- Peritoneal mesothelium produces *hyaluronic acid*
- Hyaluronic acid is expressed along the cell membrane and contributes to the pericellular matrix
- Major component of the extracellular matrix ground substance
- *CD44* is the principal receptor for hyaluronic acid
- Involved in binding of gastric cancer and ovarian cancer cells to mesothelium
- Endometrial stromal end epithelial cells express CD44
- Hyaluronidase pretreatment of mesothelial cells decreases the binding of endometrial stromal and epithelial cells to mesothelium

- Invasion follows initial adhesion
- Matrix metalloproteinase (MMP) enzymes - implicated
- MMPs (and inhibitors) play a significant role in normal endometrial remodeling that accompanies menses
- MMP family contains several structurally related Zn^{++} dependent endopeptidases
- Responsible for the degradation of various extracellular matrix components
    - collagen
    - gelatins
    - proteoglycans
    - laminin
    - fibronectin
    - elastin

TGF-β

- Produced by endometrial tissue in response to progesterone
- TGF-β suppresses expression of MMP-7
- Antibody to TGF-β abolishes this suppression
- Blocking the action of TGF-β opposes progesterone-mediated suppression of MMP-3 and MMP-7
- Blocks the ability of progesterone to prevent experimental endometriosis
- TGF-β alone does not lead to sustained suppression of MMPs
- Possibly because of resumption of MMP production in the absence of progesterone
- Consistent with the fact that peritoneal fluid levels of TGF-β are elevated in endometriosis

IL-1α

- Potent stimulator of MMP-3 in proliferative phase endometrium
- Progesterone exposure in vivo reduces the IL-1α stimulation of MMP-3 in secretory phase tissue
- IL-1α stimulation of MMP-3 is restored in a dose-dependent manner with progesterone withdrawal
- Cultured endometriotic cells obtained from a rat endo model express higher levels of MMP-3 mRNA than eutopic rat endometrial stromal cells when treated with progesterone
- Elevated and persistent MMP-3 expression by endometriotic stromal cells cultured in the presence of progesterone correlates with elevated levels of IL-1α mRNA detected in the endometriotic stromal cells
- Production of IL-1α by the endometriotic lesions - overcomes the progesterone-induced suppression of MMP-3
- IL-1α - related mechanism promotes MMP-3 production by endometriotic cells even in the presence of progesterone

Contributes to pain and infertility

- Cytokines
- Prostaglandins
- Dyspareunia
- Chronic pelvic pain
- Inflammation --> Infertility
- adhesion formation
- scarring
- disrupt fallopian tube patency
- impair folliculogenesis
- fertilization
- embryo implantation

Hormonal Dependence

- Endometriosis is an estrogen-dependent disorder
- Aberrant estrogen synthesis and metabolism –
- Aromatase catalyzes the synthesis of estrone and estradiol from androstenedione and testosterone, respectively
- Expressed by many human cell types
- Ovarian granulosa cells
- Placental syncytiotrophoblasts
- Adipose cells
- Skin fibroblasts
- Estrogen produced by aromatase activity in the cytoplasm of leiomyoma smooth muscle cells or endometriotic stromal cells
- Disease-free endometrium and myometrium lack aromatase expression

Aromatase

- Cultured stromal cells derived from endometriotic implants and incubated with a CAMP analog display extraordinarily high levels of aromatase
- Growth factors, cytokines - possible inducers of aromatase
- Prostaglandin E2 was identified as the most potent inducer
- Estrogen - up-regulates prostaglandin E2 formation
- Stimulates cyclo-oxygenase type 2 enzyme in endometrial stromal cells
- Positive feedback loop for continuous local estrogen and prostaglandin E2 production
- Possible genetic defect in aromatase expression in endo
- Androstenedione of adrenal and ovarian origins – premenopausal women
- Adrenal androstenedione in postmenopausal women
- Estrone - weakly estrogenic
- Must be converted to estradiol
- 17α-hydroxysteroid dehydrogenase (17α-HSD) type 1 is expressed in endometriosis
- In contrast 17α-HSD type 2 inactivates estradiol by catalyzing its conversion to estrone in eutopic endometrial glandular cells during the luteal phase
- Progesterone induces the activity of 17α-HSD
- Inactivation of estradiol to estrone one of the anti-estrogenic properties of progesterone
- 17α-HSD type 2 is absent from endometriotic glandular cells

FUDAMENTAL QUESTIONS

1. What are the three theories of the development of endometriosis?
2. Where is endometriosis most commonly found?
3. What clinical impact does endometriosis have on the woman?
4. What treatment modalities are available for endometriosis?
5. Other than steroid hormones, what causes endometriosis to grow and proliferate?
6. Discuss in some detail, the steroidal environment that alters endometriotic development?
7. Describe the histology of endometriosis?

IN SUMMARY
NORMAL MENSTRUAL CYCLE

MENSTRUAL CYCLE OVERVIEW

- Purpose: production of a fertilizable egg and development of appropriate uterus for implantation
- Controlled by hypothalamic pulse generator in arcuate nucleus
- Pulse generator signal = Gonadotropin releasing hormone (GnRH)

GnRH

- Release is pulsatile in nature, once every 75 minutes
- GnRH causes pituitary to release follicle stimulating hormone (FSH) and luteinizing hormone (LH)
- GnRH and LH one-to-one: each pulse of GnRH causes simultaneous pulse of LH
- FSH more complex, regulated by both GnRH and ovarian peptide inhibin

FOLLICULAR DEVELOPMENT

- Resting stage = primordial follicle
  - Consists of oocyte in meiosis I with single layer of granulosa cells
  - Maturation initially FSH and LH independent
- Follicular maturation characterized by increase in number of granulosa cells
- Almost all follicles undergo atresia due to lack of rescue by gonadotropins
  - Depends on number of FSH receptors
  - Contains no LH receptors until final development stage
- During menstrual cycle, several follicles will develop to be sensitive to FSH = “recruited,” but only one will gain dominance
  - Due to positive feedback within granulosa cells
  - FSH induces its own receptor
  - Estrogen dependent process (FSH → estradiol → FSH receptor)
- Estradiol is inhibitory to FSH release from pituitary
  - Deprives less mature follicles of FSH → only one dominant follicle is produced
- Note: in vitro fertilization administers excess FSH to produce multiple follicles for pregnancy enhancement

FOLLICULAR PHASE

- First half of menstrual cycle
- Estradiol levels increase 10-fold due to activity of dominant follicle
- Estradiol → increased LH synthesis, but inhibition of release → “reserve pool” is saturated → massive release of LH over 36-hour period = LH surge → trigger for ovulation (end of follicular phase)
- Estrogen → hypertrophy of endometrium (proliferative phase of endometrial cycle)
- Prior to LH surge, pulsatile release of LH acts on second ovarian cell type, the theca cell
- Theca cell synthesizes androstenedione → substrate for granulosa cell estradiol production via aromatization from aromatase enzyme
  - Aromatase activity induced by FSH action on granulosa cell
- LH receptor present at all time on theca cells; high levels of FSH on *granulosa* cells (a few days prior to LH surge) induces LH receptor
- LH surge typically occurs 14 days into menstrual cycle (counted from first day of bleeding), but can vary 9-17 days
- Second half of cycle, luteal phase, is more precise = 14 +-2 days

OVULATION

- LH surge → proteases digest capsule of ovary → extrusion of oocyte and granulosa cells (cumulus oophorus)
- Oocyte proceeds to meiosis II, arrested until fertilization
- LH surge → partial 17 hydroxylase block by remaining theca and granulosa cells → progesterone production → yellow color of remaining dominant follicle → corpus luteum (yellow body)

NORMAL MENSTRUAL CYCLE

LUTEAL PHASE

- Second half of menstrual cycle
- Estrogen *and* progesterone dominated
- Premenstrual breast tenderness, bloating, affective lability are progesterone induced
- Progesterone → convert endometrium into secretory pattern (secretory phase of endometrial cycle)
  - Day 21 abundant secretion, optimal for implantation of fertilized egg
- Without pregnancy, estrogen and progesterone production from corpus luteum declines → spasm of endometrial vasculature → breakdown of endometrium → menses
- In pregnancy, embryo produces human chorionic gonadotropin (hCG)
  - Identical first 121 amino acids to LH
  - Maintains corpus luteum → no menses

FUNDAMENTAL QUESTIONS

1. What is the arcuate nucleus and what does it produce?
2. Describe the wave form of GnRH? LH?
3. What does inhibin do and where does it come from?
4. What is a dominant follicle?
5. How many follicles attempt maturation each month?
6. What is produced by the corpus luteum?
7. What is a basal body temperature and how does a patient go about measuring this?
8. Describe what happens in theca cells ? Granulosa cells?
9. When in the cycle does the LH surge occur?
10. Describe the process of mitosis, meiosis one, meiosis two.
11. At what point is there a reduction in the number of chromosomes?
12. What happens to the endometrium during the follicular phase? The secretory phase?
13. Why does the endometrium shed? What happens to the vessels underlying it?

IN SUMMARY
FIBROIDS

FIBROIDS

- Smooth muscle tumors of the uterus ,very common;
- Occur in more than one third of women over the age of 35
- Complaints include discharge, bleeding from the vagina, pain, pressure
- Circumscribed - but not truly encapsulated
- Tumor can be readily "shelled out." - is glistening gray
- Composed of interdigitating bundles of smooth muscle.
- Incidence in black women three times greater than in white women.
- Strong hormonal relationship

LOCATION

- Intramural, subserosal, and submucosal
- Subserosal and submucosal leiomyomas may become pedunculated
- Submucosal leiomyomas most important - bleeding symptoms may occur
- May occur in the cervix and broad ligaments as well
- Few mitoses are present
- Their spindle shape is readily apparent.
- When the cells are cut across the nuclei appear round
- Frequently undergo degeneration. - hyaline and cystic
- Presence of large amounts of connective tissue - known as fibromyomas or "fibroids"
- Bleeding symptoms caused in part by thinning of the overlying endometrium
- Vessels are not capable of retracting in the usual manner
- No basal zone from which the overlying thin layer of endometrium can regenerate
- Submucous, pedunculated leiomyomas may prolapse
- Necrosis in intramural leiomyomas - Only one artery supplies the leiomyoma
- May calcify or undergo "red" degeneration

ATYPICALITY

- Atypicalities occur in leiomyomas - may be confused with leiomyosarcomas
- Mitotic rate is characteristically less than 5 per 10 high-power fields
- Intravascular leiomyoma - rare tumor -nodular masses of histologically benign smooth muscle growing within veins

INCIDENCE & ETIOLOGY

- Most common solid pelvic tumors in women
- Clinically apparent in 20% to 25% of women during the reproductive years
- Pathologic inspection of the uterus - present in more than 80%
- Leiomyomas are clonal in origin
  - Classic paradigm – caused by and stimulated to grow by
  - o Estrogen
  - o Progesterone

It is now clear that the following are responsible for fibroid growth

- o Transforming growth factor-s
- o Basic fibroblast growth factor
- o Somatic mutations of genes such as HMGI-C

Fibroids are characterized by their location in the uterus

- o Subserosal leiomyomas
- o Intramural leiomyomas
- o Submucous leiomyomas

- Few leiomyomas are actually of a single "pure" type
- Most leiomyomas are hybrids that span more than one anatomic location
- Increased incidence of leiomyomas in women of color
- Risk is increased in women with greater body mass index
- Decreased in women who smoke or who have given birth

- Good epidemiological evidence to suggest that use of oral contraceptive
- Birth control pills decreases the risk for leiomyomas
- 20% and 50% of women with leiomyomas have tumor-related symptoms

- Fibroids often cause
  – Abnormal uterine bleeding
    Prolonged menstrual flow (menorrhagia)
    Submucous leiomyomas appear to be particularly prone
  – Pelvic pressure.
  – Increase in uterine size
  – Pressure of particular myomas on adjacent structures
    Colon - constipation
    Bladder - urinary frequency.
    Ureters - hydronephrosis
  – Recurrent miscarriage
  – Infertility
  – Premature labor
  – Fetal malpresentation
  – Complications of labor

DIAGNOSIS

- Easily determined by bimanual examination
  – Uterus is enlarged
  – Mobile
  – Irregular
  – Palpated abdominally above the symphysis
- Ultrasonography most common method for diagnosis
  – Submucous fibroid can be missed on traditional ultrasonography

- Magnetic resonance imaging (MRI)
  – Electron spin characteristics can often distinguish
    • Leiomyomas
    • Adenomyomas
    • Leiomyosarcomas
- Primary therapy for patients with large or symptomatic leiomyomas is surgery
- Hysterectomy is the most often
- United States: more than 175,000 hysterectomies are performed yearly for leiomyomas
  - Diagnosis of leiomyoma the most common indication for this procedure
  - Hysterectomy, the only true "cure" for leiomyoma, is a surgical option when women are no longer interested in future pregnancies
- Subtraction angiography
  - Easily visualize the fibroids and also embolize them in order to cause infarction
  - May dramatically reduce bleeding as well as size.
- Myomectomy
  - 18,000 myomectomies are performed yearly
  - Myomectomy diminishes menorrhagia in roughly 80%
  - Significant risk for recurrence of leiomyomas
  - Ultrasonography evidence of recurrence in 25% to 51% of patients
  - 10% require a second major operative procedure

- GnRH agonists (Lupron, Naferelin, Gosserelin)
  - Induce a hypo estrogenic pseudo menopausal state
  - Fibroids are dependent on estrogen for their development and growth
  - Hypo estrogenic state causes shrinkage
  - Uterine volume has been shown to decrease 40% to 60% after 3 months

- Induces amenorrhea
  – increase iron stores and hemoglobin concentrations
- Cessation of GnRH agonist treatment results in rapid re-growth
- GnRH agonist treatment is useful as a pre-surgical treatment
- Not a long-term treatment option

- Androgenic agents
  – Danazol
  – Gestrinone
- Progestins
  – Medroxyprogesterone acetate (Provera)
  – Depo medroxyprogesterone acetate (Depo-Provera)
  – Norethindrone
- Do not consistently decrease uterine or fibroid volume
- Mechanism of action is thought to be the induction of endometrial atrophy
- Often not successful in controlling significant menorrhagia
- Somatic mutation is the **initial event** in most tumorigenesis
- Somatic mutations include a variety of chromosomal aberrations

Point mutations or Chromosomal loss or gain.

- Large chromosomal abnormalities such as **translocations and deletions** often detected with standard cytogenetic karyotypes
- Independent **monoclonal origin** of individual myomas
- Suggests somatic mutations offer a **selective growth advantage** to the mutated myocyte

Variety of chromosomal rearrangements

- Most common **12q14-15 and 7q22**
- **Heterogeneity of the cytogenetic abnormalities**
- Different somatic mutations may be involved in myoma tumorigenesis
- Unique somatic mutations in individual myomas
- Biologic basis for the **differential responsiveness of individual myomas** to a variety of growth-promoting agents

SOME THOUGHTS ON THE DEVELOPMENT OF FIBROIDS

- **Clonal proliferation** precedes the development of cytogenetic rearrangements
  - Somatic mutations which **cannot be detected cytogenetically** with the light microscope are the initial events in myoma tumorigenesis.
  - Explains the **absence of cytogenetic abnormalities** in a large proportion of myoma specimens.
- ER alpha and ER beta mRNA expressed in leiomyoma and normal myometrium.
- Expression of ER alpha higher than ER beta in both leiomyoma & myometrium
- ER alpha expression is increased in leiomyoma compared to that the adjacent normal myometrium
- ER beta expression is same or lower in leiomyoma than in the adjacent normal myometrium
- Estrogen considered the **major promoter of myoma growth**
- The long-term administration of a gonadotropin-releasing hormone **(GnRH)** agonist associated with both **hypoestrogenemia** and a reduction in myoma volume
- Concentration of **estradiol significantly higher in myomas** than in normal myometrium.
- L**ower conversion of estradiol to estrone in myomas than** myometrium.

- Significantly **increased concentration of estrogen receptors in myomas** compared with autologous myometrium
- Observations suggest intramyoma hormonal milieu is **hyperestrogenic**
- No evidence that estrogen directly stimulates myoma growth
- Mitogenic effects of estrogen are likely **mediated by other factors** and their receptors.
- Several estrogen-regulated genes have been confirmed
- Evidence to suggest that **estrogen stimulates**
    - progesterone receptor
    - epidermal growth factor
    - insulin-like growth factor-I
- Regulation of myoma **extracellular matrix**
- Estrogen directly stimulates **collagen types I and III m-RNA**
- Stimulates expression of gap junction protein **connexin-43**
- Stimulates local production of the **parathyroid hormone related peptide**
- Expression of these estrogen-regulated genes appears to be **greater in uterine myomas** than in the adjacent myometrium.
- **Hypersensitivity** to estrogen may be important in the pathogenesis of myomas.
- Ultrastructural features of cultured smooth muscle cells from uterine myomas and normal myometrium
- Myoma and myometrial cells in estrogen and progesterone media more active under EM than cells in estrogen only medium.
- Progesterone exposure -> increased number of myofilaments with dense bodies (suggesting that progesterone is involved in myoma differentiation)
- Increased progesterone receptor m-RNA expression
- Increased progesterone receptor protein levels in myoma tissue compared with adjacent myometrium

FUNDAMENTAL QUESTIONS

1. What constitutes the female reproductive tract?
2. Discuss the histologic makeup of the uterus.
3. Where does the ureter lie with respect to the uterus and why is this important?
4. Describe the histology of the ovary. What cell lines are present?
5. What is a fibroid?
6. What hormones make fibroids grow?
7. Discuss the genetics of fibroids?
8. What are the clinical manifestations of fibroids?
9. How may fibroids be treated?

OVARIAN PATHOLOGY

Types of Ovarian Tumors

- *Epithelial tumors:* Serous, mucinous, endometrioid, clear cell, Brenner-transitional cell, mixed mesodermal, and undifferentiated tumors are considered epithelial tumors and account for approximately 70% of all ovarian neoplasms.
- *Stromal tumors:* Approximately 5% to 10% of all ovarian neoplasms are derived from ovarian stromal cells. These include granulosa stromal cell tumor, Sertoli stromal cell tumor, sex cord tumor with annular tubules, Leydig cell tumor, lipid cell tumor, and gynandroblastoma.
- *Germ cell tumors:* Approximately 15% to 20% of all ovarian neoplasms are of germ cell origin. This includes dysgerminoma, endodermal sinus tumor, embryonal carcinoma, polyembryoma, choriocarcinoma, teratoma, mixed forms, and gonadoblastoma.
- *Metastatic tumors:* Approximately 5% of ovarian malignancies are metastatic, most commonly from the breast or bowel.
- *Other:* A small but significant number of ovarian "neoplasms" result from ovarian soft tissue or nonneoplastic processes.

Fibrothecomas

- Benign ovarian tumors
- Thecoma component of the neoplasm gives the tumor a yellowish cast
  - Lipid content
- Can also produce estrogen.
- Arise from the ovarian stroma
- Bilateral in only about 10% of cases
- Right-sided hydrothorax in association with this tumor = Meig's Syndrome
- Presence of a benign pelvic tumor (almost always an ovarian fibroma), ascites, and a pleural effusion (usually right-sided
- Disappearance of the ascites and effusion after removal of the tumor
- Due to transudation of edema fluid from the surface of the tumor

Hemorrhagic Cyst (often corpus luteum)

- Due to hemorrhage within a normal corpus luteum
- Common event each month
- Histologically shows dark red-black hemorrhagic region surrounded by a thin rim of yellow corpus luteum
- Hemorrhage can follow torsion
- Ovary is dark and enlarged from hemorrhage following torsion
- Torsion is uncommon but may occur in adults in conjunction with benign ovarian cysts or neoplasms
- In children or infants spontaneously
- Presentation like that of acute appendicitis
- Adnexal mass may be palpable.

Ovarian surface epithelial tumors

- Tumor of ovarian surface epithelium
- Most common ovarian neoplasms
- Lined by epithelium that is serous or mucinous
- Serous cystadenoma filled with pale yellow serous fluid in only a single cavity
- Mucinous tumors are filled with sticky mucin and tend to be multiloculated
- Benign epithelial ovarian tumors are bilateral in about 20% of cases

Serous cystadenoma

- Can reach massive proportions
- May exhibit multiloculation
- The inner surface is for the most part smooth
- Solitary or occasional papillations

Papillary serous cystadenocarcinoma

- Many papillations on the inner surface
- Composed of solid tissue
- Invades outside of the ovary
- Papillations seen over the surface
- Often no early signs or symptoms with masses in the ovary
- many ovarian tumors have metastasized by the time they are detected
- Characteristically spread by "seeding" along peritoneal surfaces
- "Borderline" lesions - not clearly malignant
- Treated as though they could be
- Microscopically - papillary projections of epithelium extending into the lumen
- No invasion of the stroma or capsule

KEY ISSUES

- Effective screening for ovarian cancer in the general population is not yet possible.
- Genetic tests can be performed to detect hereditary ovarian cancer syndrome (BRCA1 and BRCA2). These tests identify patients more likely to benefit from ovarian cancer screening.
- Decreasing a patient's risk for ovarian cancer is possible through the use of oral contraceptives, prophylactic oophorectomy, and possibly tubal ligation.
- Follow-up observation for patients who have undergone surgery and chemotherapy for primary ovarian cancer includes rectovaginal pelvic examination and CA-125 testing every 3 to 4 months for the first 2 years, with a decrease in frequency in subsequent years.

Epidemiology

- Epithelial ovarian carcinoma is the second most common cancer of the female reproductive tract
- The leading cause of death from gynecologic malignancies in the United States
- Diagnosed in 25,000 to 30,000 women annually
- 15,000 die of it in that time
- annual lifetime risk for ovarian cancer in US is 1.4 per 100 women
- Wide variation in global incidence rates for ovarian carcinoma
- Highest rates are observed in Scandinavia, Israel, and North America,
- Lowest rates are observed in developing countries and Japan

Associations

- *Family History*
  - Epithelial ovarian carcinoma develops sporadically in more than 95% of patients
  - Variable most strongly associated with it is family history
  - Three hereditary syndromes
    - Site-specific ovarian cancer syndrome
    - Breast-ovarian cancer syndrome
    - Hereditary
- *Infertility and Fertility Drugs*
  - Data are less consistent
  - (1992) Nonstatistically significant increased risk among nulliparous women with clinical diagnoses of infertility
  - (1994) Increase in ovarian cancer among a cohort of infertile women
  - Both studies suggest a higher risk for women with infertility that results from an ovulation related cause

Staging of Ovarian Cancer

Stage I	Growth limited to the ovaries
Stage IA	Growth limited to one ovary; no ascites
	No tumor on the external surfaces; capsules intact
Stage IB	Growth limited to both ovaries; no ascites
	No tumor on the external surfaces; capsules intact
Stage IC	Tumor either stage IA or IB, but with tumor on surface of one or both ovaries; or with capsule ruptured; or with ascites containing malignant cells; or with positive peritoneal washings
Stage II	Growth involving one or both ovaries with pelvic extension
Stage IIA	Extension or metastases to the uterus or tubes
Stage IIB	Extension to other pelvic tissues
Stage IIC	Tumor either IIA or IIB but on the surface of one or both ovaries; or with capsule(s) ruptured; or with ascites containing malignant cells; or with positive peritoneal washings

Stage III	Tumor involving one or both ovaries with peritoneal implants outside the pelvis or positive retroperitoneal or inguinal nodes; superficial liver metastasis equals stage III; tumor is limited to the true pelvis, but there is histologically proven malignant extension to small bowel or omentum

Stage IIIA	Tumor grossly limited to the true pelvis with negative nodes but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces
Stage IIIB	Tumor involving one or both ovaries with histologically confined implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter; nodes are negative
Stage IIIC	Abdominal implants larger than 2 cm in diameter or positive retroperitoneal or inguinal nodes
Stage IV	Growth involving one or both ovaries with distant metastases; if pleural effusion is present, there must be positive cytology to categorize as stage IV

INTRAOPERATIVE FINDINGS CONSISTENT WITH INVASIVE OVARIAN MALIGNANCY

- Bilateral
- Adherent to adjacent organs
- Surface excrescences
- Ruptured capsule
- Ascites
- Peritoneal implants
- Hemorrhage and necrosis
- Solid areas
- Intracystic papillations

Time Line For Therapy

- Stage.
- Cytoreductive surgery
- Postoperative therapy
- Radiation therapy
- Intraperitoneal therapy
- Systemic chemotherapy.
- Second-look procedure
- Tumor marker CA-125.

Germ Cell Tumors

- Usually occur in children and young adults
- May not be discovered until later in life
- After the menopause in rare cases

Classification of Germ Cell Tumors

- DYSGERMINOMA
- YOLK SAC TUMOR (ENDODERMAL SINUS TUMOR)
- EMBRYONAL CARCINOMA
- POLYEMBRYOMA
- CHORIOCARCINOMA
- TERATOMAS
  - Immature
  - Mature
    - Solid
    - Cystic (dermoid cyst)
    - With secondary tumor (specify type)
  - Monodermal
- MIXED (specify types)

What is a Teratoma ?

- Composed of tissues derived from usually all three germ cell layers
- Occasional teratomas contain tissues from only two germ layers
- Rare teratomas contain tissues from only one germ layer (monodermal teratomas).
- Most common example of the latter in the ovary is struma ovarii (thyroid)
- Most common ovarian tumor
- 40% of all primary ovarian tumors
- 60% of benign forms
- Usually occur in children and young adults
- May be encountered throughout reproductive life
- Dermoid cyst, show squamous epithelium, sebaceous glands, fat, and cartilage

Complications of Dermoid Cysts

(a) Torsion that may lead to one or more of infarction, perforation, hemoperitoneum, and autoamputation

(b) bacterial infection of the cyst

(c) spontaneous perforation into the peritoneal cavity or a hollow viscus; a sudden rupture may lead to an acute abdomen, whereas a slow leak may lead to a granulomatous peritonitis that can mimic metastatic carcinoma or tuberculosis at operation

(d) hemolytic anemia that disappears after removal of the tumor.

>95% of dermoid cysts are benign, but rarely they are complicated by the development of a cancer that can arise from any component of the dermoid cyst (or a mature solid teratoma), but in most cases it is a squamous cell carcinoma that arises from the squamous lining of the cyst showing both in situ [right] and invasive [left] squamous cell carcinoma arising in a dermoid cyst). The risk of malignant transformation of a component of a dermoid cyst increases with the age of the patient, and is most likely to be found in a dermoid cyst in a postmenopausal woman. In such cases, the dermoid cyst probably arose years earlier, but did not clinically present until after the menopause. If the cancer has spread beyond the ovary at the time of its removal, the prognosis is poor.

Mixed Germ Cell Tumors (malignant)

- 10% of ovarian germ cell tumors
- Dermoid cyst, for example, may be associated with a malignant germ cell tumor Most common of such tumors is the dysgerminoma
- Ovarian analogue of the testicular seminoma
- Sheets of tumor cells resembling primordial-type germ cells
- Separated by a stroma rich in lymphocytes
- Dermoid cysts need to be examined carefully
    - Associated malignant germ cell tumor (usually in a child or young adult),
    - Cancer arising from a component of the dermoid cyst, usually squamous cell carcinoma, in an older patient.

Sex Cord Tumors

- From the sex cord-type cells in the ovary
- Granulosa cells - giving rise to granulosa cell tumors - estrogen producing
- Theca cells - giving rise to thecomas - estrogen producing
- Fibroblastic stromal cells of the ovary - giving rise to fibromas – no func.
- Rare tumors exhibit differentiation into testicular type cells - Sertoli-Leydig cell tumors - androgenic.

Metastatic tumors to ovary

- Uncommon
- Large mass and resembles a primary tumor - "Krukenberg" tumor
- Signet ring histologic pattern
- Usually is metastatic from a primary in gastrointestinal tract
- Usually GI tract (especially colon and stomach) and breast, but almost any primary tumor in the body can spread to the ovaries
- History of a previous cancer
- Bilateral ovarian involvement
- Multinodular growth pattern
- Implants of tumor on the serosal surface of the ovarian tumors
- Histologic resemblance of the ovarian tumor to the primary tumor

FUNDAMENTAL QUESTIONS

1. What ovarian tumors produce estrogen?
2. What ovarian tumors may produce testosterone?
3. Name the common epithelial ovarian tumors. Name the common stromal tumors?
4. What is a “borderline” ovarian tumor? How is it treated?
5. How can one tell a benign from a malignant ovarian tumor grossly?
6. What is CA-125? What, other than ovary produces CA-125?
7. How is ovarian cancer staged? Why does one stage tumors?
8. What is a “dermoid”? What cell lines does it contain? Are any of these cell lines ever malignant?
9. Where else beside the ovary may dermoid tumors be found?
10. Are there any medical risk to a woman who has a benign dermoid?
11. What percent of ovarian tumors are bilateral?
12. What drains the peritoneal cavity and why is this of importance surgically when treating ovarian cancer?
13. How is ovarian cancer treated?
14. Discuss the genetics of ovarian cancer? What is BRCA?

NON-HORMONAL CONTRACEPTION

HISTORY

- Ebers Papyrus (1550 BC)
- Pliny the Elder (23-79 AD)
- Dioscorides (58-64 AD)
- Soranus (100 AD)
- Al-Razi (923 AD)
- Ali ibn Abbas (994 AD)
- Avicenna (1037 AD)

Lactational Control of Fertility

- Darwin (1809-1882)
- Koran (2:223)
- "Mothers shall give suck to their offspring for two whole years if they desire to complete the term"
- Stillborn → pregnancy interval reduced
- Higher fertility rates among users of "wet nurse"
- Patterns changed around 1750' when breast feeding started to decline

Reasonable at Time

- Wiping out the vagina
- ? Reasonable but ineffective
- Honey, alum, lactic acid, pepper as pessaries and barriers
- Totally unreasonable and ineffective
- Holding breath at time of ejaculation
- Jumping backwards 7 times after coitus

Contraception

- (Population control ???)
- Infanticide
- Opiates, gin, too little food, smothering (common in 18th century)
- Abandonment
- Foundling Hospital (Coram 1741)
- 10,204/14934 infants admitted then died
- Majority were illegitimate children
- 50% of all legitimate births admitted were of mothers with 6 or more children

IUD History

- 1909 Richter used silkworm gut
- 1909 Graefenberg used silkworm gut and silver wire
- 1960 Lippes Loop, Margulies Spiral, Saf-T-Coil
- 1960's Zipper used copper devices
- Tail-less steel ring used in China by 45 million
- About 10% of contraceptors in USA
- Progesterone impregnated
- Copper coated (various shapes)
    - 10 year use

IN SUMMARY
NON-HORMONAL CONTRACEPTION

CONDOMS

Condom History

- Used for centuries
- Pregnancy protection
- Infection protection
- Stimulation
- Decoration
- 1350 BC Egyptians wore decorative covers for penis
- 1564 Fallopius described linen sheaths
- 1700's animal intestines:
- Given name "Condom": Protection from venereal disease and numerous bastard offspring
- 1798 Casanova (from his memoirs)

Barrier Methods

Dr. Condom - English physician
Charles II had many children
1800 in brothels all over Europe
English Cape
French letters
1844 Vulcanization of rubber
1850 rubber condoms in USA
1880 Diaphragm in Germany

Sponge

Real sponges used for centuries
June 1983 "TODAY" (Two Day)
2 inches long by 1.25 inches thick
Polyurethane - 1 gram nonoxynol-9
125-150 ug per 24 hours (10-15%)
Rare toxic shock
Bacteriocidal/virocidal/spermicidal
Not carcinogenic
Remove 6 hours after the last coitus

FUNDAMENTAL QUESTIONS

1. What are condoms made of?
2. Which sexually transmitted disease do the various types of condoms protect against and why?
3. What is a contraceptive sponge made of? How effective is it?
4. How does the IUD work?
5. What are the different types of IUD available?
6. What are life spans of the various types of IUD's in current use?
7. What non-contraceptive benefits does the MIRENA have?
8. What is the causative organism of "toxic shock syndrome"? What are the clinical manifestations of this syndrome?
9. Discuss the history of the IUD.

CERVICAL CAP

- Thousands of years old
- Molded opium into cup-like devices
- Placed over the cervix
- Beeswax and oiled paper - shaped into "thimbles"
- Casanova, - lime or lemon and cut it in half.
- Acid of the juice - spermicidal agent
- Skin of the fruit - cap to cover the cervix
- Modern cap **(The Prentif Cavity-Rim Cervical Cap)** - latex rubber
  most exotic of all caps were made of gold!

ADVANTAGES

- One small application of spermicide
- Less messy than the diaphragm
- more aesthetic for the user.
- Smaller than a diaphragm
- Less noticeable to either partner.
- May use with poor vaginal muscle tone.
- Assist in avoiding urinary tract infections associated with diaphragm use.
- Sturdier than a diaphragm.
- Less spermicide is used - more pleasant oral sex.
- Fewer and less serious side effects than the pill or IUD.
- Protection against some sexually transmitted diseases.
- No reports of cases of toxic shock
- Can be left in place for up to 48 hours

MAY NOT BE USED WITH

- An unusually long or short cervix precludes use
- A history of cervical lacerations or scarring.
- Current cervicitis.
- An unusually shaped or asymmetrical cervix.
- Current vaginal infections.
- Unresolved Abnormal Pap smears

PROBLEMS

- Sometimes more difficult to insert or remove than a diaphragm.
- Can be dislodged from the cervix during sex.
- Development of abnormal PAP smear

VAGINAL SPONGES

- Polyurethane material
- Impregnated with nonoxynol-9
- TODAY - effectiveness due to release of spermicide not as a barrier
- Left in place 6 hours
- Wear up to 48 hours
- 6 hours without replacement
- Collagen Sponge (traps sperm, absorbs semen, is large, grows bacteria, TSS enhancement)

IN SUMMARY
NON-HORMONAL CONTRACEPTION

FUNDAMENTAL QUESTIONS

1. What is a cervical cap ?
2. What is the contraceptive efficacy ?
3. What is the mechanism of action of the cap ?
4. What are cervical caps made of and why ?
5. What is a contraceptive sponge ?
6. What spermicide is used in the sponge ?
7. Are there any risks in using the sponge ?

FEMALE CONDOMS

ADVANTAGES

- Effective immediately
- Do not affect breastfeeding
- Do not interfere with intercourse (may be inserted up to 8 hours before)
- Can be used as backup to other methods
- No method-related health risks
- No systemic side effects
- No prescription or medical assessment necessary
- Controlled by the woman
- May provide protection against STDs
- May help prevent cervical cancer

DISADVANTAGES

- Expensive (at this time)
- Moderately effective (5-21 pregnancies per 100 women during the first year1)
- Effectiveness as contraceptives depends on willingness to follow instructions
- User-dependent (require continued motivation and use with each act of intercourse)
- Disposal of used condoms may be a problem
- Adequate storage must be available at the client's home
- Supplies must be readily available before intercourse begins
- Resupply must be available

FOR THESE

Women

- Who prefer not to use hormonal methods or cannot use them (e.g., smokers over 35 years of age)
- Who prefer not to use IUDs
- Who are breastfeeding and need contraception
- Who want protection from STDs and whose partners will not use condoms

Couples:

- Who need contraception immediately
- Who need a temporary method while awaiting another method (e.g., implants, IUD or voluntary sterilization)
- Who need a backup method
- Who have intercourse infrequently
- In which either partner has more than one sexual partner (at high risk for STDs, including HBV and HIV/AIDS), even if using another method

PROBLEMS

- Diminished sexual pleasure:
- Condom breaks or breakage suspected (before intercourse):
- Condom breaks or slips off during intercourse

FUNDAMENTAL QUESTIONS

1. Of what are female condoms made?
2. What is the efficacy of the female condom?
3. Do female condoms protect against STD's?
4. Who might be a good candidate for the female condom?

SPERMICIDES

NONOXYNOL-9

- Spermicide used in contraceptive products
- Mixture of compounds having the general formula C15H23(OCH2CH2)nOH
- Average of nine ethylene oxide groups per molecule .
  STD Protection
- Choice of contraception can affect risk for HIV
- Some contraceptives containing nonoxynol-9 (N-9) might increase the risk for HIV sexual transmission.
- Three randomized controlled trials of N-9 failed to demonstrate any protection against HIV infection
- One trial showed an increased risk
- N-9 contraceptives also failed to protect against infection with Neisseria gonorrhoeae and also Failed to protect against Chlamydia trachomatis in two randomized trials
- The 2002 STD treatment guidelines state that condoms lubricated with spermicides are no more effective than other lubricated condoms in protecting against the transmission of HIV infection and other STDs
- CDC recommends that previously purchased condoms lubricated with N-9 spermicide continue to be distributed provided the condoms have not passed their expiration date. The amount of N-9 on a spermicide-lubricated condom is small relative to the doses tested in the studies in Africa and the use of N-9--lubricated condoms is preferable to using no condom at all.

FUNDAMENTAL QUESTIONS

1. What is the most widely used spermicide worldwide?
2. How does nonoxynol-9 work?
3. Are there any deleterious effects of nonoxynol-9?

HORMONAL CONTRACEPTION

HISTORY

- In 1938, a gram of natural cortisone was worth $100 (1938 dollars!)
- For many years ethnobotanists had known that certain plants had been used by primitive peoples as fish poisons, soaps, and shampoos
- Produce foam in water and are called **saponins**, which are steroids
- Saponin destroys red blood cells on contact
- Causes the fish to be stunned and then float to the surface
- Fish are not poisonous to humans when eaten.
- Some of these saponin-bearing plants were also used by societies as oral contraceptives
- Mexico and Central America, several plants were taken to stop female ovulation
- Matto Grosso Indian women in Brazil took plant products daily to prevent pregnancy
- A company called Syntex was then organized to make steroids from Mexican yams, using the syntheses invented by Marker
- About 66 pounds of fresh yams could yield one pound of diosgenin and two ounces of cortisone
- In the 1960s, a gram of cortisone cost two dollars to produce

PHARMACOLOGY OF STEROID COMPOUNDS

- Estradiol is most potent natural estrogen
- Inactive when given orally
- Ethinyl group at 17 position made it orally active (EE)
- Older pills had used 3-methyl ester (mestranol) of ethinyl estradiol
- Mestranol will not bind to receptor and must be converted to EE
- Currently used compound is EE

PHARMACOLOGY OF STEROID COMPOUNDS

- Metabolism of EE
  - –Varies from person to person
  - –Varies with sampling time in the same person
  - –Same does may cause different side effects in different individuals
  - –Thrombosis is related to estrogen dose

PHARMACOLOGY OF STEROID COMPOUNDS

- Success of EE led to development of ethisterone (oral derivative of testosterone)
- Removal of 19 carbon from ethisterone to form norethindrone
- Changed properties from androgenic to progestogenic
- Progestational derivatives of testosterone are referred to 19-nortestosterone
- Androgenic properties not totally eliminated

PHARMACOLOGY OF STEROID COMPOUNDS

- Norethindrone can be converted to EE in small amounts
- Also has very weak binding to estrogen receptor
- Serious side effects related to high dose of progestational agents now minimal
- Another compound (Norgestrel) is racemic mixture of d- and l- norgestrel (only d-norgestrel is active)

IN SUMMARY
HORMONAL CONTRACEPTION

Northethindrone Family
- Norethindrone
- Norethynodrel
- Norethindrone acetate
- Lynestrenol
- Norgestrel
- Norgestimate
- Desogestrel
- Gestodene

Desogestrel & Norgestimate
- Two degradative steps before it expresses progestational properties
- Active metabolite is 3-keto-desogestrel
- Differs from levonogestrel by a methylene group in 11 position
- Several metabolites contribute to norgestimate activity
- Considered a second generation progestational agent because of metabolite (levonorgestrel)

Definitions of Dose
- Low-Dose Oral Contraceptives
- Product containing <50 ug EE
- First Generation Oral Contraceptives
- Products containing 50ug or more EE
- Second Generation Oral Contraceptives
- Products containing levonorgestrel, norgestimate and other norethidndrone family and 30 or 35 ug EE
- Third Generation Oral Contraceptives
- Product containing desogestrel or gestodene with 20 or 30 ug EE

Potency
- Historically used
- Different responses
  - Uterus
  - Breast
  - Liver
- Animal and human responses differ
- ***Biologic efficacy*** of various agents is the same
  - Clinical characteristics
  - Efficacy
  - Side effects
  - Risks
  - Benefits

New Progestins
- Old belief – androgenic progestins caused heart disease
  - (actually due to coagulation facilitation by estrogen)
- New Progestins
  - Desogestrel
  - Gestoden
  - Norgestimate
- All are comparable to old products
  - Cycle control, bleeding, amenorrhea
- They produce increased SHBG
  - Reduced free testosterone
  - Use for acne and hirsuitism

IN SUMMARY
HORMONAL CONTRACEPTION

Formulations

- Attempt to reduce side effects, BTB
- No real difference noted over monophasic
- 7 day pill free
- 4 days pill free
- 2 days pill free
- Estrophasic approach – low early estrogen
  - Reduced nausea initially
  - As estrogen rises the SHBG rises
  - Reduced androgenic effects

Mechanism of Action

- Combined pill is given daily for 3 weeks of 4
- Prevents ovulation
  - Pituitary
  - Hypothalamic
- Progestational effect supresses LH (no surge)
- Estrogenic effect suppresses FSH (no dominant follicle)
- Even if follicle developed there would be sufficient inhibition to prevent ovulation (minipill)
- Purpose of the estrogenic component
  - Stability to the endometrium
- Prevent irregular shedding (breakthrough bleeding)
  - Potentiates the action of progesterone
- Allowed reduction in dose of progestational agents
- Probably increases intracellular expression of P receptors
- Minimal level of estrogen is required to maintain efficacy of combined pill
  - Effect of progesterone always exceeds that of estrogen
- Endometrium (decidualized, exhausted glands)
- Cervical mucous (thick and impervious to sperm)
- Tubal function (? Reduction in motility – alteration in tubal fluid)

Efficacy of Oral Contraceptives

- Most failures occur because of delay in initiation of next cycle
  - Allow *escape ovulation*
  - Use of placebo pills to avoid “forgetting to restart” is a good idea
- Most prevalent problem associated with failure
  - Vomiting
  - Diarrhea
  - Use backup method after bout of gastroenteritis
    - Place pill in vagina
- .1% failure rate if motivated, 7.6% during first year

David Wagner - Package Designer

28-day menstrual cycle and would encourage women to view the method as “natural.”

IN SUMMARY
HORMONAL CONTRACEPTION

Benefits of OC

- Decreased cancer
- Ovarian and endometrial cancer risk decreases by 40% after 1 year of total OCP pill use and 80% reduction after 10 years of use
    - Decreased pelvic inflammatory disease
    - Decreased rheumatoid arthritis
    - Regulates and reduces menstrual bleeding
    - Decreased endometriosis
    - Decreased osteoporosis
    - Decreased anemia
    - Decreased menstrual cramps, ovulation pain & premenstrual tension
    - Decreased acne and hirsutism
    - Can adjust menses for vacations or if conditions require amenorrhea
    - No interference with coitus
    - Decreased benign ovarian tumors and cysts

FUNDAMENTAL QUESTIONS

1. How do oral contraceptives work?
2. Why is estrogen added to combined OC?
3. Name the common chemical compounds used in modern OC's?
4. List the major risks of taking OC's.
5. What are the benefits of OC's other than contraception?
6. What is the rationale for taking active OC pills for 21 days?
7. What is the effect of the combined OC on the endometrium?
8. What is the "morning after pill"?
9. What is a triphasic OC?
10. To whom would you not prescribe oral contraceptives? Why?

IN SUMMARY
HORMONAL CONTRACEPTION

PHYTOESTROGENS

Dietary Supplements
- 1994 Dietary Supplement and Health Education Act (DSHEA)
- Don't have to be proven safe
- Don't have to be proven effective
- No guarantee that drug is what the label states
- May not be removed from market
- FDA must prove it will create medical problem
- No manufacturing standards
- No processing, harvesting, packaging standards

Anatomy of Requirements
- Identity
- Net quantity of contents
- Structure-function claim
- Directions for use
- Supplemental facts panel
- Name and place of business of manufacturer

Definition of a Dietary Supplement
- Any product intended for ingestion as a supplement to the diet
- Minerals
- Vitamins
- Herbs
- Botanicals and other plant derived substances
- Amino acids
- Metabolites, constituents and extracts of these

Definition of Drug
- Diagnose
- Cure
- Mitigate
- Treat
- Prevent

Clinical studies
- Efficacy
- Safety
- Interactions
- Dosages

Nutrition Support Claim
- Describe link between nutrient and deficiency disease
    - Calcium builds strong bones
    - Antioxidants maintain cell integrity
    - Fiber maintains bowel regularity

Structure-Function Claims
- This statement has not been evaluated by the FDA
- This product is not intended to diagnose, treat, cure, or prevent any disease.
- Based on review of literature and interpretation of scientific findings
- May be used without FDA authorization
- Must be true and not be misleading

IN SUMMARY
PHYTOESTROGENS

Ginkgo Biloba

- Oldest living tree species
- 150-200 million years
- Chinese monks kept as sacred herb
- Brought to Europe in 1700's
- One of the best researched herbs in the world
- Over 300 studies showing possible benefits
    - Enhances memory
    - Vertigo
    - Tinnitus
    - Neurological and circulatory ailments
    - Mental fatigue
    - Increases circulation in brain and boosts oxygen consumption
    - Increases cardiac output

- Diseases that are treated
    - Alzheimer's
    - Lack of concentration
    - Absentmindedness
    - Confusion
    - Lack of energy
    - Depression
    - Anxiety
    - Dizziness
    - tinnitus

GINSENG

- Family Araliacae Genus Panax
- 55 genera and 700 species
- Herbs, trees and shrubs
- Tropical and temperate climates
- High conc. In American tropics and Indo-Malaysia
- Native to China
- Eastern and central North America
- Used by American Indians
- "Wonder of the World"
- Panax derived from the Greek Panakos (a panacea)
- Virtue ascribed to it by the Chinese who used it for almost anything

PHYTOESTROGENS

- Non-steroidal compounds found in plants
- Ability to bind and activate estrogen receptors
- Activity weak
- Isoflavones
- Lignans
- Coumestans

IN SUMMARY
PHYTOESTROGENS

- ***BLACK COHOSH***
- Cimifuga racemosa
- Black Snakeroot
- Bugbane
- Rattleroot
- Rattleweed
- Squawroot
- USA and Canada
- Root and rhizome – dried and not fresh
- Anti-spasmodic, emmenagogue, hypertensive
- Extract of dry rhizomes and roots of Cimicifuga recemosa (Actaea recemosa)
- Native to eastern North America
- Cimex (latin) – a kind of bug
- Fugare – "to put to flight"
- Bugbane
- Strong odor
- Placed in mattresses and pillows in Eurasia
- Cohosh – Algonquin – "rough" – lumpy rhizomes

Estrogenic Effects

- Binds directly to estrogen receptors
- Suppresses LH
- May not suppress FSH
- No advantage over commercial estrogens

Soy & Cholesterol

- NO influence of endothelial function or lipid profile
  - Soy free animals had larger testes and greater sperm development
  - Genistein reversed effect of soy free diet
  - Plasma FSH levels were proportional to spermatogenesis
- No significant effect on bone turnover
- No change in breast epithelial proliferation, E & P receptors, mitosis
- NO EFFECTS ON VAGINAL CYTOLOGY OR ENDOMETRIUM

FUNDAMENTAL QUESTIONS

1. Describe the basic types of phytoestrogens?
2. Name some commonly used estrogenic plants.
3. How effective are phytoestrogens in relieving hot flushes?
4. What is the difference between a "drug" and a "food additive"?
5. Why may some phytoestrogens preparations work in one woman and not another?
6. What are the known risks of taking phytoestrogens?
7. Describe the history of phytoestrogen use?
8. What is black cohosh? Where does it come from? Does it work?

IN SUMMARY
PLACENTATION

PLACENTA

General facts

- Mammalian organ vital for fetal life
- Acts as interface between fetal and maternal environments
- Source of pregnancy-associated hormones and growth factors
- Protects the fetal "allograft" from maternal immune system

Development of the human placenta

- The placenta forms from zygotic tissues and the first cell type to differentiate is the trophoblast
- By the end of the third week the placenta is functioning
- The human placental cell types include:
  - –Cytotrophoblast
  - –Synctiotrophoblast
  - –Intermediate trophoblast
  - –Stroma - macrophages (Hofbauer cells), fibrocytes, Wharton's jelly
  - –Vascular structure - endothelium

Implantation

- After ovulation
  - –glands become tortuous
  - –spiral arteries form
  - –endometrium thickens
- pinopods, microvilli, apical protrusions present at 20-23 days
- implantation occurs during this time
- pinopods
  - –absorb molecules and fluid from lumen
  - –increase apposition of embryo and endometrium
  - –reduce potential cavity

- Ovum captured in distal portion of tube
- Fertilization occurs
- Enters uterus 4 days after ovulation
- Cleavage occurs during these 4 days but no change in size (no cell growth) - 150um
- Inner cell mass --> embryo
- Trophoblast ---> placenta
- Cell growth then begins

- Invasion
  - –between uterine epithelium (intrusive penetration)
    - •ferret, guinea pig, rhesus monkey
  - –replace epithelial cells (displacement penetration)
    - •mouse, rat
  - –fuse with epithelial cells (fusion penetration)
    - •rabbits
- Human mode unknown but probably intrusive
- attaches to epithelium and heads for decidua
- Cytotrophoblasts fuse to form syncytium
- Penetration continues for 4 days
- Stroma undergoes changes (not just displaced)

- Implantation occurs 7-8 days after ovulation

IN SUMMARY
PLACENTATION

- Embryo “targets” hypoepithelial vessels on surface of endometrium
- Surface is made receptive for the embryo
- Immediate
  - –human
- Delayed
  - –facultative (rat, red kangaroo only during stress)
  - –facultative (Alaska fur seal, mink, bear always)
  - –free floating morula/blastocyst with min. met. act.
  - –Light cycle control duration of delay

Immature Chorionic Villi

Trophoblast cell types

- Cytotrophoblast
  - –Stem cell
  - –Mononuclear/euploid
  - –epithelial
- Intermediate trophoblast
  - –Invasive/implantation site cell
  - –Mono-multinucleate
  - –hPL>hCG
  - –proteases
- Syncytiotrophoblast
  - –Terminally differentiated
  - –Polynucleated-polyploid
  - –hCG> hPL
  - –transport

- Murine placental development
- E3.5 trophectoderm (epithelial cells) differentiates:
  - –Extraembryhonic ectoderm
  - –Extraplacental cone
  - –Trophoblast giant cells

Trophectoderm differentiation

- Control over the initial cell fate decision is unclear
- After blastocoel forms, signalling between the ICM and the trophectoderm are critical
  - –Fgf4/Fgfr2

IN SUMMARY
PLACENTATION

Early trophectoderm differentiation

- Trophectoderm spatially most distant from ICM become TGC
- ICM expresses Fgf4, trophectoderm expressed Fgfr2
- Fgf4 ko embryos are lethal YET Fgf4 -/- stem cells are viable
- Oct4 ko lack ICM but make trophectoderm if Fgf4 given
- Fgfr dominant negative constructs block trophoblast proliferation
- Trophoblast "stem cells" require Fgf4, withdrawn will differentiate to TGC

Branching

- As the trophoblast grows it branches and increases its surface area
- Molecules involved in this process are same as involved in branching morphogenesis in other organs (e.g. lung, kidney)
- Initiation of branching - Gcm1
- Branching morphogenesis - Fgf and others downstream/ signal transduction: Grb2, Gab1, Sos1 Mek1
- Wnt canonical pathway via Tcf1 and Lef1

Vasculogenesis

- Coordination of vascular pattern with villous pattern varies amongst species
  - Murine the events are simultaneous
  - In human, the villi form first then vessels "come in"
- Eptihelial-mesenchymal interactions are key
- Trophoblast factor - Esx1
- Hypoxia inducible factors important
  - Hif1b1
  - Vegf
  - Epas

Immunobiology of pregnancy

- Mother's immune system has to protect the mother and fetus against pathogens and prevent the rejection of the semi-allergeneic feto-placental unit
- Potential for repeated pregnancies with same paternity potentially triggering immune memory

Innate vs Adaptive Immunity

- Fetal alloantigens are recognized by maternal adaptive immune system
- Innate immune response during implantation involves complement activation and neutrophil infiltration

Controlled complement activation

- Complement regulatory proteins prevent overdrive of complement activation in successful pregnancies
- Complement mediated cell lysis is regulated by factors:
  - DAF/CD55
  - MCP/CD46
  - Crry

IN SUMMARY
PLACENTATION

Complement

- C3 deposition at maternal-fetal interface results in hemorrhagic necrosis and neutrophil infiltration leading to fetal resorption
- Crry -/- abort
- Crry -/- crossed with C3 -/- produce viable fetuses
- C3 deficient mothers have no fetal injury even when injected with aPL which results in antibody deposition in the decidua

T-Cell dependent inflammation inhibited by IDO

- Tryptophan is needed for cell proliferation and is lower in pregnant than in non-pregnant women
- IDO degrades tryptophan
- IDO is expressed by syncytiotrophoblast, macrophages, and antigen presenting cells
- Macrophages suppress T cell activity due to degradation of tryptophan by IDO
- Treatment with an IDO inhibitor leads to inflammation, complement deposition and hemorrhagic necrosis at fetomaternal interface

Role of HLA-G

- Nonclassical HLA class I molecule expressed in trophoblasts, amnion, and thymic epithelial cells
- 7 distinct protein isoforms each encoded by a specific alternatively spliced transcript
- 4 are membrane bound and 3 are soluble
- Inhibit NK cell mediated cytolysis and Ag specific CD8+ T cell mediated cytolysis

HLA-G and Implantation

- Normally highly expressed by invading trophoblast
- Greatly reduced in trophoblasts from spontaneous abortions and in patients with pre-eclampsia
- Zygotic expression of soluble HLA-G is required for blastocyst attachment

Adhesion molecules

- Integrins
  - cell to cell adhesion receptors
  - cell-to-cell matrix reactions
  - 21 different types
  - anchor cells to specific locations
  - transmit information to cells
- Immunologic homing
- Metastatic spread
- Healing

- –Heterodimers composed of α and β subunits
- –Ligand specificity a function of $\alpha\beta$ heterodimer
- –May bind to several ligands
- –Ligand may bind to several heterodimers

•constituitivly expressed
•phasic and hormonally regulated

- –infertility ? Related to reduced endometrial expression of β_3
- –defect in embryonic expression of β_1 causes involution of inner cell mass but preserves trophoblast
- –polarity

•Distribution of the integrins either promotes or retards attachment

- –Switching

•Early trophoblast exhibits $\alpha_5\beta_1$ but later changes to $\alpha_1\beta_1$
•May regulate invasiveness

FUNDAMENTAL QUESTIONS

1. What cell types are seen in the human placenta?
2. How does a human placenta differ from that of a dog? rabbit?
3. Describe the process of implantation?
4. How is invasion accomplished?
5. What types of trophoblast are seen?
6. Why is the placenta not rejected?
7. Describe the branching process of the placenta?
8. What is an integrin? How do integrins act in placental development?

PERINATAL PLACENTAL PATHOLOGY

Categories of Perinatal Pathology

- Infertility
- Abortions and ectopic pregnancies
- Placentas
- Gravid hysterectomies
- Fetal deaths
- Neonatal deaths
- Maternal deaths

What should one look for in the placenta?

- Relevance:
  - –Timing of stress/insult
  - –Diagnosis of specific etiologies
  - –Diagnosis of zygosity
  - –Improved management for future pregnancies
  - –Assessment of newborn risk

When should one request placental pathology

- Maternal indications
- Obstetric indications
- Pediatric indications

Basically - all placentas should receive a pathologic examination except:

–Term singletons to normal healthy moms that had prenatal care and delivered in the hospital by a certified clinician and went home with mom on the appropriate d/c day!!

What do we look for?

- What is important in the gross exam
  - –Cord insertion
  - –Color
  - –Weight
  - –Gross parenchymal lesions
- What is important in the histologic exam
  - –BOTH maternal and fetal side tissues!
- Findings with immediate prognostic implications for **infant**
  - –Congenital infections
  - –Meconium myonecrosis
  - –Fetal vasculopathy
  - –Evidence of anemia
  - –Evidence of prolonged oligohydramnios

Congenital Infections

- Acute chorioamnionitis most common
  - –Cervicovaginal flora
  - –Preterm rupture of membranes
- Transplacental infections rarer
  - – Viral

Fetal response

- Fetal response to acute chorioamnionitis includes:
  - Inflammatory cells migrating from fetal vessels
- Umbilical cord
- Chorionic plate
- "Vasculitis" is a risk factor for neurodevelopmental delay/cerebral palsy

Transplacental Infections

- Maternal sepsis
- Maternal viremia
- Histology:
  - –Chronic villitis

Chronic Villitis

- Most are non-infectious (Villitis of unclear etiology - VUE)
  - –~Host v. Graft
  - –~1/3 recur and if recur associated with ~2/3 risk of IUFD or IUGR
- Infectious causes:
  - –CMV
  - –HSV
  - –Toxoplasmosis

Congenital CMV

- Fairly common infection
- Occurs in primary or recurrent infection in Mom
- Rarely causes fetal/infant problems
  - –IUFD
  - –IUGR
  - –Congenital deafness
  - –Poor neurodevelompent

CMV Placentitis

- Chronic villitis
- Stromal expansion of villi
- Inclusions
- Hemosiderin

Congenital Listeria Infection

- Maternal ingestion of contaminated foods
- Mild maternal disease
- Often lethal fetal disease
- Treatable for mom and infant/fetus
- ACUTE villitis and acute chorioamnionitis
  - –Macro and micro abscesses

Meconium

- Common after 40 weeks NOT a sign of fetal distress
- Before 40 weeks can implicate fetal distress
- Histologic findings can be correlated with length of time of exposure
- Problems arise with:
  - –Aspiration
  - –Prolonged exposure - myonecrosis

Timing meconium exposure

- Takes AT MINIMUM 1 hour to see meconium grossly or in the amnion
- Takes AT MINIMUM 3 hours to see pigment in chorion
- Takes AT MINIMUM 6 hours to see ulceration of amnion
- Takes AT MINIMUM 12 hours to see in Wharton's jelly of umbilical cord
- Takes AT MINIMUM 18 hours to see myonecrosis

Meconium myonecrosis

- Post-dates
- Low Apgar scores
- Severe neurodevelopmental delay

Fetal Vasculopathy

- Thrombosis in fetal vessels of placenta
- Visceral infarcts
- Differential diagnosis:
  - –Heart failure
  - –Anatomic disorder of placenta
  - –Sepsis
  - –Vascular damage due to fetal inflammation
  - –Maternal diabetes
  - –Herited hypercoaguable state
  - –Meconium myonecrosis

RARE but WOW

- Metastatic malignancies
- Inborn errors of metabolism

Placental findings with prognostic implications for mother

- Villitis of unclear etiology
- Massive chronic intervillositis
- Malignancies
- Maternal floor infarct
- Decidual vasculopathy

Maternal floor infarct

- Rare disorder associated with:
  - –IUGR (24-100%)
  - –IUFD (13-50%)
  - –Cerebral palsy
  - –Recurrence (12-78%)
  - –Elevated MSAFP
- Diagnosis depends on gross and histologic exam
  - –"orange rind" like maternal floor
  - –Basal villi of entire maternal floor encased by perivillous fibrinoid of $\geq$ 3mm thick

FUNDAMENTAL QUESTIONS

1. What are the indications for placental pathologic examination?
2. What gross features are commonly looked for in a placental examination?
3. What is meconium? Why does it occur? What can the placenta tell us about meconium?
4. What are some infections that can affect the fetus and placenta in utero?
5. What is villitis? Chorioamnionitis?

TROPHOBLASTIC NEOPLASIA

Types of trophoblast neoplasia

- Molar (always gestational)
  - villi
- Non-molar (can be non-gestational)
  - No villi
- Molar gestations
  - Errors in fertilization or meiosis
  - Abnormal paternal contribution to the zygote
- Two categories:
  - Complete hydatidiform mole
  - Partial hydatidiform mole

Partial Hydatidiform Mole

- Excess tissue:
  - Occasionally large villi are grossly identifiable but should be < 1cm in greatest dimension
- Fetal development is possible with characteristic anomalies:
  - IUGR
  - 3-4 syndactyly - hands
  - 2-3 syndactyly - feet
  - Renal, cardiac, neural structural anomalies
- Histology:
  - Two populations villi - large + cisterns, small
  - Irregular outlines of villi
  - Villous syncytiotrophoblastic inclusions
  - Excess and atypical villous synctyiotrophoblast
  - “molar” implantation site
  - Embryonic/fetal development

- Characterized by focal villous hydrops
- Focal trophoblastic hyperppasia
- Two populations of villi
  - large and hydropic
  - background of small, sclerotic and normal-sized villi
  - scalloped villous outlines
  - tangential sectioning of the villi results in stromal trophoblastic inclusions
  - villous surfaces may have many tiny projections of syncytiotrophoblast forming notches
- Focal trophoblast hyperplasia
- Mounds of syncytiotrophoblast
- Nuclear atypia infrequent
- Villi vessels contain nucleated RBS and often fetal tissue found
- Triploid
- Extra haploid DNA is paternal

Complete hydatidiform mole

- Villi grossly identifiable, often >1cm in greatest dimension
- No fetal development
- Excess tissue
- Villous hydrops
- Trophoblast hyperplasia
- Cistern formation
- Blood vessels lacking
- Mounds of mitotic cytotrophoblast
- Lacy proliferation of syncitiotrophoblast
- Extravillous trophoblast
- Cytologic atypia
- Diploid
- Nuclear DNA **androgenically** derived

Molar gestations

- Complete moles - choriocarcinoma, recurrence
- Partial moles - rarely persist

Imprinting

- Molar gestations are evidence of difference between maternal and patnernal DNA
- Molar pregnancies are due to a overabundance of paternal DNA
- Paternal DNA preferentially makes extraembryonic
- Maternal DNA preferentially makes embryonic tissues

Choriocarcinoma

- Malignancy of all trophoplast lineages
- Gestational or non-gestational
- Presents with bleeding, toxemia
- Widely metastatic
- Gestational is chemosensitive
- Followed with bHCG and imaging
- Histology of choriocarcinoma
- Biphasic trophoblast
- Hemorrhage and necrosis

Choriocarcinoma in situ

- Can present as mass-like lesions in the placenta
- Can cross the placenta to metastasize to fetus
- Silent at placental presentation or widely metastatic

Placental site trophoblastic tumor

- Neoplasm of intermediate trophoblast
- Locally invasive, rare metastases
- Mild symptoms of persistent pregnancy
- No good serum markers
- Therapy is hysterectomy

Histology of PSTT

- Mono or binucleate trophoblast
- Pushing border
- Massachusetts

FUNDAMENTAL QUESTIONS

1. Define gestational trophoblastic disease.
2. Describe the histology of a complete mole. A partial mole.
3. What is the karyotypes of a complete mole ? A partial mole?
4. What is the malignant potential of the partial mole? The complete mole?
5. How does one follow a patient who has had a molar pregnancy?
6. What is the treatment for persistent trophoblastic disease?

IN SUMMARY
CERVICAL NEOPLASIA AND PAP SMEAR

CERVICAL NEOPLASIA

Appearance of Cervix

- The normal appearance of the cervix - central cervical os and the smooth, glistening epithelial surface
- The normal appearance of the cervical epithelium - orderly maturation of the basal cells along the basement membrane
- demarcation between the normal cervical mucosa - dysplastic epithelium
- koilocytotic change -human papillomavirus infection - vacuolization of epithelial cells.
- Cytologic features of dysplasia
- Increased nuclear/cytoplasmic ratio
- Darker and more irregular nuclei
- Large amount of cytoplasm and small pyknotic nuclei
- Fungating, exophytic tumor - typical for invasive squamous cell carcinoma.
- Nests of tumor cells have broken through the basement membrane - invade underlying stroma

What is a Pap Smear ?

- Exfoliated cells can be obtained from various body sites
- Many cells and tissues - constant process of maturation/death/regeneration
- Cells that die slough off or exfoliate
- Proliferation and maturation leads ultimately to exfoliation of cells
- Collect exfoliated cells - primarily from epithelial surfaces
- Mechanically enhance the exfoliation process - spatulas or brushes
- Single cells or small tissue fragments

Infections Detected by Pap Smear

- Chlamydia
- Gardnerella vaginalis
- Trichomonas vaginalis
- Neisseria gonorrhea
- Group B Streptococcus
- Candida albicans
- Herpes simplex
- Treponema pallidum (syphilis)
- Human papillomavirus

Cervical Intraepithelial Neoplasia (CIN) – Risk factors

- Sexual intercourse at a young age
- Multiple sexual partners
- Intercourse with a high risk male
- History of HPV infection

INTERPRETATION

- ASC-US (undetermined significance)
- ASC-H (cannot exclude HSIL)
- ALL ASC is suggestive of SIL
- Some cases of ASC-US may represent CIN-2 or 3

PURPOSE OF THE PAP SMEAR

- Detection of occult pathologic abnormalities of the uterine cervix in asymptomatic women
- Detection of recurrence of known pathologic abnormalities of the uterine cervix
- Evaluation of a suspected hormonal abnormality

•Monitoring of hormonal therapy

Role of HPV

- World-wide, genital warts (condylomata acuminata) is one of the most common sexually transmitted diseases
- Genital warts is one of the most common new diagnoses made at genito-urinary clinics
- Highest rates occur in men and women aged 18-28 years
- The highest rates of genital HPV infection are consistently found in sexually active women <25 years of age
- In developed countries, genital HPV infection has increased steadily since the 1950s
- About 1% of all sexually active adults (15-49 years of age) either have had or have genital warts
- Only a very small percentage of those infected with the HPV virus actually develop genital warts.
- An overall estimate is that 15% of this population (at least 20 million adults) is infected
- The prevalence of genital warts is higher in certain populations, especially those attending STD clinics.
- Global prevalence of condylomata is between 1-2% of the sexually active population 15-49 years of age.

Risk Factors Associated with Acquiring HPV Infection

- The risk of acquiring HPV infection increases with increasing numbers of partners, increasing frequency of intercourse, and having sex with infected partners
- Studies looking at the use of condoms have been inconclusive
- Similar rates of HPV infection are found in pregnant and non-pregnant women
- Highest rates of genital HPV infection are consistently found in sexually active women <25 years of age
- Initiation of sexual intercourse at an early age
- Infection with other STDs
- Increased frequency of sexual intercourse per week
- Oral contraceptives may slow disease progression in women already infected with HPV
- Correlation between smoking and malignant manifestations of HPV disease
    - Deficiency of cell-mediated immunity will create a risk factor transplant patients
    - diabetes mellitus
    - drugs such as steroids and chemotherapy
    - cancer

- More than 150 HPV types have been identified
- More than 90% of genital wart lesions examined are associated with HPV types 6 and 11
- The risk of genital tract cancer from HPV types 6, 11 or 42 - 44 is considered low or negligible
- HPV types 16 and 18 have been strongly implicated in cervical and other anogenital cancers
- 99.8% of patients who develop CIN are infected with the HPV virus

What are the signs of cervical cancer?

- Early stages of cervical cancer usually do not have any symptoms.
- Abnormal bleeding
- Bleeding after sexual intercourse - in between periods
- Heavier/longer lasting menstrual bleeding
- Bleeding after menopause
- Abnormal vaginal discharge (may be foul smelling)
- Pelvic or back pain
- Pain on urination
- Blood in the stool or urine
- Non-specific, and could represent a variety of different conditions

Staging of Cervical Cancer

- Stage IA - microscopic cancer confined to the uterus
- Stage IB - cancer visible by the naked eye confined to the uterus
- Stage II - cervical cancer invading beyond the uterus but not to the pelvic wall or lower 1/3 of the vagina
- Stage III - cervical cancer invading to the pelvic wall and/or lower 1/3 of the vagina and/or causing a non-functioning kidney
- Stage IVA - cervical cancer that invades the bladder or rectum, or extends beyond the pelvis
- Stage IVB - distant metastases

Type of Cancers

- **Cervical intraepithelial neoplasia (CIN).** This is a term used to describe abnormal changes on the surface of the cervix after biopsy. CIN — along with a number (1, 2 or 3) — describes how much of the lining of the cervix contains an abnormal growth of cells. Another term for this condition is dysplasia.

- **Carcinoma *in situ* (CIS).** This cancer involves cells on the surface of the cervix that haven't spread into deeper tissues. Treatment to remove the cancer is necessary and highly successful.

- **Cervical cancer.** Abnormal cells will eventually invade deeper tissues and may spread into blood vessels and the lymphatic system, where they can be carried to distant sites. Both squamous and glandular cancers can arise in the cervix. Most of the time, abnormal Pap tests pick up precancerous tissue that can be treated before these more dangerous diseases arise.

Treatments

- **Conization.** This is a procedure in which your doctor removes a cone-shaped piece of cervical tissue containing the abnormal area.

- **Laser surgery.** In this procedure, your doctor uses a laser to kill precancerous cells.

- **Loop electrosurgical excision procedure (LEEP).** This is a technique in which a wire loop with an electrical current running through it is used like a surgeon's knife to remove abnormalities.

- **Cryosurgery.** Your doctor may use this technique to freeze and kill precancerous cells.

- **Hysterectomy.** This is the surgical removal of precancerous areas, including the cervix and uterus

Cervical Cancer Therapy

- SURGERY
    - Radical hysterectomy
    - Pelvic lymph nodes
- RADIOTHERAPY
    - External Beam
    - Brachytherapy HDR, LDR
- CHEMOTHERAPY
    - Cisplatin
    - 5-FU
    - Hydroxyurea
    - Ifosfamide
    - Paclitaxel

FUNDAMENTAL QUESTIONS

1. What is the difference between histologic and cytologic diagnostic methods?
2. Describe the appearance of a dysplastic cell?
3. What is the role of HPV in the development of intraepithelial neoplasia?
4. Describe the HPV in terms of structure, replication and classification?
5. Discuss the epidemiology of cervical cancer?
6. How is cervical cancer staged?
7. What are risk factors for the development of cervical neoplasia?
8. How are Pap smears classified?
9. Describe the different methods of cervical cancer treatment and discuss which treatments are optimal for a given patient.

Title: Fetal Sex Differentiation – Postnatal Diagnosis and Management of Intersex Abnormalities

By: Patricia K. Donahoe, M.D.
Marshall K. Bartlett Professor of Surgery, Harvard Medical School
Director, Pediatric Surgical Research Laboratories, MassGeneral Hospital *for* Children

1. Embryonic development of the urogenital ridge
   - A. Congenital abnormalities produced by molecular defects

2. Germ cell development
   - A. Germ cell selection from embryonic stem cells
   - B. Development, migration, and homing of germ cells
   - C. Meiosis and mitosis of XX and XY germ cells
   - D. Somatic cell nuclear transfer (SCNT) using oocytes
   - E. Oocytes from embryonic stem cell lines; presidential/Melton
   - F. Male germ cell development from embryoid bodies
   - G. Epigenetic erasure (methylation of DNA; acetylation of histones)

3. Development of the reproductive tracks (Mullerian and Wolffian)
   - A. Role of Mullerian Inhibiting Substance

4. Development of external genitalia
   - A. Role of 5α reductase and dihydrotestosterone

5. Pathogenesis (molecular defects) of intersex abnormalities
   - A. Excessive androgen syndromes
     Congenital Adrenal Hyperplasia – Female pseudohermophroditism
   - B. Chromosomal abnormalities
     1. pure gonadal dysgenesis
     2. mixed gonadal dysgenesis
     3. true hermophroditism
   - C. Deficient Androgen Syndromes - Male pseudohermaphroditism
     1 testosterone deficiency
     2 androgen receptor deficiency
     3 5 Alpha reductase deficiency

6. Female reconstruction

7. Male reconstruction

SEXUAL DIFFERENTIATION

NORMAL SEXUAL DIFFERENTIATION

- Embryo has bisexual potential, each gonad can develop into either testis or ovary
- Orderly sequence needed for normal sexual differentiation of gonads, internal ductal system, and external genitalia
- Psychosocial issues in intersex disorders may have more serious ramifications than physical ambiguity and sterility → delivering child with ambiguous genitalia should be considered medical emergency
- Differences between male and female fetuses:
  - Testicular determinant genes must be present and active for testicular development *to begin*; ovarian determinant genes must be present at *later stages* → absence of testicular determinants, ovarian development will begin regardless of genetic sex of embryo
  - Male sexual differentiation depends on endocrine function of testes (testosterone, DHT, and MIS); female internal and external genital development occurs in absence of ovarian hormones
- Note: Mullerian female; Wolffian male

MALE SEXUAL DIFFERENTIATION

- Initial switch seems to be Y-chromosome SRY gene
- Primordial germ cells travel to genital ridge, become enveloped by developing seminiferous tubules
- Sertoli cells at base of seminiferous tubules
- Sertoli cells produce MIS (Mullerian inhibiting substance) → arrest of development of Mullerian system
- Leydig cells appear and produce androgens
- DHT has significantly greater potency than testosterone
- Wolffian development due to testosterone because no 5-alpha-reductase to convert to DHT
- External genital masculinization due to DHT (5-alpha-reductase present)
- Competent androgen receptor needed for masculinization
- Mullerian and Wolffian systems are local and unilateral
  - Remove one testis, that side develops Mullerian
- External genital development begins week 9, completed in early middle trimester
- Testicular descent week 32, gubernaculums “pulls” testis toward scrotum

FEMALE SEXUAL DIFFERENTIATION

- Ovarian development begins in absence of testicular or ovarian determinant genes
- Mullerian development begins and continues unless arrested by MIS
- External genitalia will feminize in absence of androgens
- Ovaries contain 6 million germ cells by 20 weeks

DELETION SYNDROMES
- Turner's syndrome: X-chromosome deletion, 45X
- Missing important ovarian determinant genes necessary for preservation of ovarian follicles
- Bilateral streak gonads
- Normal Mullerian and external genital development
- Associated with coarctation of aorta and horseshoe kidney
- 46XY gonadal dysgenesis (Swyer's syndrome):
  - phenotypically normal female, delayed puberty, taller

MULLERIAN INHIBITING SUBSTANCE DEFICIENCY
- Testicular development and androgen production otherwise normal, normal penile development
- Phenotypic males with uni- or bilateral cryptorchidism, testis contains Mullerian elements
- Sterility may result
- Agonadia (testicular regression syndrome):
  - 46XY, phenotypic female, normal MIS
  - blind vaginal pouch, absent internal genitalia and gonads = "empty pelvis syndrome"
  - defect in sexual differentiation after MIS elaboration, androgens not produced
  - likely environmental insult or vascular accident

DEFECTS IN ANDROGEN BIOSYNTHESIS
- 46XY, normal TDF (testicular determining factor) genes and MIS expression → normal testes, Mullerian regression
- Steroid enzyme deficiency → suboptimal testosterone or DHT
  - Wolffian system development stimulated by testosterone
  - External genitalia depends on 5a-reductase and androgen receptor stimulation by DHT
  - → under masculinization and ambiguity
- Sex of rearing usually female
- 5a-reductase deficiency: female sex of rearing at birth, then masculinization at puberty because of high testosterone
- 17a-hydroxylase deficiency may develop gynecomastia because of conversion of weak androgens to estrogen

ANDROGEN INSENSITIVITY SYNDROME
- Normal TDF genes, testicular development, MIS production, Mullerian regression, testicular androgen biosynthesis
- Androgen receptor defect prevent end-organ masculinization in utero and at puberty
- Mutation of X-located androgen receptor gene
- Resistance to androgens → absent masculinization, vaginal pouch, spontaneous breast development at puberty, diminished pubic hair
- Large breasts because no androgens to limit breast size
- Tall stature

TRUE HERMAPHRODITISM
- Usually 46XX, less frequency 46XX/46XY (chimera), rarely 46XY
- Usually unilateral ovary and contralateral testes
- Chimera has two or more cells lines originating from different embryos
- Ovarian component more developed
- External genitalia under masculinized, breast development and menstruation common, should be raised as female

SEX-REVERSED MALE
- 46XX with Y DNA sequences translocated to X chromosome

- Translocation of SKY, not genes controlling sperm production
- Male phenotype, infertile

CONGENITAL ADRENAL HYPERPLASIA

- Most common and serious abnormality of sexual differentiation, seen in 46XX
- Deficiency of 21-hydroxylase or (less commonly) 11b-hydroxylase or 3b-hydroxysteroid dehydrogenase
- Cortisol production limited → ACTH overproduction → accumulation of cortisol precursor and androgens
- High fetal adrenal androgens adversely influence external genitalia in female but not male fetuses → virilization
- Ranges from clitoromegaly and mild labioscrotal fusion through hypospadias
- Bilateral undescended gonads with ambiguous penile development, think CAH
- Tall children, short adults because of premature epiphyseal fusion
- Skin hyperpigmentation because of high melanocyte stimulating hormone (MSH)
- 21-OH deficiency:
  - Salt wasting and simple virilization
  - Males infants more likely to be undiagnosed and develop salt-wasting crisis than females, because normal phenotype at birth

ANDROGEN EXCESS SYNDROME

- In utero exposure to endogenous maternal or exogenous androgens can cause masculinization of the external genitalia
- Placenta can aromatize native androgens, except DHT, to estrogen and prevent masculinization
- Luteoma of pregnancy: androgen producing tumor

IDENTIFICATION AND EVALUATION OF INTERSEX DISORDERS

- Sex of rearing = label of male or female given at birth
- Gender identity = one's own feelings of sexuality
- Ambiguous genitalia caused by intersex disorder may include: minimal clitoromegaly, isolated cryptorchidism, hypospadias, overt ambiguity
- If gender is in question, best to withhold decision on sex of rearing
- Can say "sexual development of this infant is incomplete; after a few studies it will be easy to determine whether the baby is a boy or girl"

FUNDAMENTAL QUESTIONS

1. How does the genital tract of a female fetus develop?
2. How does the genital tract of a male fetus develop?
3. What is the role of MIS?
4. What is the role of SRY?
5. How does mullerian agenesis occur?
6. What is a pseudohermaphrodite?
7. What is the role DHT in male development?
8. What is the androgen insensitivity syndrome?
9. What can congenital adrenal hyperplasia cause? Why?
10. What is the workup for CAH?
11. What is a true hermaphrodite? A pseudohermaphrodite?
12. What is androgen excess syndrome?

IN SUMMARY
SEXUAL DIFFERENTIATION

IN SUMMARY
SEXUAL DIFFERENTIATION

INFERTILITY

Defined as 1 year of unprotected intercourse during which a pregnancy is not achieved

- United States - 15% to 20% of all couples are infertile
- Higher in older couples
- Rate has remained relatively stable since the early 1980s
- Primary - neither partner has achieved a successful pregnancy
- Secondary - couples who have achieved a pregnancy previously but are having difficulty currently with conception

Estimated 25% of women will experience infertility during their childbearing years

- Societal trend toward delayed marriage and childbirth
- Greater awareness of treatment options
- Increased societal acceptance of infertility as a medical condition

Fecundability is the probability of achieving a pregnancy within 1 menstrual cycle

- Fecundability of a “normal” couple (<35 years) is approximately 25%
- 3 months, 57% of couples achieved pregnancy
- 6 months, 72%
- 12 months, 85%
- 24 months, 93%

Initial assessment - thorough patient history and physical examination
Evaluate for evidence of systemic disease, genetic abnormalities, or androgen dysfunction.

Female partner - specific areas requiring extra attention

- Body mass index
- Distribution of body fat
- Breast formation and galactorrhea,
- Hair pattern (hirsutism or virilization)
- Focal neurologic findings (anosmia, visual field impairments)
- Assessment of the pelvis
    - Anomalies of the external genitalia, vagina, or cervix
    - Should include a rectovaginal examination for endometriosis of the
    - uterosacral ligaments and an assessment of the uterus and adnexa

The causes of infertility include abnormalities of any portion of the male or female reproductive system.

- Specific cause can be identified in approximately 80% of couples
- Normal menstrual cycles likely to be ovulatory
- 21 to 35 days
- Associated with symptoms consistent with the premenstrual syndrome

Male partner

- Hair pattern (degree of virilization)
- Breast abnormalities (gynecomastia and/or discharge)
- Focal neurologic findings (anosmia, visual field impairments)
- Assessment of the genitalia.
    - Size and location for the urethral meatus and prostate
    - Bilaterally descended testes of normal size (≥4 cm in the long axis or ≥20 mL volume)
    - Presence of a varicocele

- Frequency of intercourse (every 1 to 2 days around the time of expected ovulation)
- Risk of using commercially available lubricants (spermotoxic)
- Rationale for subsequent laboratory testing
    - Complete blood cell count
    - Urinalysis
    - Screening for sexually transmitted diseases
    - Sensitive thyrotropin
    - Semen analysis
        - Oligospermia or azoospermia
        - Disorders of sperm function or motility (asthenospermia)
        - Abnormalities of sperm morphology (teratospermia)

Chromosomal abnormalities in men - approximately 7%

- Klinefelter syndrome the most common

Male infertility is caused most frequently by a varicocele

- Palpable varicoceles have a more notable effect on fertility
- 13% of men with nonobstructive azoospermia have been shown to have Y chromosome microdeletions
- Congenital bilateral absence of the vas deferens - approximate 70% chance of being carriers of cystic fibrosis mutations
- High levels of reactive oxygen species (oxidants) may affect sperm quality and function

IN SUMMARY
INFERTILITY

Medical history and review of systems	General health and erectile function, degree of virilization, history of sexually transmitted diseases, testicular infections (such as mumps), genital trauma, or undescended testicle, pubertal development, history of exposure to high temperatures (hot baths, construction work sites) or recent fever, current medical problems, vaccinations, allergies, and use of certain prescription antihypertensives (ie, calcium channel blockers)
Surgical history	History of hernia, testicular, or varicocele surgery
Sexual history	Previously fathered a pregnancy, history of contraception use and infertility in a previous relationship, and excessive use of lubricants.
Social history	Use of alcohol, tobacco, caffeine, recreational drugs; exposure to chemotherapy or radiation
Family history of genetic diseases	Ancestry-based genetic diseases, eg, cystic fibrosis, sickle cell disease, Tay-Sachs disease, and thalassemia; family history of male infertility

Female Evaluation

- Complete blood cell count
- Urinalysis
- Screening for sexually transmitted diseases (as indicted by risk factors in the medical history)
- Confirmation of rubella and varicella immunity
- Papanicolaou smear
- Ovarian Reserve Testing (Aging woman)
    - Decline in fertility rates - an increase in women's age
    - Spontaneous abortion
    - Chromosomal nondisjunction
    - Associated aneuploidy
    - Serum FSH and estradiol test is performed on day 3 of the menstrual cycle
        - 10 IU/L - referred to a reproductive endocrinologist

Medical history and review of systems	Abnormal hair growth, weight gain, breast discharge, hypothyroid or hyperthyroid symptoms, history of diabetes mellitus or current symptoms, current medical problems and medications, vaccinations, allergies
Surgical history	Fallopian tube surgery, ectopic pregnancy, appendectomy or other pelvic surgery
Menstrual cycle and developmental history	Menarche, breast development, dysmenorrhea, history of sexually transmitted diseases, use of prior contraception, history of diethylstilbestrol (DES) exposure, and history of abnormal results from Papanicolaou smear and subsequent treatment
Sexual history	Frequency of sexual intercourse, timing of intercourse with basal body temperature charting or ovulation predictor kits, dyspareunia, use of lubricants
Infertility history	History of infertility treatment in pregnancy, duration of current infertility
Social history	Use of alcohol, tobacco, caffeine, recreational drugs; exposure to chemotherapy or radiation; exercise; excessive stress
Family history of genetic diseases	Ancestry-based genetic diseases, e.g. cystic fibrosis, sickle cell disease, Tay-Sachs disease and thalassemia

Tubal and Pelvic Pathology

- Patency of the fallopian tubes
  - Hysterosalpingography (HSG)
  - Injection of dye into the uterus via an intracervical catheter
  - Dye outlines the endometrial cavity and fallopian tubes
  - Eventually spills freely into the peritoneal cavity
  - Laparoscopy with intraoperative injection of tubal dye
- Hysteroscopy
- Cervical Mucus Factors
- Postcoital test shown to be of limited value
- Cervical surgeries
- Cryotherapy
- Loop electrosurgical excision
- Consideration of treatment by intrauterine insemination (IUI)

Ovulatory Dysfunction

1. Anovulation or inconsistent ovulation
2. History of irregular menses (<21 days or >35 days)
3. Nonbiphasic basal body temperature pattern
4. Absence of a luteal phase elevation of temperature of approximately 1degree F for at least 10 days
    a. Observation of several cycles is recommended
    b. Confirmation by abnormally low serum progesterone levels
    c. <5 ng/mL during the midluteal phase
    d. 7 days before anticipated menses

Unexplained Infertility

- Intrauterine insemination (IUI)
    - Reasonably free of hazards.
- Clomiphene Citrate
    - Multiple pregnancy in 8% to 10% of cases and ovarian cysts in 5% to 10% are the most common
    - Clomiphene use may be associated with increased ovarian cancer risk after 12 cycles of use
- Clomiphene Citrate and IUI
- Gonadotropin therapy
    - Multiple pregnancy
    - Ovarian hyperstimulation syndrome
- Assisted reproductive technology (IVF, GIFT, etc)
    - Multiple gestation pregnancy
    - Ovarian hyperstimulation
    - Increased pregnancy losses

IN SUMMARY
INFERTILITY

Figure removed due to copyright restrictions. Please see:
Basal Body Temperature (BBT) Chart in Frey, K. A., and K. S. Patel. "Initial Evaluation and Management of Infertility by the Primary Care Physician." *Mayo Clin Proc.* 79, no. 11 (November, 2004): 1439-43, quiz 1443.

FUNDAMENTAL QUESTIONS

1. What is a basal body temperature chart and why does the temperature change?
2. What is the definition of infertility?
3. List 10 causes of primary or secondary infertility in women.
4. Describe how a hysterosalpingogram? Is performed.
5. What is Clomiphene citrate and how does it work?
6. List 8 causes of infertility in males.
7. What is a varicocele? How does it cause oligspermia?
8. What occurs during an IVF cycle?
9. How does ovarian hyperstimulation occur? What are the risks?

MATERNAL PHYSIOLOGY

Cardiovascular System

- Heart size increases 12%
- Murmurs (systolic and diastolic)
- ECG changes similar to ischemia but due to positional changes
- Extrasystoles, supraventricular tachycardia
- Output rises 1.5 liters/minute
- Heart rate rises from 70 to 85 beats per minute

- Stroke volume rises from 63 to 70 ml
- A-V oxygen difference drops near end of first trimester from 44 to 33 ml/l but then rises again
- Blood pressure
  - Systolic unchanged
  - Diastolic drops in mid-pregnancy
  - Increases again near term

- Pulse pressure higher
- Venous pressure unchanged in the arms and raised in the legs
- Peripheral resistance drops
- Pulmonary pressure unchanged
- Circulation time unchanged

Figure removed due to copyright restrictions. Please see:

Elkayam, Uri, and Norbert Gleicher, eds. *Cardiac Problems in Pregnancy : Diagnosis and Management of Maternal and Fetal Disease.* 2nd ed. New York, NY: Liss, 1990, p. 61. ISBN: 0471505005.

Figure removed due to copyright restrictions.
[bar graph of Cardiac Output (L/min) vs Weeks Gestation]

- Cardiac output +43%
- SVR -21%
- PVR -34%
- HR +17%
- Stroke index +17%
- MAP -4%
- Osmotic pressure -14%

Pulmonary

Figure removed due to copyright restrictions.
[graph showing Lung Volume Compartments corresponding to Months Pregnant]

- Inspiratory capacity goes up
- Vital capacity unchanged
- Functional residual capacity goes down
- Mother notes subjective dyspnea as pregnancy advances
- Seen as early as 12 weeks
- Progesterone effect

Glucose Metabolism

- Mean blood glucose drops
- Basal and total insulin goes up
- Daily glucose excretion goes up due to elevated GFR and fixed rate of reabsorption
- Oral 100 gram glucose tolerance test or 50 gram 1 hour glucose challenge test
- Hemoglobin A1c is good measure of long term control
- A1c related to incidence of congenital malformations
- Malformations
    - Cardiac
    - Gastrointestinal
    - Genitourinary
    - Central nervous system

Laboratory parameter effects of pregnancy

- Lower hematocrit (although elevated red cell mass)
- Creatinine clearance rises (creatinine falls)
- BUN falls
- Plasma volume goes up
- Serum sodium falls
- Estrogen levels (all three estrogens) rise
- LH, FSH fall
- Prolactin rises
- TSH unchanged

FUNDAMENTAL QUESTIONS

1. Describe the changes in cardiovascular parameters in pregnancy?
2. What changes in pulmonary function are to be expected? Why do these occur?
3. Describe the changes in renal function as pregnancy progresses.
4. What happens to serum glucose and insulin as pregnancy advances?
5. What risks does diabetes confer on the fetus? Why?
6. What normally happens to the hematocrit and plasma volume in pregnancy?
7. What laboratory parameters rise and which ones fall in a normal pregnancy?
8. What is the most optimal physiologic position for a woman to labor?
9. Describe the changes in plasma glucose and insulin as pregnancy advances.
10. What happens to serum binding proteins in pregnancy? Why?

IN SUMMARY
PREGNANCY INDUCED HYPERTENSION

Classification of Hypertensive Disorders of Pregnancy

- Gestational hypertension (6-7%)
  - Onset of HTN without proteinuria after 20wks of gestation with resolution to baseline by 12wks postpartum
- Preeclampsia (5-8%)
  - Hypertension plus proteinuria
  - 140/90 on two occasions six hours apart
  - 0.3 gm/dl in 24hrs or 1+ on urine analysis

- Chronic hypertension (3-5%)
  - HTN prior to pregnancy
  - Gestational HTN which does not resolve within 12 wks of delivery
- Superimposed preeclampsia (25% of CHTN)
  - Chronic HTN plus new onset proteinuria or other signs or symptoms of preeclampsia

ACOG Jan. 2002

Figure removed due to copyright restrictions. Please see:

Berg, C. J., H. K. Atrash, L. M. Koonin, and M. Tucker. "Pregnancy-Related Mortality in the United States, 1987-1990." *Obstet Gynecol*. 88, no. 2 (August, 1996): 161-7.

Classification of Preeclampsia

- Mild preeclampsia
  - BP 140/90
  - 300mg of proteinuria in 24hrs
- Severe preeclampsia (any of these)
  - BP 160/110
  - 5gm of proteinuria in 24hrs
  - Oliguria or <500 ml in 24hrs
  - Cerebral of visual disturbances
  - Pulmonary edema or cyanosis
  - RUQ tenderness
  - Fetal growth restriction
  - Thrombocytopenia
  - Impaired liver function
- Eclampsia
  - Presence of new-onset grand mal seizures in a woman with preeclampsia

ACOG Jan. 2002

RISK FACTORS

Nulliparity	3:1
Age >40 y.o.	3:1
African-American race	1.5:1
Family history	5:1
Chronic Renal disease	20:1
Chronic hypertension	10:1
Antiphospholipid syndrome	10:1
Diabetes mellitus	2:1
Twin gestation	4:1
High body mass index	3:1
Angiotensiongen gene T235	
Homozygous	20;1
Heterozygous	4:1

Adopted from ACOG Technical Bulletin 219, Washington, DC 1996

Measurement of Blood Pressure

- Comfortable sitting position
- Korotkoff V th sound should be used
- If the V th sound is not present, use the IV th but should note as such
- Some automated BP cuffs use the IV th sound
- In serial readings use the higher set of values
- Relative BP change of 30mmHg/15mmHg is no longer used as hypertension

Clinical Predictors of Eclampsia

- 254 Women with Eclampsia
- No edema: 80 (32%)
- No HTN: 58(23%)
- No proteinuia: 49 (19%)

Sibai 1990

- 383 Eclampsia collected from 279 Hospitals in UK
  - ✓ No proteinuria: 71 (22%)
  - ✓ No proteinuria or HTN: 36 (11%)
  - ✓ No hypertension: 32 (10%)
  - ✓ Headaches: 188 (50%)
  - ✓ Visual disturbance: 72 (19%)
  - ✓ Epigastric pain: 71 (19%)

Douglass, 1994

"A disease of theories that have not stood the test of time"
1000 BC: First description of eclampsia is found in Kahun papyrus from Egypt
1800: An association between onset of hypertension and proteinuria and seizure during pregnancy was recognized
1950: A distinction between primary renal disease, chronic hypertension, epilepsy and preeclampsia/eclampsia became widely accepted

Figure removed due to copyright restrictions.

EVIDENCE FOR TROPHOBLASTIC INVASION

Clinical

- Predisposing factors include
  - Vascular disease (SLE, diabetes, chronic HBP))
  - Multiple gestation
  - Hydatidiform mole

Animal Studies

- Most successful models have induced utero-placental ischemia in rabbits, dogs and primates

Human Studies

- Histopathology of the placental bed
- Doppler of uterine artery

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Figure removed due to copyright restrictions.

Adhesion Molecules Expressed by Cytotrophoblasts

Villous Cytos	Invasive Cytos
α6/β4	α1/β1
E-cadherin	αv/β3, β6
	VE-cadherins
	PECAM
	VCAM

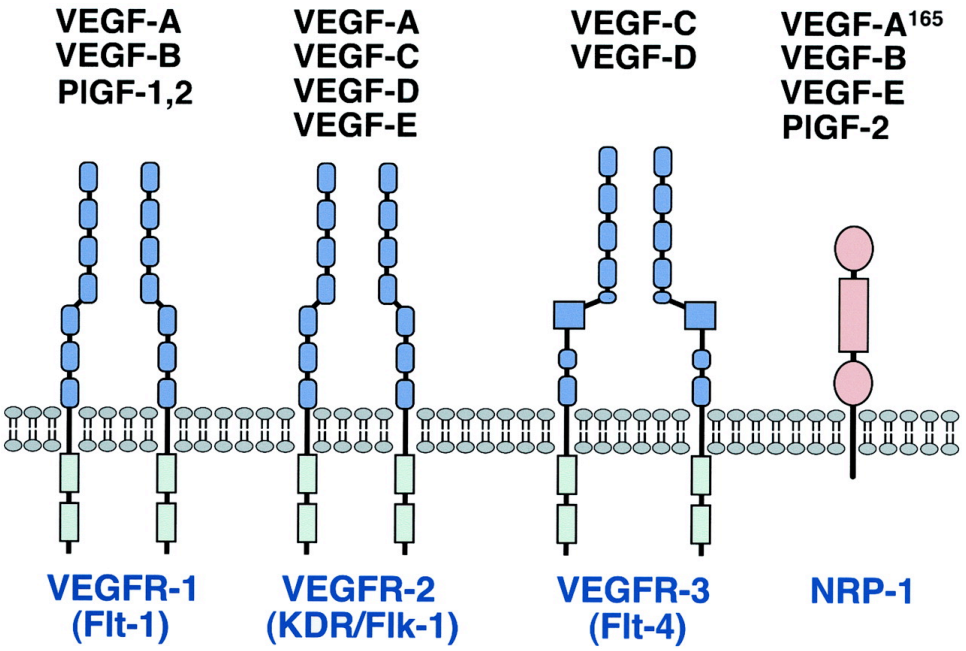
Invasive Cytotrophoblasts in Preeclampsia (Lim et al Am J Path 152 (6) 1997

Figures removed due to copyright restrictions. Please see:

Lim, K. H., Y. Zhou, M. Janatpour, M. McMaster, K. Bass, S. H. Chun, and S. J. Fisher. "Human Cytotrophoblast Differentiation/Invasion is Abnormal in Pre-eclampsia." *Am J Pathol*. 151, no. 6 (Dec 1997): 1809-18.

CIRCULATING TOXIC FACTORS

- Cytokines
- IL-1
- IL-6
- TNF-alpha
- Free fatty acid
- Antioxidants
- Angiogenic factors



VEGF

- Promotes angiogenesis
- Induces nitric oxide and prostacyclin
- Glomerular healing

Anti-VEGF

- Increases apoptosis
- Impairs glomerular capillary repair
- Increases proteinuria in rat model of messangio-proliferative nephritis
- Increases proteinuria in experimental thrombotic microangiopathy

Figure removed due to copyright restrictions.
[Structure of sVEGFR-1 (sFlt-1).]

First described by Kendall et al.(1993) and has been localized to trophoblast by Clark et al.1998.
Has been shown to be produced in greater amount in trophoblasts isolated from preeclampsia (Zhou et al. 2002)
Its role in distal organs and in various pathologic state is unknown
Hypoxia may increase its production in trophoblast

Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia

See the related Commentary beginning on page 600.

Sharon E. Maynard,[1,2] Jiang-Yong Min,[1,2] Jaime Merchan,[1,2] Kee-Hak Lim,[2,3] Jianyi Li,[2,4] Susanta Mondal,[1,2] Towia A. Libermann,[1,2] James P. Morgan,[1,2] Frank W. Sellke,[2,4] Isaac E. Stillman,[2,5] Franklin H. Epstein,[1,2] Vikas P. Sukhatme,[1,2] and S. Ananth Karumanchi[1,2]

[1]Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA
[2]Harvard Medical School, Boston, Massachusetts, USA
[3]Department of Obstetrics and Gynecology,
[4]Department of Surgery, and
[5]Department of Pathology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA

See the related articles beginning on pages 649 and 707.

Soluble VEGF receptor Flt1: the elusive preeclampsia factor discovered?

Aernout Luttun and Peter Carmeliet

The Center for Transgene Technology and Gene Therapy, Flanders Interuniversitary Institute for Biotechnology, Katholieke Universiteit Leuven, Leuven, Belgium

J. Clin. Invest. 111:600–602 (2003). doi:10.1172/JCI200318015.

Figure removed due to copyright restrictions. Please see:
Figure 1 in Luttun, A., and P. Carmeliet. "Soluble VEGF receptor Flt1: The Elusive Preeclampsia Factor Discovered?" *J Clin Invest*. 111, no. 5 (March, 2003): 600-2.

Serum Levels of sFlt-1 Prior to onset of Preeclampsia

Figures removed due to copyright restrictions.

Serum Levels of PIGF Prior to onset of Preeclampsia

Figures removed due to copyright restrictions.

Parenteral Antihypertensive Agents in Pregnancy

Drug	Dose	Onset	Duration	Side Effects
Hydralazine	**5-10 mg IV**	**10-20 min**	**3-6 hrs**	**Tachycardia Headache, flushing, angina**
Labetalol	**20-80 mg IV**	**5-10 min**	**3-6 hrs**	**Scalp tingling, vomiting, heart block**
Sodium Nitroprusside	**.25-10 ug/kg/min**	**Immediate**	**1-2 min**	**N/V, thiocyanate toxicity**
Nicardpine	**5015 mg/h IV**	**5-10 min**	**1-4 hrs**	**Tachycardia,headaches, phlebitis**

Magnesium Sulfate

- Seizure Prophylaxis
  - Intravenous
    - Loading dose: 4-6 g in 100ml over 15-20 min
    - Maintenance: 2 g per hr. (target 4-8 mEq/L
  - Intramuscular
    - Loading dose: 10 g $MgSO_4$ as 50% sol
    - For severe PE and eclampsia: 4g in 20% solution at 1g/hr (IV)
    - Maintenance: 5g $MgSO_4$ in 50% sol. Q4hr

Eclampsia

- Reversible posterior leukoencephalopathy
  - Renal insufficiency and hypertension
  - Immunosuppressive therapy
  - Eclampsia
  - Subcortical edema in the posterior circulation
    - Presents with blurred vision, cortical blindness, headaches, vomitting, confusion and seizures
    - Resolution of neurologic deficits in 2wks
    - Anterior circulation may be more protective
- MRI findings
    - Cerebral ischemia
    - Cerebral edema
    - Local hemorrhagic infarcts
- Pathophysiology
    - Local cerebral vasoconstriction--cytotoxic edema
    - Loss of autoregulation--over perfusion -- vasogenic cerebral edema

Pulmonary Edema

- Cardiogenic
  - Systolic dysfunction
  - Diastolic dysfunction
  - Combined
- Noncardiogenic
  - Increased capillary permeability
  - Narrowed COP-wedge pressure gradient
    - Decreased COP
    - Delayed mobilization of extravascular fluid
    - Iatrogenic fluid overload

FUNDAMENTAL QUESTIONS

1. What happens to blood pressure as pregnancy advances?
2. What happens to blood volume in pre-eclampsia?
3. When does one typically develop pre-eclampsia?
4. List 5 risk factors for pre-eclampsia?
5. What are the clinical manifestaton of pre-eclampsia?
6. What happens in eclampsia? What are the changes one may see in the CNS?
7. What is VGEF?
8. What is sFlt1? What role might it play in pre-eclampsia?
9. Describe the alterations in trophoblastic invasion in pre-eclampsia.
10. What is the most effective therapy for pre-eclampsia?
11. Name some drugs used to control blood pressure in pre-eclampsia.

IN SUMMARY
OVARIAN FAILURE

OVARIAN FAILURE

Possible Menstrual Changes During the Perimenopause

- *Cycle shorter than 28 days*
- *Bleeding for more days than usual*
- *Bleeding for fewer days than usual*
- *Heavier bleeding*
- *Lighter bleeding*
- *Skipped periods*

Causes of Ovarian failure

Genetic factors	- e.g. micro deletions X-chromosome, mosaic 45X0/46XX
	- e.g. mutation in FSH receptor gene
Viral factors -	e.g. mumps
Iatrogenic factors	- surgery (e.g. oophorectomy, hysterectomy)
	- chemotherapy (e.g. for breast cancer, lymphoma)
	- radiotherapy (e.g. for cervix cancer, Hodgkin's)
Life style factors	- e.g. cigarette smoking, vegetarian diet
Other factors	- e.g. autoimmune diseases (myasthenia gravis)
	e.g. low body weight

Menopause

Three phases over about 10 years

- *Perimenopause (mid forties)*
  - Irregular cycles
  - Hot flushes
  - Mood changes
  - Sleep disturbance
- *Menopause*
  - 12 months without a period
  - Not pregnant
  - Age 48-55
- *Postmenopause*
  - No periods

- Onset of menopause determined by the ovary
- Other functional body changes secondary
- Loss of the capacity of the ovary to sustain ovulation
- Nearly complete loss of ovarian follicles
- Minimum 1000 follicles has to be present for ovulation
- Birth - a few million primordial follicles are present
- Menarche - around 250,000 follicles

- 500 will reach the stage of a Graafian follicle
- Process of apoptosis and atresia
- 38 years - disappearance of follicles becomes accelerated
- Mid-thirties - the duration of the menstrual cycle gradually declines
- 4 to 6 years before menopause - women start to notice changes in their menstrual cycle
- Accompanied by
  - night sweats
  - hot flushes
  - vaginal dryness

- Ovulatory cycle remains intact until the mid-forties,
- 5 years before menopause - three-quarters of all women, mean cycle length Gradually increases from 28 days (range 26 - 32 days) to 60 days (range 35 - >100 days)
- Hormone levels may fluctuate - highly variable between cycles
- Estradiol tends to stay within the normal fertile range (400 - 600 pmol/L)
- Levels below 200 pmol/L at one year after menopause

- Postmenopausally - non-ovarian tissues
  - fat
  - liver
  - kidney

- Peripheral conversion of androgens
- Obese postmenopausal women
  - Higher circulating estradiol
  - Less oestrogen bound to SHBG

- Estrone may rise
- Secretion of androgen by the ovary is reduced
- Decline of peripheral androgen levels by 20 - 40 percent
- Increased androgen to estrogen ratio
  - Androgen-associated facial hair pattern
  - Deepening of the voice

The hypothalamic-pituitary-ovarian axis

- Growing hypothalamic-pituitary stimulation
- Early follicular phase FSH levels (cycle day 3 FSH)
  - Rise typically 10 years before the menopause

- Ovaries become also progressively less responsive to exogenous gonadotrophins
- Refractory to stimulation with exogenous gonadotrophins

- Secretion of FSH is influenced
    - Estradiol
    - Inhibin
        - Products of the ovarian granulosa cells
- Both suppress the pituitary secretion of FSH
- LH serum levels remain unchanged

Symptoms of Menopause

- *Hot flush*
  - *30-80 percent of postmenopausal women*
  - *Sudden sensation of heat rising to the top of one's body*
  - *Shiver at conclusion*
  - *Disruptive to sleep patterns*
  - *Cross-cultural differences*

May last from 1 month to 7 years

What's Hot Flush Really About ?

- *75% of women experience hot flushes*
- *10-15% seek physician help for this*
- *Highest during first 2 years*
- *85% for more than 1 year*
- *20-50% for up to 5 years*
- *Oophorectomy flashes are more severe and frequent*
- *2/3 premenopausal women*
- *May continue to have periods*

Description of Hot Flush

- *Visible redness of upper chest, neck, face*
- *Perspiration in that area*
- *Finger temperature rises p to 6 degrees C.*
- *Temperature drops after sweating*
- *Night sweats*
  - *More severe*
  - *Awaken from sleep*
    - Fatigue, irritability, inability to concentrate, impaired memory

Awakened *before* temperature changes

Ovarian function reduced

- *6AM to 8 AM & 6 PM to 10 PM*
- *Most women have one a day*
- *Some have many or a few as one weekly*
- *Triggers*
  - *Stress*
  - *Warm weather*
  - *Hot drinks, alcohol*
  - *Aura*
    - Anxiety attack
    - *Uniformity of Experience But not Frequency*

Treatment
- *Evaluate severity - keep a chart*
- *Pinpoint triggers*
- *Evaluate therapy*
- *ESTROGEN WITHDRAWL IS TRIGGER*
- *90+% effective*
  - *Pills*
  - *Patches*
  - *Creams*
  - *May take up to four weeks*
- *Other Causes*
  - *Thyroid*
  - *Carcinoid*
  - *Diabetes*
  - *Alcoholism*

Vaginal Dryness
- *Gradual process*
  - *May take years to develop*
- *Estrogen replacement*
  - *Systemic*
  - *Local*
- *Topical lubricants*
- *Uncomfortable*

Linked to "decline" in sexual functioning

Menopausal Therapeutics
- *35%-40% of women ever start conventional ERT*
- *Conventional ERT Benefits*
  - *Osteoporosis risk reduced 50%*
  - *Cardiovascular disease reduced ???*
  - *Reduced menopausal symptoms*
  - *Memory loss*
  - *Alzheimer's disease*
  - *Tooth loss*
  - *Colon cancer reduction*
- *Conventional ERT Risks*
  - *Breast cancer*
  - *Vaginal bleeding*
  - *Endometrial cancer (negated by progesterone)*

Bone Density Evaluation

* *DXA (Dual Energy X-ray Absorptiometry) measures the spine, hip or total body;*
* *pDXA (Peripheral Dual Energy X-ray Absorptiometry) measures the wrist, heel or finger;*
* *SXA (single Energy X-ray Absorptiometry) measures the wrist or heel;*
* *QUS (Quantitative Ultrasound) uses sound waves to measure density at the heel, shin bone and kneecap.*
* *QCT (Quantitative Computed Tomography) most commonly used to measure the spine, but can be used at other sites;*
* *pQCT (Peripheral Quantitative Computed Tomography) measures the wrist;*
* *RA (Radiographic Absorptiometry) uses an X-ray of the hand and a small metal wedge to calculate bone density;*
* *DPA (Dual Photon Absorptiometry) measures the spine, hip or total body (used infrequently);*
* *SPA (Single Photon Absorptiometry) measures the wrist (used infrequently);*

Estrogen Replacement Therapy (ERT) and Hormone Replacement Therapy (HRT)

- *Estrogen replacement therapy (ERT) is approved for the prevention and management of osteoporosis. ERT has been shown to reduce bone loss, increase bone density in both the spine and hip, and reduce the risk of hip and spinal fractures in postmenopausal women.*

- ***Alendronate Sodium (brand name Fosamax®)***
  - *Alendronate is a medication from the class of drugs called bisphosphonates.*
- ***Risedronate Sodium (brand name Actonel®)***
  - *Risedronate is also from the bisphosphonate family. Taken daily, 5 mg of risedronate slows bone loss, increases bone density and reduces the risk of spine and non-spine fractures.*
- ***Raloxifene (brand name Evista®)***
  - *Raloxifene, 60 mg a day, is approved for the prevention and treatment of osteoporosis. It is from a new class of drugs called Selective Estrogen Receptor Modulators (SERMs) that prevent bone loss at the spine, hip, and total body. Raloxifene produces increases in bone mass.*
- ***Calcitonin (brand name Miacalcin®)***
  - *Calcitonin is a naturally occurring hormone involved in calcium regulation and bone metabolism. In women who are at least 5 years beyond menopause, calcitonin slows bone loss, increases spinal bone density,*

Osteoporosis Detection

- *Ultrasound measures the heel*
- *DEXA (Dual Energy X-ray Absorptiometry) measures the spine, hip or total body*
- *SXA (single Energy X-ray Absorptiometry) measures the wrist or heel*
- *PDXA (Peripheral Dual Energy X-ray Absorptiometry) measures the wrist, heel or finger*
- *RA (Radiographic Absorptiometry) uses an X-ray of the hand and a small metal wedge to calculate bone density*
- *DPA (Dual Photon Absorptiometry) measures the spine, hip or total body*
- *SPA (Single Photon Absorptiometry) measures the wrist*
- *QCT (Quantitative Computed Tomography) measures spine or hip*

Women's Health Initiative

- 7.7% of the study participants reported having had cardiovascular disease at enrollment.
- Not intended to measure the effect of HT on vasomotor symptoms
- Women were discouraged from participating in the study if they reported moderate or severe Menopausal symptoms during a 3-month period prior to the study
- Only approximately 500 women aged 50-54 with moderate -to -severe symptoms at study entry were included in the WHI
- Women randomized to the use of estrogen plus progestin were significantly more likely than women randomized to placebo to report a reduction in hot flushes and night sweats.
- Likewise had an improvement in sleep disturbances but no other benefit in terms of the other quality-of-life outcomes reported.
- Continuous conjugated equine estrogen (0.625 mg/d) and medroxyprogesterone acetate (2.5 mg/d) (Prempro®)
- Women aged 50-79 years at enrollment,
- Similar numbers of women in each age category (50-59 years, 60-69 years, and 70-79 years)
- Mean age of study participants was 63 years,
- Most women experience natural menopause around age 50.
- HT is primarily prescribed to women aged 50-59 years,
- 33% of study participants were aged 50-59 years
- Absolute risks for women taking HT starting at age 50 are substantially lower than the comparable risks for women starting use at age 65

Cardiovascular Disease

- No benefit for the prevention of coronary heart disease
- 22% increased risk of cardiovascular disease
- 29% increased risk of coronary heart disease
- 7 more coronary heart disease events (37 versus 30) per 10,000 women per year, and this risk is cumulative over time

Breast Cancer

- Confirm an observed trend reported in previous research
- Increased risk of breast cancer with HT use
- 26% increased risk of invasive breast cancer
- 8 additional new cases (38 versus 30) per 10,000 women per year
- Breast cancer takes several years to develop
- Became clinically apparent at four years
- Survival rates are higher in women diagnosed with breast cancer who have taken hormone therapy than those who have not

Stroke and Pulmonary Embolism

- 41% increased risk of
- 8 additional cases of stroke (29 versus 21)
- 8 additional cases of pulmonary embolism (16 versus 8) per 10,000
- Risk is cumulative
- Elevated risk of stroke appears in the second year - continues through year five

Fractures

- Support previous research - decreased risk of vertebral and other osteoporotic fractures
- Hip fracture were reduced by 34%
- 5 per 10,000 women per year (10 versus 15)

Colon Cancer

- Suggest a benefit in the prevention of colorectal cancer
- Reduced by 37%
- 6 fewer cases (10 versus 16) per 10,000 women per year

Cognitive Function

- Subset of 4532 women aged 65 years or older
- Higher incidence of probable dementia - (66% versus 34%)
- Increase of 23 cases per 10,000 women
- Not helpful in preventing mild cognitive impairment

Recommendations For Hormone Replacement Therapy Use

- Encouraged to take it for as short a time as needed
- Lowest effective dose.
- Long-term use should be counseled about the risks and benefits
- Talk about available alternatives.
- Women who want to continue taking HT
- May do so provided they understand the potential risks

HT has been shown to be the most effective treatment for symptomatic relief of vasomotor symptoms including hot flashes

Nonhormonal alternatives such as selective serotonin reuptake inhibitors, may be helpful for this indication

Combined continuous estrogen and progestin therapy is no longer recommended for the prevention of cardiovascular disease

Alternatives for improved cardiovascular health, including lifestyle modifications such as exercise, smoking cessation, and weight loss, should be encouraged for all women. The use of cholesterol-lowering medications such as statins and the need for treatment of hypertension should be evaluated for each individual patient.

Figures removed due to copyright restrictions.
[Kaplan-Meier Estimates of Cumulative Hazards for Breast Cancer;
Kaplan-Meier Estimates of Cumulative Hazards for CHD;
Disease rates for women as shown by WHI study results]

Figures removed due to copyright restrictions.
[Kaplan-Meier Estimates of Cumulative Hazards for Hip Fracture;
Kaplan-Meier Estimates of Cumulative Hazards for Total CVD;
Kaplan-Meier Estimates of Cumulative Hazards for the Global Index]

FUNDAMENTAL QUESTIONS

1. Define menopause. What is the average age for natural menopause?
2. What happens to the number of viable oocytes as a woman ages?
3. Describe 7 physiologic changes seen in women during the menopausal period.
4. What happens to estradiol, FSH, LH and androgens as the ovaries age?
5. What is osteoporosis and how is it measured? Describe the techniques?
6. Why are some older women “hairy”?
7. How can one treat the vasomotor symptoms other than with estrogen?
8. What hormones are frequently used as HRT?
9. What are the proven benefits of HRT?
10. How do bisphosphanates work?
11. What are the risks of combined HRT?
12. What are the risks of estrogen only HRT in women with a uterus? Without a uterus?
13. What are the alternative therapies available to treat
    a. Hot flushes
    b. Vaginal dryness
    c. Skin changes
14. What advice would you give women who insist on HRT because her menopausal symptoms make it impossible to function normally?

Functional classification of Cell junctions

Occluding junctions
Anchoring junctions
- Actin filament attachment sites
  - Cell to cell (adhesion belts)
  - Cell to matrix (focal contact)
- Intermediate filament attachment sites
  - Cell to cell desmosomes
  - Cell-matrix (hemidesmosomes)

Communicating junctions
- Gap junctions
- Chemical synapses
- Plasmodesmata (plants only)

The gap junction

- 2 plasma membranes connected by a seres of structures made up of connexons
- connexon has six subunits
- 2 connexons in register forming open channels between the two membranes
- Channel is 1.5 nm in diameter
- Gap between membranes is 2-4 nm
- Electrical resistance is determined by number of junctions that are expressed
- Resistance (measure of gap junction density)
  - 100 ohm/cm non-pregnant
  - 50 ohm/cm at term
    - 100 ohm/cm for stomach
    - 250 ohm/cm for taenia caeci
    - 400 ohm/cm for bladder
- Junctions increase at time of parturition
- Connexin-43 has high turnover rate in labor
- Estradiol and oxytocin increase levels
- Progesterone and hCG decrease levels

Signaling in myometrium

- Action potentials = electro-mechanical coupling
- Receptor stimulation = pharmaco-mechanical coupling
  - Hormones
    - Oxytocin, epinephrine
  - Classic transmitters
    - 5-HT, acetylcholine
  - Lipid mediators
    - prostanoids

Role of calcium

- Good temporal relationship between intracellular Ca++ and development of force
- Ca++-calmodulin activate myosin light chain kinase (MLCK)
- This phophorylates a serine residue (Ser 19) in the 20 kDa light chain of myosin (MLC20)
- Phosphorylated myosin interacts with actin and causes a contraction
- When Ca++ is removed phosphorylation ceases and the muscle relaxes

Uterine Smooth Muscle

- Bundle of myometrial cells embedded in a matrix of connective tissue
- Matrix enables transmission of individual contractile forces
- ripening
  - Increased collagen solubility
  - Alteration in ground substance
- Ripening occurs in the cervix and corpus
- Cytoplasm
  - Myosin (thick filaments)
    - Hexamer
      - 2 identical 200kDa heavy chains
      - 4 light chains (two 20 kDa chains and two 15-17 kDa chains)
    - Enzyme capable of converting ATP → mechanical energy
    - Head
      - Actin and myosin interact
      - ATPase sites located
    - Tail
      - Formation of myosin filaments

Figure removed due to copyright restrictions. Please see: Figure 3-5 in Speroff, Leon, Robert H Glass, and Nathan G Kase. "Structural similarities of Oxytocin and Vasopressin." In Clinical Gynecologic Endocrinology and Infertility. Baltimore, MD : Williams & Wilkins, 1989. ISBN: 0683078976.

Oxytocin

- Nonapeptide as shown
- First peptide ever synthesized - Nobel Prize
- High affinity, low capacity receptors
  - 80-fold increase in number by term
  - marked increase in sensitivity
- Act by raising intracellular free calcium levels
- Calcium stimulates actomyosin formation and muscular contraction
- Maternal and fetal source

- High affinity, low capacity receptors
  - 80-fold increase in number by term
  - marked increase in sensitivity
- Act by raising intracellular free calcium levels
- Calcium stimulates actomyosin formation and muscular contraction
- Maternal and fetal source

Uterus

Oxytocin receptors distributed in a gradient from fundus (maximum) to cervix (few)
Receptor concentration begins to rise early in pregnancy and exponentially increases to term

Prostaglandins

- Prostaglandins (PG) and thromboxane
- Products of arachidonic acid metabolism.
- Produce numerous physiologic and pathophysiologic effects
- Regulating cellular processes in nearly every tissue
- Local hormones
  - act in the vicinity of their site of production
  - Function in an autocrine and/or paracrine manner
- Widely distributed
- Can be formed by nearly every tissue and cell type
- Ability to provoke different responses in various tissues
- Five physiologically important prostanoids
  - PGE2
  - PGF2a
  - PGI2
  - TXA2
  - PGE2
- Wide spectrum of physiological and pharmacological actions
  - Immune
  - Endocrine
  - Cardiovascular
  - Renal
  - Reproductive
  - Contraction and relaxation of smooth muscle

- Exert their effects through GTP-binding protein (G protein)-coupled, rhodopsin-type receptors
- Receptors for PGE2 are termed EP, which include EP1, EP2, EP3 and EP4 subtypes
- High specific PGE2 binding has been observed in the brain, kidney, uterus, liver, thymus and the adrenal medulla
- PGE2 has versatile and opposing actions due to multiple EP receptor subtypes and the coupling of EP receptor isoforms to a variety of signal transduction pathways

G-proteins

- adrenaline
- glucagon
- luteinizing hormone (**LH**)
- parathyroid hormone (**PTH**)
- adrenocorticotropic hormone (**ACTH**
- prostaglandins
- Evidence suggests
  - PGE2 affects gene transcription
  - Regulates growth and cell proliferation.
- Endogenous PGE2 regulates the growth of epithelial cells
- PGE2 exhibits mitogenic activities in bone cells and stimulates DNA synthesis
- PGE2 is involved in the growth and metastasis of tumors
- Inhibition of prostaglandin synthesis has been shown to result in growth retardation of tumors in experimental animals
- Decreased risk of colon cancer with ASA

Cervical Collagen

- Major protein of the extracellular matrix
  - 25% of mammalian protein
  - Stiff triple stranded helical structure
  - α chains (1000 amnio acids long)
  - Proline and glycine bonds create the left handed helical configuration
  - More than 20 types of collagen
  - I, II, III are fibrillar collagens
  - Assemble into fibrils and then fibers

- Collagen polypeptides formed on membrane bound ribosomes and injected into the ER as pro-α chains (have extra amnio-acids called pro-peptides at ends)
- In lumen of ER hydroxyproline allows bonding of three pro-α chains to form procollagen
- Propeptides of type I, II, and III removed by extracellular enzymes converting them to tropocollagen (1.5 nm diameter)
- Tropocollagen assembles to form fibrils (10-200 nm)
- Type I and type III
- Spaces between bundles dilate @ 8-14 weeks
- Total collagen increases but concentration goes down 30-50%
  - Water and non-collagen proteins increase
  - Fibrils decrease in size
- Stains for collagen polymer show reduced amount (lower numbers of intact fibers)
- Spaces between bundles dilate @ 8-14 weeks
- Total collagen increases but concentration goes down 30-50%
  - Water and non-collagen proteins increase
  - Fibrils decrease in size
- Stains for collagen polymer show reduced amount (lower numbers of intact fibers)

Ground substance

- Glucosamine - naturally occurring amino sugar found in glycosoaminoglycans (mucopolysaccharides)
- Integral components of the proteoglycans
- Proteoglycans - large carbohydrate rich structures
  - Resiliency
  - Load distribution
  - Shock-absorbing
  - Compressive
  - Lubricating
- Dietary glucosamine
  - An immediate precursor for glycosaminoglycan synthesis
  - Stimulates incorporation of other precursors into the connective tissue matrix

- GAG (glycosaminoglycan)
  - Total increases throughout pregnancy
  - Concentration remains constant
  - Helps loosen the collagen network
- HA (hyaluronic acid)
  - Increases 12 fold at 2-3 cm dilation
  - HA may bind water and help hydration of tissue and hence deformability
- CS (chondroitin sulfate)
  - Decrease
  - Results in decreased rigidity of cervical tissue

- Elastase acts on the telopeptide non-helical domains of collagen

  - Can degrade collagen, elastin, and proteoglycans
  - Synergistically with collagenase
  - Polys from blood
  - Cervical fibroblasts
- Amount of soluble collagen (degraded) increases in parallel with enzyme activity
- More immature crosslinks
- Collagen with many x-links replaced with collagen with fewer x-links
- Up to several thousand sugar residues
- Repeating sequence of non-sulfated disaccharide units
- Variable amounts in all tissues (esp embryos)
- Earliest evolutionary form of glycosaminoglycans (GAG)
- Has some function in cell migration

Cervical ripening

- Decrease in total collagen content
- Increase in collagen solubility
- Increase in collagenolytic activity
  - Collagenase
  - Leukocyte elastase
- Rapid turnover of extracellular matrix
- Strong correlation free hydroxyproline
- Similar to inflammatory response
  - IL-8, other cytokines, eosinophils, mast cells, macrophages, neutrophils

- Sex steroid hormones
  - Estrogen
    - Collagen degradation
    - IV estradiol produces cervical ripening
- Progesterone
  - Blocks estrogen induced collagenolysis
  - Antagonists produce ripening
  - IL-8 in rabbits is down regulated by progesterone
- Changes are gradual and antecede labor by several weeks

FUNDAMENTAL QUESTIONS

1. Describe the types of junctions seen between cells.
2. What kinds of junctions are seen in myometrial cells?
3. What is the structure of a gap junction?
4. What happens to the expression of oxytocin receptors as gestation advances?
5. What type of connexon is seen in myometrium?
6. Describe the signaling mechanisms in the myometrium.
7. What is oxytocin, its structure and function?
8. What is the role of prostaglandin in myometrial contractility?
9. How do G-proteins work?
10. Describe the cervical ground substance. How is it suited for labor?
11. What factors influence how soft the cervix becomes before the onset of labor?
12. Describe the process of “ripening” in detail.

POLYCYSTIC OVARIAN SYNDROME

Presence of oligo- or anovulation in combination with hyperandrogenism.

- Chronic anovulation may present as irregular menstrual periods or amenorrhea.
- It is not essential to document anovulation by ultrasonography or progesterone measurements in the presence of a clear clinical history
- PCOS occurs in 85 to 90% of women with oligomenorrhea and in 30-40% of women with amenorrhea
- Diagnosed either by clinical (hirsutism) or laboratory (elevated testosterone or androstenedione)
- Should not be diagnosed if evidence of other causes of oligo-ovulation and hyperandrogenism(ovarian androgen secreting tumor or nonclassical adrenal hyperplasia)
- Prevalence of PCOS is approximately 5–7% of women of reproductive age
- 50% of PCOS women are obese and tend to have an android pattern of obesity

Elevated levels of serum LH - increased LH pulse amplitude and LH pulse frequency

- Steady-state levels of gonadotropins and ovarian steroids
    - “chronic estrous state”
    - Proliferation and hyperplasia of the endometrium
    - Can lead to unpredictable bleeding episodes
    - Unopposed estrogen exposure -confirmed by progesterone withdrawal test

- Women with PCOS have higher mean concentrations of LH
    - Increased bioactivity of LH
    - Low to low-normal levels of follicle stimulating hormone

- Obese PCOS women do not have elevated LH levels
    - normal LH level or normal LH/FSH ratio does not rule out PCOS
    - LH/FSH ratio is now not included in the diagnostic criteria of PCOS

- In research studies almost all women with PCOS have elevated LH secretion.
- In clinical practice - difficult to use a single measurement of LH to diagnose PCOS,
        - LH is secreted in a pulsatile manner
        - Normal range of serum LH concentration decreases with increasing body mass index (BMI)

Association between hyperinsulinemia and PCOS

- First noted by 1980

- Significant positive correlation between insulin, androstenedione and testosterone levels among PCOS women

- It is estimated that 20-40% of PCOS women have impaired glucose tolerance
    - Seven-fold higher than the rates in age and weight-matched women
    - Prevalence of type 2 diabetes mellitus is also increased in PCOS women (15% versus 2.3% in normal women)
    - Lean PCOS women have lower rates of carbohydrate intolerance
    - Lean PCOS women still have higher rates than age and weight-matched controls. PCOS is associated with insulin resistance independent of total or fat-free body mass
    - Obese PCOS women are more insulin resistant than obese non-PCOS or non-obese PCOS women

- Pancreatic beta cell secretory dysfunction in a subset of PCOS women
    - Probably has the highest risk of developing carbohydrate intolerance
    - Type 2 diabetes.
    - Oral glucose tolerance tests recommended for obese PCOS patients

- PCOS in 8 out 30 premenopausal women with type 2 diabetes
    - Insulin resistance is characterized by post-receptor defect in the action of insulin
    - Cause of this defect is still being elucidated

Action of insulin

- Binds to the cell-surface receptor
- Receptor undergoes auto-phosphorylation on specific tyrosine residues
- Accomplished by activation of insulin receptor tyrosine kinase
- Activated receptor then activates insulin receptor substrates (IRS-1,2 and 3)
- Binds to signaling molecules such as PI3 kinase
- Activates downstream signaling
- Leads to insulin-mediated glucose transport

PCO insulin resistance

- Abnormalities in both insulin receptor tyrosine kinase
- Mediators distal to the receptor are present in insulin resistance states
- Adipocytes from women with PCOS
- Adipocyte insensitivity to inhibition of lipolysis by insulin
-
- Occur in PCOS in the absence of obesity
- Decreased insulin receptor auto-phosphorylation in 50% of fibroblasts removed from PCOS women
- Due to increased receptor serine phosphorylation
- Serine phosphorylation - associated with decreased insulin receptor tyrosine auto-phosphorylation

- In vitro human theca cell studies
  - Insulin has direct stimulatory effects on ovarian steroidogenesis
  - Insulin produced a greater increase in androgen production by theca cells in PCOS than in cells obtained without PCOS
  - Effect is mediated specifically through insulin receptor
  - Insulin enhances the effect of LH on preovulatory ovarian follicles
  - Premature activation and subsequent follicle arrest
  - hyperinsulinemia (due to insulin resistance) drives the LH effect on ovarian theca cells
    - Causes androgen excess which are intrinsically programmed to produce more androgen
    - Excess androgens are known to interfere with the process of follicular maturation
    - Inhibiting ovulation
    - Producing more arrested follicles

- 1983 it was proposed that severe hyperinsulinemia caused by insulin resistance results in ovarian hyperandrogenism (Barbieri & Ryan)
- PCOS is associated with insulin resistance independent of total or fat free body mass
  - Hyperandrogenism, insulin resistance and acanthosis nigricans syndrome

- Cause of the insulin resistance – germ line mutation in the insulin receptor gene
  - Prevents normal function of the insulin receptor.

- Puberty: LH secretion rises
  - Severe insulin resistance
  - LH stimulation → hypersecretion of testosterone by the ovary
  - Often present with severe insulin resistance and hyperandrogenism including virilization and amenorrhea

- Acanthosis nigricans
  - Dermatologic manifestation of the hyperinsulinemia
  - Hyperandrogenism

- Clinical criteria suggestive of insulin resistance
  - BMI greater than 27 kg/m2
  - Waist-to-hip ratio greater than 0.85
  - Presence of acanthosis nigricans

- Laboratory criteria
  - Elevated fasting insulin concentration
  - Elevated glucose-to-insulin ratio

- Must have 2 of the following 3 manifestations
  - Irregular or absent ovulation
  - Elevated levels of androgenic hormones
  - Enlarged ovaries containing at least 12 follicles each

- Polycystic ovaries are defined - on ultrasound
  - To contain 12 or more follicles
  - Measuring 2 to 9 mm in diameter
  - Increased volume of 10 mL or greater
  - Only one ovary fulfilling these criteria is enough

- Polycystic ovaries are not necessary feature of PCOS
  - Many women with polycystic ovaries do not have PCOS
  - Should not be considered to have PCOS unless there is corroborating clinical evidence of the syndrome.

- Treatment of insulin resistance
  - Can reduce ovarian androgen secretion and
  - Cause the resumption of ovulatory menses.
  - Cause–effect relationship between insulin resistance and hyperandrogenism–anovulation.

- Typical Presentation
  - Chief compliant of hirsutism
  - Irregular menses
  - Infertility

- Treatment of Hirsutism
  - Combination of an estrogen–progestin contraceptive
  - Antiandrogen (spironolactone)

- Standard treatment for infertility
    - Clomiphene citrate and weight loss

Treatment with insulin sensitizers, metformin (biguanide which reduces plasma glucose concentrations in type 2 diabetes) and thiazolidinediones (Troglitazone and Rosiglitazone), improve both metabolic and hormonal patterns and also improve ovulation in PCOS

- Metformin
    - Does not lead to weight gain
    - Can induce weight loss
    - Predominantly works by reducing hepatic glucose production
    - Inhibiting gluconeogenesis both directly and indirectly (by decreasing free fatty acid concentrations)
    - May slightly improve peripheral insulin sensitivity in PCOS
    - Reductions in androgen levels
    - Improvements in ovulation
    - Reduce the high rates of gestational diabetes in PCOS

- Thiazolidinediones (TZDs)
    - Decrease peripheral insulin resistance
    - Enhancing insulin action
        - Skeletal muscle
        - Liver
        - Adipose tissue

Mechanism of action
- Binding and modulating the activity of a family of nuclear transcription factors
    - Peroxisome proliferator-activated receptors (PPARS)

- Shown an improvement of the androgen levels
    - Ovulation rate
    - Enhanced insulin sensitivity
    - No reduction in the weight of subjects
    - Decrease testosterone, androstenedione, DHEA
    - Increase in SHBG
        - Thereby causing a decrease in free testosterone levels
        - Improvement in insulin sensitivity
    - Improved both spontaneous and clomiphene-induced ovulation rates
    - Independent effects on ovarian steroidogenesis
    - Direct effect of TZD apart from improvement of insulin resistance ?

- PCOS women have higher circulating levels of inflammatory mediators
  - C-reactive protein
  - Tumor necrosis factor
  - Tissue plasminogen activator
  - Plasminogen activator inhibitor-1 (PAI-1)

- Hirsutism occurs in approximately 80% of PCOS women
- Documented by measuring androgen levels in the blood
  - Free testosterone
  - Total testosterone
  - Androstenedione
  - Dehydroepiandrosterone (DHEA)

- In obese PCOS women
  - Sex hormone binding globulin (SHBG) levels are decreased
  - Leads to an increase in free testosterone levels
  - Insulin is a negative regulator of the production of SHBG by the liver
  - SHBG levels are decreased in hyperinsulinemic conditions
  - Concentrations of sulfated DHEA (DHEAS) are also increased
    - Secreted exclusively by the adrenal glands
    - Mechanism of increased DHEAS production by the adrenals unknown
      - Insulin ?
      - IGF-1 ?

- Under influence of low but constant levels of FSH
  - Multiple follicles of the ovary are stimulated
  - Do not achieve maturation
  - Lifespan of the follicles may extend over several months
  - Leading to multiple follicular cysts
  - Luteinized in response to constant and relatively high LH levels
  - “arrested” follicles provide a constant supply of steroids
  - Atretic follicle becomes an androgenic follicle
  - Atretic follicles are deficient in aromatase activity

- Follicular cells from the small follicles of polycystic ovaries
  - Produce small amounts of estradiol
  - Show a dramatic increase in estrogen production when stimulated by FSH or IGF-1
  - FSH therapy induces a larger cohort of follicles to develop in women with PCOS
  - deficient in vivo ovarian response to FSH
  - Due to impaired interaction between signaling pathways associated with FSH and IGF1 ?

Figure removed due to copyright restrictions.

References:
Dhindsa G, Bhatia R, Dhindsa M, Bhatia V. Insulin resistance, insulin sensitization and inflammation in polycystic ovarian syndrome. J Postgrad Med 2004;50:140-144 **** Excellent review of topic of PCO – most of above abstracted from this paper ******

Robert L. Barbieri,
Metformin for the Treatment of Polycystic Ovary
Syndrome
New Eng;land Journal of Medicine
Vol. 101, No. 4, April 2003 ***** Excellent review of use of Metformin *****

FUNDAMENTAL QUESTIONS

1. Describe the PCO syndrome.
2. Describe the relationship of PCO with carbohydrate metabolism.
3. What is metformin? What benefit does it confer when given to women with PCO?
4. Where are androgens produced in the ovary?
5. What are some clinical problems associated with PCO?
6. What happens under the influence of sustained low levels of FSH?
7. Name some inflammatory mediators?
8. What is the role of obesity in PCO?
9. What happens to SHBG in obese women with PCO?
10. What is acanthosis nigricans?
11. Is hirsuitism common in PCO?

TOXOPLASMOSIS

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Types of Hosts

Intermediate Hosts

•Hosts in which asexual replications occurs

•If no replication occurs, the intermediate host is known as a transport or paratenic host
- e.g. *Toxoplasmosis gondii* - various vertebrates
Plasmodium Spp. - vertebrate blood and tissues

Definitive Hosts

•Hosts in which the sexual cycle occurs
- e.g. *Toxoplasmosis gondii* - wild and domestic cats
Plasmodium spp. - mosquitoes (Diptera)

Major "Groups" of Apicomplexa

•Gregarines - septate & aseptate (considered "primitive") - parasites of invertebrates - extracellular

•Coccidia - homoxenous and heteroxenous species
–eimerid coccidia (*Eimeria, Lankesterella,* etc)
–isosporoid coccidia (*Sarcocystis, Toxoplasmosis, Neospora*, etc.)

•*Cryptosporidium* spp.

•Haemosporinids - malarial parasites and their relatives

•Piroplasms - *Babesia, Theileria* and their relatives

•Haemogrgarines - *Haemogregarina, Heptozoon*, etc.

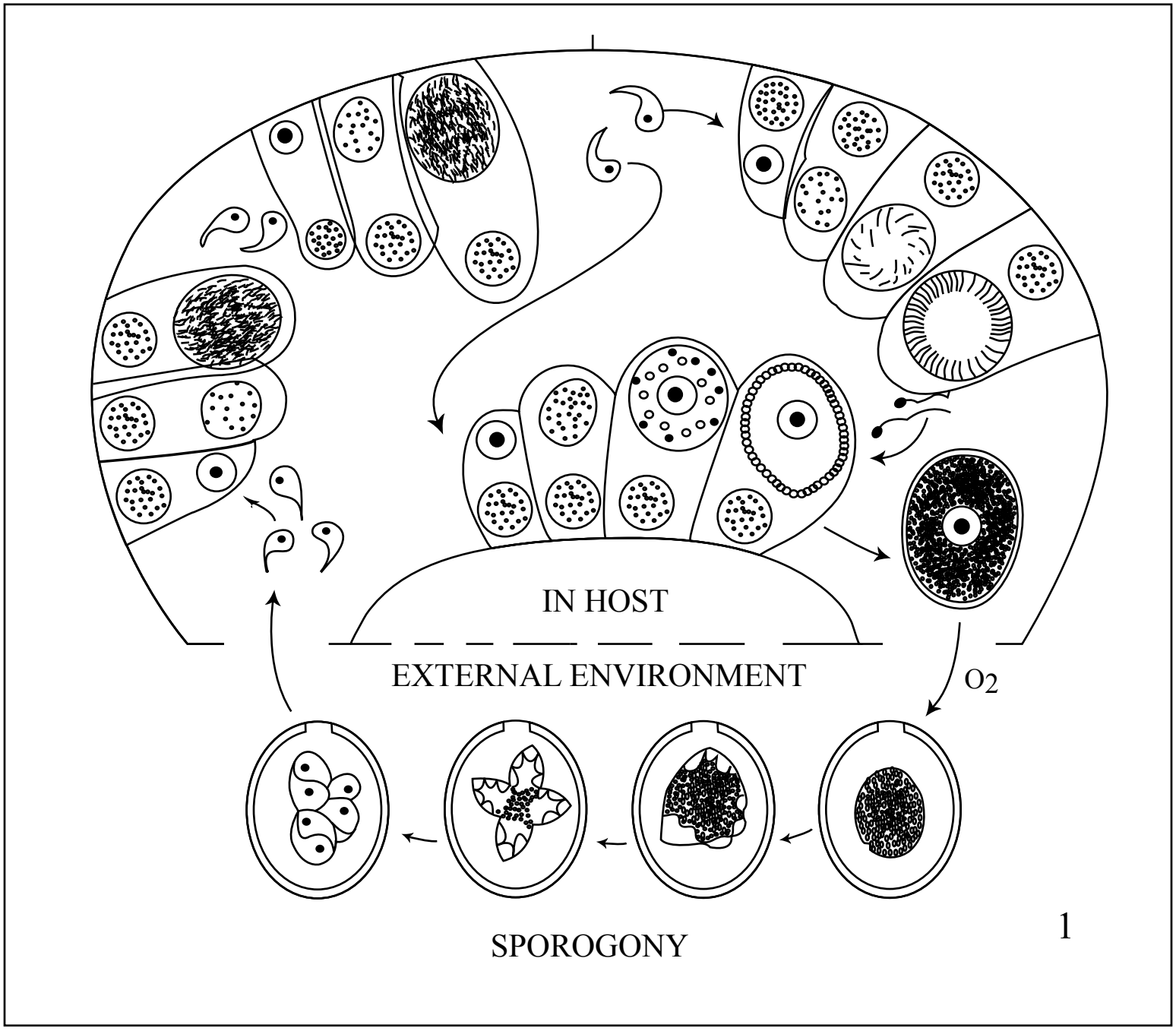

Illustration courtesy of MIT OCW.

Apicomplexa Protista

- All members of the phylum Apicomplexa
- Single-celled organisms
- Possess a more or less developed "apical complex"
- Possess flattened subpellucular vessicles
  (share this feature with the dinoflagellates and ciliates)
- Usually complex life cycles

Typical Apicomplexan Zoite

- Eukaryotic, apical complex, trilaminate pellicle

Figure removed due to copyright restrictions.

Figure removed due to copyright restrictions. Please see:

Figure 3 in Schematic drawings of a tachyzoite (left) and a bradyzoite (right) of *T. gondii*.
The drawings are composites of electron micrographs.

Dubey, J. P., D. S. Lindsay, and C. A. Speer. "Structures of Toxoplasma Gondii Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts."
Clin Microbiol Rev. 11, no. 2 (April, 1998): 267-99.

- Tachyzoites can move by gliding, flexing, undulating, and rotating
- No visible means of locomotion such as cilia, flagella, or pseudopodia
- Functions of the conoid, rhoptries, micropores, and micronemes are not fully known
- Probably associated with host cell penetration
- The conoid can rotate, tilt, extend, and retract as the parasite probes the host cell plasmalemma immediately before penetration

- Rhoptries have a secretory function associated with host cell penetration, secreting their contents through the plasmalemma just above the conoid to the exterior
- Tachyzoites multiply asexually within the host cell by repeated endodyogeny
- Specialized form of reproduction - two progeny form within the parent parasite consuming it
- Golgi complex divides first - becoming two complexes at the anterior end of the nucleus The host cell ruptures when it can no longer support the growth of tachyzoites
- After the ingestion of tissue cysts by cats, the cyst wall is dissolved by proteolytic enzymes in the stomach and small intestine.
- Released bradyzoites penetrate the epithelial cells of the small intestine and initiate the development of numerous generations of *T. gondii*
- Five morphologically distinct types of *T. gondii* develop in intestinal epithelial cells before gametogony begins

Figure removed due to copyright restrictions. Please see:

Figure 1: Life cycle of *T. gondii*. in
Dubey, J. P., D. S. Lindsay, and C. A. Speer. "Structures of Toxoplasma Gondii Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts."
Clin Microbiol Rev. 11, no. 2 (April, 1998): 267-99.

Factors Determining Expression of Disease

- The susceptibility of the host
- Age of the host
- The strain of the pathogen
- The degree of acquired immunity

Prevalence

- Overall world prevalence ~13%
- Diet Uncooked or poorly cooked meat is eaten
- Immuno-compromised host
    - Patient is undergoing suppressive therapy
    - Transplant patients
    - HIV

Spread by the Cat

- Only members of the cat family shed oocysts
- Cats become infected by ingesting
    - Oocysts from fecal contamination
    - A cat can excrete 107 to 109 oocytes per day
    - Tissue cysts present in the flesh of eaten animals

Digestive enzymes release the organisms
Invade the feline small intestine
The organisms reproduce

- Producing millions of noninfectious, unsporulated oocysts
- Excreted in feces of cats for up to 2 weeks

Outside the cat's body

- Sporogony occurs for up to 21 days
- Development of infectious oocysts
- Warm, moist soil - oocysts viable for more than 1 year

Intermediate Hosts

- Herbivores/omnivores -Type 1 Host
- Carnivores - Type 2 Host

Distinctive Differences in the life cycle stages

Herbivore/omnivore - intermediate host

- Hosts infected by eating sporulated oocysts
- Each oocyst contains 2 sporocysts with 4 sporozoites

Liberated sporozoites infect extra-intestinal sites

- Acute phase
  - Mesenteric lymph nodes
  - Liver
- Chronic
  - Brain
  - Heart
  - Skeletal muscles

Sporozoites - infect the intestinal epithelial cells
undergo schizogony

Only in the cat

- Sexual phase of the parasite (*gametogony*) observed
- Poduces a single celled oocyte
- Cycles through the cat rapidly (3-5 days)
- Cat does not appear to suffer much discomfort
- Quickly replaces damaged epithelial cells
- Kittens - more susceptible and some die of the infection.

Sporozoites divide by *endodyogeny*
[*Endodyogeny - Formation of daughter cells each surrounded by its own membrane, while still in the mother cell*]

Form pseudocysts

- Contain merozoites (**tachyzoites)**
- Can infect other cells and form **tissue cysts**.

Pseudocyst and Tissue cysts

Require the host to be devoured by a carnivore (type 2 host) to continue life cycle

Both are infective to cats.

Human Spread

Spread of the merozoites usually prevented by the host immune response

- Predominately a cellular one
- Persisting as long as the host remains infected
- Usually for life

If tissue cysts eaten by a carnivore other than a cat

- Released merozoites
- Penetrate extra intestinal cells
- Form pseudocysts
- Produce more merozoites or **bradyzoites**
- Lead to the production of tissue cysts
- Transmission via the placenta in females to the fetus occurs

Insects

- Flies and cockroaches - can act as transport hosts
- Disseminating the oocysts

Review of Basic Facts About Toxoplasmosis

- *Toxoplasma gondii* - obligate intracellular parasite
- Properties similar to the pathogen that causes malaria
- One of the most common human parasites in the world
- First discovered in the gundi, a North African rodent
- Members of the cat family are the definitive hosts
- Domestic cats play a major role in transmission
- Congenital infection is the only form of human-to-human transmission.

Presenting History

- Acquired toxoplasmosis can present with a range of clinical manifestations, from **subclinical lymphadenopathy** (the most common presentation) to **fatal, fulminant disease**
- In healthy adults, infection with *T gondii* **usually is subclinical**.
- In the immunocompetent host, infection with *T gondii* may be indistinguishable from **infectious mononucleosis**.
- In immunocompromised hosts, toxoplasmosis may **mimic** other opportunistic infections, such as tuberculosis or infection with *P carinii.*
- In patients with **AIDS, CNS** involvement is the most common manifestation, followed by pulmonary involvement.

Physical Findings

- Toxoplasmosis cannot be diagnosed on clinical grounds alone
- May mimic a variety of other diseases.
- No clinical features are pathognomonic for toxoplasmosis
- Lymphadenopathy is the most common finding

Laboratory Studies

- The immunoglobulin (IgM) immunofluorescent antibody test (IgM-IFA)
- An IgM-IFA titer of 1:160 or greater
- IgM-enzyme-linked immunosorbent assay (IgM-ELISA) titer of 1:256 or greater
- Considered diagnostic of recently acquired *T gondii* infection

Imaging Studies

- MRI
  - Considered best diagnostic imaging modality
  - May detect lesion not visualized on C-T
  - May find single or multiple lesions
- Ring enhancing
- Basal ganglia
- Cortico-medullary regions

Other Tests

- Cerebrospinal fluid
- Mononuclear pleocytosis
- Elevated protein
- Normal glucose
- Presence of tissue cysts is diagnostic for toxoplasmosis
- Does not distinguish between acute and chronic infection
- Presence of either tachyzoites or toxoplasmal antigens in tissue or smears confirms acute infection
- Brain biopsy
- May reveal presence of tachyzoites

Histologic Diagnosis

- Tachyzoites in tissue sections or smears of body fluid
  - Difficult to demonstrate tachyzoites in conventionally stained tissue sections immunoperoxidase technique
    - Uses antisera to *T. gondii*,
    - Sensitive and specific
    - Presence of the parasite in the central nervous system (AIDS)
    - Applicable to unfixed or formalin-fixed paraffin-embedded tissue sections
    - A rapid and technically simple method
  - Air-dried, Wright-Giemsa-stained slides
  - Sediment of CSF
  - Brain aspirate
  - Impression smears of biopsy tissue
  - The presence of multiple tissue cysts

Ocular Toxoplasmosis

•Acquired toxoplasmosis may be seen
•Majority of cases are felt to be a reactivation of a congenital toxoplasmosis
•Characterized by an acute retino-choroiditis
•Marked vitreous reaction overlying the active infection
•"headlight in the fog"
•Healed lesion leads to a large scar

Sabin-Feldman Dye Test

IgG antibodies are primarily measured by the Sabin-Feldman Dye Test Sensitive and specific neutralization test
Live organisms are lysed
- Presence of complement
- The patient's IgG *T. gondii*-specific antibody

•Becomes positive 1 to 3 weeks after infection
•Titer increases for many months
•Gradually decles over a period of 5 years or more
•May remain positive for life
•Titers of 1:4 are significant
•Titers of 1:8 or 1:16 usually should be considered as positive.
Rising titers - 2 weeks apart indicate recently acquired infection

IgG antibodies

- Usually appear within 1 to 2 weeks of the infection
- Peak within 1 to 2 months
- Fall at variable rates
- Persist for life
- Titer does not correlate with the severity of illness

A positive DT = patient has been exposed to the parasite

A negative DT essentially rules out prior exposure to *T. gondii*

Small number of patients

- IgG antibodies might not be detected within 2 to 3 weeks
- Toxoplasmic chorioretinitis
- Toxoplasmic encephalitis) in immuno-compromised patients

Differential Agglutination Test

- Also known as the "AC/HS test")
- Two antigen preparations
  - Early following acute infection (AC antigen)
  - Later stages of infection (HS)
- Ratios of titers using AC versus HS antigens
  - Acute (may persist for one or more years)
  - Equivocal
  - Non-acute
- Useful in helping differentiate acute from chronic infections
- Best used in combination with a panel of other tests

Avidity Testing

- Functional affinity of specific IgG antibodies
- Initially low after primary antigenic challenge
- Increases during subsequent weeks and months
- Protein-denaturing reagents (urea) used to dissociate the antibody-antigen complex.
- Avidity result is determined using the ratios of antibody titration curves of urea-treated and untreated serum.

Used as additional confirmatory diagnostic tool

- patients with a positive and/or equivocal IgM
- acute and/or equivocal pattern in the AC/HS test

- Confirmatory test
- Not the ultimate test for decision-making
- Low or equivocal IgG avidity antibody results should not be interpreted as diagnostic of recently acquired infection
- Low or equivocal avidity antibodies can persist for months to one year or longer.

IgM Antibodies

- Measured by the"double-sandwich" or "immuno-capture" IgM-ELISA
- Avoids false positive results
  - Rheumatoid factor
  - Antinuclear antibodies
- In recently acquired infection
- IgM *T. gondii* antibodies are detected initially
- Become negative within a few months
- Occasionally positive IgM *T.gondii*-specific titers can be observed during the chronic stage of the infection
- IgM antibodies have been reported to persist as long as 12 years
- Persistence has no clinical relevance – chronically infected
- The FDA recommends: sera with positive IgM test results obtained at non-reference laboratories should be sent to a Toxoplasma reference laboratory (Such as Remington or CDC Labs)

Polymerase Chain Reaction (PCR)
Used to detect *T. gondii* DNA in body fluids and tissues
- Congenital
- Ocular
- Disseminated

Performed on amniotic fluid
- Revolutionized the diagnosis of fetal *T. gondii* infection
- Enabling an early diagnosis to be made
- Avoiding the use of more invasive procedures (PUBS)

Allowed detection of *T. gondii* DNA in brain tissue
Cerebrospinal fluid (CSF)
Vitreous and aqueous fluid
Bronchoalveolar lavage (BAL) fluid
Urine
Amniotic fluid
Peripheral blood.

Treatment
- Pyrimethamine (Daraprim)
- Folic acid antagonist
- Selectively inhibits plasmodial dihydrofolate reductase
- Highly selective against plasmodia and *T gondii*.
- Does not destroy gametocytes
- Arrests sporogony in mosquito
- Possesses blood schizonticidal and some tissue schizonticidal activity against malaria parasites of humans.
- Extend regimens to include suppressive cure through any characteristic periods of early recrudescence and late relapse for at least 6-10 wks in each case.

Treatment
- Spiramycin - macrolide antibiotic
    - Used effectively in the treatment of pregnant women
    - Now is recommended for congenital infection
- Clindamycin plus pyrimethamine
- Clarithromycin
- Azithromycin
    - More potent than spiramycin and clindamycin

Drugs with Human Activity in Human Toxoplasmosis

- Pyrimethamine (inhibits DHFR)
- Sulfadiazine (inhibits dihydropterolate synthetase)
- Trimethoprim-sulfmethoxazole (Bactrim)
- Clindamycin (binds to ribosome - inhibits protein synthesis)
- Spiramycin (binds to ribosome - inhibits protein synthesis)
- Atovaquanone (inhibits mitochondrial cytochromes)
- Azithromycin, Clarithromycon, Trovafloxacin

Treatment is a Function of Immune Status

- Infants
  - –Normal immune system
- Only with lymphadenopathy
- No treatment is required
- Organ damage
  - –Pyrimethamine initiated in loading dose
  - –Maintenance
  - –Potent folic acid antagonist
- Bone marrow suppression
- Leukopenia
- Anemia
- Thrombocytopenia
- Leucovorin (Folinic acid)

Serologic

- Indirect fluorescent antibody (IFA)
  - –Measures the same antibody as Sabin-Feldman Dye Test
  - –IFA titers of 1:8 or 1:16 are believed to represent infection
- IgM fluorescent antibody (IgM-IFA)
  - –Based on the known early rise of IgM
  - –Precedes IgG
  - –Used for early diagnosis of congenital infection
  - –Acute infection in the acquired disease
  - –Reactivation of latent toxoplasmosis
  - –Titers of 1:10 to 1:1000 or higher are seen
  - –May persist for a variable period
  - –Usually becomes negative by the third of fourth month
  - –Persists as low titers for a year

Table removed due to copyright restrictions.
[Multivariate analysis of risk factors for *Toxoplasma gondii* infection adjusted for age, location, period between diagnosis of infection and interview, and all other exposures shown]

FUNDAMENTAL QUESTIONS

1. Why is toxoplasmosis important to a pregnant woman?
2. List all the known intermediate hosts for toxoplasmosis?
3. What should a pregnant woman be told about toxoplasmosis avoidance?
4. What effect does Toxo have on a fetus?
5. Describe the life cycle of Toxo?
6. What drugs may be used in a pregnant woman who has acquired Toxo? How do they work?
7. What laboratory tests had been used to diagnose toxo until recently?
8. What is the Sabin-Feldman dye test? How is it done?
9. What is the current test used to diagnose toxo?
10. How long can toxo live in the muscle of an infected human? What conditions threaten the life of an adult patient infected with toxo?

IN SUMMARY
ISO-IMMUNIZATION

ISOIMMUNIZATION

History Of Rh Isoimmunization

- The Rh story is one of multiple foci of independent investigations
  - –Occurring at different sites
  - –Different times
- High level of competition that can develop
  - –Laboratory scientists
  - –Clinical scientists
  - –Pharmaceutical industry

Ortho Pharmaceutical Company

- Trade name RhoGAM, three decades ago
- Rh Isoimmunization affected approximately 1 percent of the pregnancies in the U.S. at the beginning of this century

Clinical findings

- Hemolytic anemia
- Edema of the fetal tissues known as **hydrops (eryhtroblastosis) fetalis**
- Autopsy evidence of proliferation of red blood cells in multiple sites
- Large of number of immature red cells

- 1930s - recognized as one clinical entity were
  - Hydrops fetalis
  - Icterus gravis neonatorum
  - Congenital anemia
  - Erythroblastosis fetalis
- World War II lead to discovery of antigenic blood factors, which might result in immunization and cause transfusion reactions.
- Major contributors
  - Alexander Wiener
  - Philip Levine,
  - Karl Landsteiner

Causes of fetal hydrops

➢ Lymphatic Abnormalities
  - Lymphangiectasia
  - Cystic hygroma
  - Turner's syndrome (XO)
  - Noonan's syndrome
  - Multiple ptergium syndrome
  - Pulmonary Malformations
  - Lymphangiectasia
  - Chylothorax
  - Cystic adenomatoid malformation
  - Hypoplasia
  - Other

IN SUMMARY
ISO-IMMUNIZATION

- Hematologic
  - Fetal hemolytic anemia
  - α-Thalassemia
  - Fetomaternal or twin-to-twin transfusion
- Congenital Infections
  - Viruses
  - Cytomegalovirus
  - Parvovirus B19
  - Toxoplasmosis
  - Syphilis
  - Chagas Disease
- Cardiovascular
  - Arrhythmias
  - Cardiomyopathy
  - Structural anomalies: lesions that result in increased right atrial pressure and volume
  - primarily with atrioventricular regurgitation
  - left sided obstructive lesions
  - Ebstein's anomaly
  - Premature closure of the foramen ovale
  - Intracardiac tumors (tuberous sclerosis)
  - Vascular malformations
  - Chorangioma of the placenta, chorionic or umbilical vessels
  - Hemangiomas (Hepatic, Klippel-Trenaunaysyndrome)

- Other Causes
  - Obstructive uropathy
  - Congenital nephrosis
  - Chromosomal abnormalities
  - Trisomy 15, 18, 21
  - XX/XY
  - Neoplasms
  - Storage diseases
  - Bone diseases
  - Placental abnormalities
  - Neurologic abnormalities
  - Idiopathic

Genetics and Biochemistry of the Rh Antigen

Nomenclature

•1940, Landsteiner and Wiener - rabbit immune sera to rhesus monkey erythrocytes
•Agglutinated the majority (85 percent) of human erythrocytes
•Named this the Rh factor.
•Agglutinated cells were called *Rh positive*
•Disease caused by antibody directed against an erythrocyte surface antigen of the rhesus blood group system.
•High degree of polymorphism

Five major antigens can be identified

- Many variant antigens
- Three Systems of Categorization
- Fisher-Race
- Wiener system
- HLA-like system of Rosenfield

IN SUMMARY
ISO-IMMUNIZATION

Fisher-Race System

- Nomenclature is best known in Obstetrics
- Presence of three genetic loci
- Each with two major alleles
- C, c, D, E, and e
- *No antiserum specific for a "d" antigen*
- *"d" indicates the absence of a discernible allelic product*
- Anti-C, anti-c, anti-D, anti-E, and anti-e designate specific anti-sera directed against the respective antigens.
- Rh gene complex described by the three appropriate letters

Rh Antigen Complexes

Eight gene complexes (decreasing frequency in humans)

Cde
cde
Cde
cDe
Cde
cdE
CDE
CdE

Table removed due to copyright restrictions.
[Rh Gene Frequencies in 2000 Unrelated Caucasian Adults]

Nomenclature

- Written in the order C(c), D(d), E(e)
- Actual order of the genes on chromosome 1 is D, C(c), E(e).
- Vast majority of Rh Isoimmunization - incompatibility with respect to the D antigen
- *Rh positive* indicates the presence of the D antigen
- *Rh negative* indicates the absence of D antigen

Weiner System

- Assumption of only one genetic locus
- Eight genotypes are designated (in decreasing order of frequency in the white population) R^1 , r, R^2 , R^0 r, r, R^Z , and r^v.

IN SUMMARY
ISO-IMMUNIZATION

Variants of D Antigen

- Unique Rh antibodies have been used to identify more than 30 antigenic variants
- Two of the most common
- C^w antigen
- D^u antigen
- Heterogeneous group of clinically important D antigen variants most often found in African Americans
- D^u -positive individuals - quantitative decrease in expression of the normal D antigen,
- Some D^u variants are significantly different antigenically
- Two cellular expressions responsible for the D^u phenotype
- Reduction in the number of D antigen sites with all epitopes represented
- Expression of only some of the various D antigen epitopes with some epitopes missing

D^u Variant

- D^u -positive erythrocytes - bind anti-D typing sera
- *In some cases only by sensitive indirect antiglobulin methods*
- At least some D^u -positive patients are capable of producing anti-D, presumably by sensitization to missing D epitopes.
- Could result in a D^u -positive mother becoming sensitized to her D-positive fetus

Genetic Expression

- Genetic locus for the Rh antigen on the short arm of chromosome 1
- Within the Rh locus are two distinct structural genes adjacent to one another,
- RhCcEe and RhD.
- Likely share a single genetic ancestor,
- Identical in more than 95 percent of their coding sequences
- First gene codes for the C/c and E/e antigens
- Second gene codes for the D antigen
- D-negative lack the RhD gene on both their chromosomes.
- D-negative patients have a deletion of the D gene on both their chromosomes 1
- Expression of the Rh antigen on the erythrocyte membrane
    - Genetically controlled
    - Structure of the antigen
    - Number of specific Rh-antigen sites (e.g., D, E, C, c, or e)
    - Relatively constant amount of Rh antigen sites available
    - About 100,000 sites per cell
    - Evenly divided between C(c), D, and E(e) antigens

Allelic Interactions

- CDe/cde express less D antigen than cDE/cde.
- CDe/cDE express less C antigen than CDe/cde

Structure and location of antigens

- The Rh antigens - polypeptides
- Embedded in the lipid phase of the erythrocyte membrane
- Distributed throughout the membrane in a nonrandom fashion
- D antigen sites - spaced in a lattice-like pattern
- 92 nm in Rh(D) heterozygotes
- 64 nm in homozygotes
- Rh polypeptides are polymorphic
- MW of the D antigen 31,900 d
- C(c) and E(e) antigen MW 33,100 d

Biochemistry & Immunology

- Rh polypeptide lies within the phospholipid bilayer of the membrane

IN SUMMARY
ISO-IMMUNIZATION

- Spans the membrane 13 times
- Short segments extending outside the red cell
- Extrude into the cytoplasm
- D antigen appears very early in embryonic life - 38-day-old fetus
- Expressed early in the erythroid cell series – pronormoblasts
- Seven different D antigen epitopes have been identified or deduced using human monoclonal anti-D antibodies, and others may exist. One hypothesis suggests that these different epitopes are part of the same protein-lipid complex more or less expressed according to the depth of polypeptide

Clinical Issues

- Anti-D antibody titer of greater than 1:4 - considered Rh sensitized
- Consider possibility that the fetus might be Rh negative
- Fathered by another partner
- Mismatched blood transfusion
- Determining the paternal Rh-antigen status is reasonable
- DNA analysis can be used to determine his zygosity
- Father homozygous - all his children will be Rh positive
- Father heterozygous - 50 percent likelihood that each pregnancy will have an Rh-negative fetus
- Cordocentesis with analysis of fetal red blood cells
- Blood sampling for fetal Rh antigen status at 18 to 20
- Increased risks of fetal loss and fetomaternal hemorrhage

Current Technology

- *The Rh locus on chromosome 1p34-p36 has been cloned*
- Polymerase chain reaction (PCR)
- Uncultured amniocytes
- 2 ml of amniotic fluid
- 5 mg of chorionic villi.

Ultrasound and Doppler Studies

- Sonographic findings that might predict the severity of Erythroblastosis fetalis
- Avoid the need for invasive assessments
    - Pre-hydropic changes
    - Polyhydramnios
    - Placental thickness
    - Pericardial effusion
    - Dilation of the cardiac chambers
    - Chronic enlargement of the spleen and liver
    - Visualization of both sides of the fetal bowel wall,
    - Dilation of the umbilical vein

Figure removed due to copyright restrictions.

[Liley curve]

SUMMARY OF IMPORTANT FACTS IN RH DISEASE

- To reduce the incidence of Rh sensitization, Rh-immune globulin should be given to Rh-negative unsensitized women at 28 weeks' gestational age and again after delivery if the newborn is Rh positive. Rh-immune globulin is also indicated for these patients in cases of miscarriage, ectopic pregnancy, chorionic villus sampling, amniocentesis, or fetomaternal hemorrhage.
- The gene coding for the D antigen has been cloned, and in the near future prenatal determination of fetal Rh status should be routinely available from uncultured amniocytes obtained at amniocentesis.
- Measurement of amniotic fluid bilirubin remains the standard for assessment of pregnancies at risk for significant fetal anemia. Neither ultrasound alone nor Doppler are adequately sensitive to identify anemic fetuses.
- The timing of the first amniocentesis is based on history, maternal anti-D titers, gestational age, and ultrasound findings. The timing of subsequent amniocenteses is based on the $DeltaOD_{450}$ values and trends.
- Analysis of amniotic fluid bilirubin before 26 weeks is controversial. Although most data suggest that $DeltaOD_{450}$ values and trends are accurate before the third trimester, more liberal use of cordocentesis may be appropriate.
- Fetal transfusion can be performed using either the intraperitoneal or intravascular route. For hydropic fetuses, intravascular transfusion is clearly superior. For non-hydropic fetuses, perinatal survival rates are similar with either method.
- With the reduction in Rh disease brought about by widespread use of Rh-immune globulin prophylaxis, sensitization to the minor or atypical antigens has become relatively more common. A number of these minor antigens can cause several fetal anemia.

FUNDAMENTAL QUESTIONS

1. Give a brief history of the discovery of the Rh antigen.
2. Where is this antigen located and what are its biochemical characteristics?
3. Name the antigens?
4. Which one is of major significance?
5. What are the clinical manifestations of isoimmunization?
6. What is hydrops fetalis and what are the causes?
7. What us a D^u variant?
8. What determines the severity of clinical disease in the fetus?
9. What is a Liley curve and how is it used?
10. What diagnostic modalities are useful in the detection and therapy of Rh disease?
11. What is Rho-gam and how is it obtained in the year 2004?
12. Are there any risks to giving Rho-gam?
13. How can one determine the amount of Rho-gam that is needed for any given patient?

IN SUMMARY
ISO-IMMUNIZATION

IN SUMMARY
ISO-IMMUNIZATION

TERATOLOGY

DEFINITION

An exposure in pregnancy that has a harmful fetal effect.

1. An increase in the frequency of an abnormal fetal effect
2. A dose-response relationship
3. Established mechanism of action, which often requires animal model
4. The proposed teratogenicity must make sense biologically
5. Identifying a genetically more susceptible group.
   Clinical epidemiologic studies
   e.g. features of exposed and controls
   Animal models
   - address issues of dose
   - determine cellular effects

POTENTIAL FETAL EFFECTS

Spontaneous abortion	Maternal diabetes
Growth restriction	Alcohol
Pattern of major and minor anomalies	Anticonvulsant drugs, Warfarin, retinoic acid
Major malformations only	Cigarette smoking
Stillbirth	Maternal diabetes
Abruptio placenta	Cocaine
Cognitive dysfunction	Retinoic acid, PCB phenobarbital, lead
Altered social behavior	Diethylstilbestrol (DES)
Cancer	DES

DISTINCTIVE PHENOTYPIC EFFECTS

- Nose hypoplasia in Warfarin-exposed
- Ear malformations in retinoic acid (Accutane)-exposed
- Severe nail hypoplasia and fused interphalangeal joints in phenytoin-exposed
- Vascular disruption defects in CVS-exposed and misoprostol-exposed

PERIOD OF GREATEST SENSITIVITY

KNOWN FOR VERY FEW HUMAN TERATOGENS
ex: THALIDOMIDE: days 20-34 post fertilization
WARFARIN: weeks 4-7 post fertilization (anticoagulant)

DOSE RESPONSE RELATIONSHIPS

- VALPROIC ACID
- MATERNAL PHENYLKETONURIA (PKU)
- ALCOHOL
- CIGARETTE SMOKING

MUST MAKE SENSE BIOLOGICALLY

Ex. EXOGENOUS SEX HORMONES

- NOT PLAUSIBLE BECAUSE FETAL TISSUES ALLEDGEDLY AFFECTED (HEART, LIMBS) HAVE NO RECEPTORS FOR HORMONES

- FDA REMOVED WARNING FROM PACKAGE INSERT

Ex. BENDECTIN (VITAMIN B6 AND ANTIHISTAMINE)
- SCIENTIFIC EVIDENCE LACKING
- DRUG RE-INTRODUCED IN CANADA

GENETICALLY MORE SUCEPTIBLE GFROUPS

1. CIGARETTE SMOKING
2. ALCOHOL
3. FOLIC ACID DEFICIENCY

Ethanol----------→Acetaldehyde----------→Acetate

Enzyme:	*Alcohol Dehydrogenase*	*Aldehyde Dehydrogenase*
Gene loci:	ADH1, ADH2, ADH3, ADH4	ALDH2
Polymorphisms:		
	$ADH2^1$	$ALDH2^1$ (active)
	$ADH2^2$ (high Km, high Vmax)	$ALDH2^2$ (inactive)
	$ADH2^3$ (high Km, high Vmax)	
	$ADH3^1$	
	$ADH3^2$	

SPINA BIFIDA

DEFINITION: Defect in closure of neural tube in lumbar or thoracic region

PREVALENCE: 0.4 per 1,000 U.S. Caucasians
0.4 per 1,000 African-Americans
0.6 per 1,000 Hispanics

ETIOLOGY: Combined effect of genetic and non-genetic factors

CANDIDATE GENES: methylenetetrahydrofolate reducate (MTHFR) [C677T]; methionine synthase, sonic hedgehog, uncoupling protein 2

ENVIRONMENTAL FACTORS: Folic acid deficiency, maternal diabetes mellitus, maternal obesity, anticonvulsant drugs (Tegretol and Depakote)

MOST EXPOSURES HAVE NOT BEEN STUDIED

- MOST STUDIES FOCUS ON MAJOR MALFORMATIONS ONLY
- LITTLE DATA ON EFFECTS ON BEHAVIOR AND I.Q.
- FEW STUDIES OF DERMAL EXPOSURES
  AIRBORNE EXPOSURES
- NEED TO ESTABLISH MOLECULAR BASIS FOR TERATOGENESIS

COUNSELING FOR EXPOSURES: IT IS NOT GENETIC COUNSELING

MICROTIA

DEFINITION: MALFORMED AND UNDERDEVELOPED EAR;
MILD TO SEVERE; USUALLY UNILATERAL
RIGHT > LEFT

ASSOCIATIONS: TYPICALLY ISOLATED;
NO INCREASE IN KIDNEY ABNORMALITIES
HEARING LOSS: 50 TO 70dB

PREVALENCE: 1 IN 10,000

GENETICS: 7% EMPIRIC RECURRENCE RISK

ACUTANE

35% Have Major Malformations

- Conotruncal Heart Defects
- Cranial Nerve Palsies
- Absence of Vermis of Cerebellum
- Moderate to Severe Mental Retardation

25% Of Children With No Malformations Are Mentally Retarded

PHYSICIAN'S DESK REFERENCE (PDR)

SECTION ON RISKS IN PREGNANCY DESIGNED TO PROTECT LIBILITY

TWO SYSTEMATIC STUDIES SHOWED POOR CORRELATION BETWEEN CATEGORIES A, B, C, D AND X WITH CLINICAL DATA AVAILABLE

STUDY OF ALL DRUGS APPROVED BY FDA 1980-2000
468 DRUGS: 80% "RISK UNDETERMINED"
USED ONLINE "TERIS" AS SOURCE

POOR CORRELATION OF TERIS RATINGS AND FDA DRUG CATEGORIES (A, B, C, D & X) FOR 163 DRUGS
KAPPA STATISTIC = 0.08 ± 0.04

OTIS

Example: Centers collaborate to identify exposed pregnancies and organize follow-up exams.
Examples: asthma medication
leflunomide (Arava)
Outcomes: body and head size, dysmorphic features, major malformations

TERATOGEN COUNSELING VS GENETIC COUNSELING

ALIKE: PREPARATION FOR MEETING
COMMUNICATION
RISK ASSESSMENT
SPERM OR EGG DONOR

DIFFERENT: PERIOD OF EXPOSURE
ALTERNATIVE TREATMENTS
EGG DONOR
PRENATAL DIAGNOSIS LIMITED
PREVENTION: AVOIDANCE

RECOGNIZED HUMAN TERATOGENS (2004)

1. DRUGS
   - Aminopterin/amethopterin
   - Androgenic hormones
   - Angiotensin converting enzyme(ACE) inhibitors
   - Busulfan
   - Carbamazepine
   - Chlorobiphenyls
   - Cocaine
   - Cyclophosphamide
   - Cyclosporin
   - Diethylstilbestrol
   - Etretinate
   - Fluconazole
   - Heroin/methadone
   - Iodide
   - Isotretinoin (13-cis-retinoic acid)
   - Lithium
   - Methimazole
   - Phenobarbital
   - Phenytoin
   - Propylthiouracil
   - Prostaglandin
   - Tetracycline
   - Thalidomide
   - Trimethadione/paramethadione
   - Valproic acid
   - Warfarin

2. HEAVY METALS

Lead
   - Mercury

3. RADIATION
   - Cancer therapy

4. MATERNAL CONDITIONS
   - Alcohol
   - Insulin-dependent diabetes mellitus
   - Iodide deficiency
   - Maternal phenylketonuria
   - Myasthenia gravis
   - Obesity, severe
   - Smoking cigarettes/marijuana
   - Systemic lupus erythematosus
   - Vitamin A deficiency

5. INTRAUTERINE INFECTIONS
   - Cytomegalovirus
   - Herpes simplex

Parvovirus
Rubella
Syphilis
Toxoplasmosis
Varicella
Venezuelan equine encephalitis Virus

6. OTHER EXPOSURES
Chorionic Villus Sampling (CVS)
Dilation and Curettage (D & C)
Gasoline fumes (excessive)
Heat
Hypoxia
Intracytoplasmic Sperm Injection (ICSI)
Methyl isocyanate
Methylene blue
Polychlorinated biphenyls
Toluene (excessive; glue sniffing)
Trauma, blunt

FUNDAMENTAL QUESTIONS

1. What is a teratogen?
2. Describe the embryologic time line for teratogenesis?
3. What are the specific abnormalities that are seen in the fetal Warfarin syndrome?
4. What are the specific abnormalities that are seen the fetal alcohol syndrome?
5. What are the specific abnormalities that are seen in the fetal hydantoin syndrome?
6. List 10 known anatomic teratogenic fetal effects of drugs?
7. Name 7 infectious diseases known to be teratogenic? In what trimester are these of greatest concern?
8. Name 7 mechanical causes of teratogenic effects?
9. What are the adverse fetal effects of prenatal cigarette exposure?
10. What are the effects of fetal exposure to Accutane? How may these be prevented?
11. What is a good reference source to use in counseling patients about teratogenic effects of drugs?

ENERGY IMPACT ON PREGNANCY

Hyperthermia

- Hyperthermia promotes in-vivo and in-vitro synthesis of PG
- During hyperthermia inhibition of PG promotes severe acidosis
- Selected PG's induce expression of heat shock proteins (HSP) and induce thermotolerance
- Molecular changes unclear
- Possible role of prostaglandins as protective
- Sheep demonstrate Increased prostaglandin plasma levels in mother and fetus
- Inhibition of PG synthesis resulted in fetal death
- Prevent aggregation in the lens (cataract)
- Effect protein folding
- Stabilization of extended chains
- Membrane translocation
- Regulation of heat shock response
- Binding and stabilization/regulation of steroid receptors
- Thermotolerance, proteolysis, resolubilization of aggregates
- Glycoprotein maturation in the ER
- Folding catalysts
- "Quality Control"

Heat Teratogenesis

Non-teratogenic doses of ASA potentiate hyperthermic teratogenesis
Arsenicals, vitamin A, ethanol, Lead
Day 8.5 in rate selected - initial phase of organogenesis
Impairments if somitogenesis (axial skeleton)
Dysraphia of rostral neuropore (exencephaly)
Dose response relationship
Axial skeleton has lower threshold (43°)
79.6% vs. 9.6%
Sensitivity of neural tubes is strain dependent

Ultrasound

- Sound absorbed differently by different media
- Process not well understood
- Temperature rise may be major effect
- Thermal conductivity
- Frequency
- Heat capacity
- Physiotherapy
- Several degrees in 10 minutes
- Total rise over 10 degrees in small volume
- Experimental Pulse Echo >250watts/cm2
- No *gross* effects on fetal development
- ? Intracellular effect
- Levels tested are 100 times greater than in clinical use
- Possible effect of U/S on DNA
- In use >40 years for fetal imaging without any obvious issues
- Most women have >2 U/S per pregnancy
- 40% of All U/S is for OBS use
- Grayscale, B-Mode, 3D, Harmonic imaging, simultaneous multigate imaging
- General belief that it is safe

- Amplitude reduction as u/s wave enters tissue
- Energy is transferred
- Absorption – conversion into heat
- Scatter – part that changes direction
- Thermal indices
- Soft tissue (TIS)
- Bone (TIB)
- Cranial bone (TIC)

Role of Bubbles

- Occurrence of gaseous bubbles in air-water interface
- Transient – violent activity with hot spots
- High temperature
- High pressure
- Both
- Short bursts (microseconds)
- Stable
- Gaseous body oscillates due to presence of US field
- Fluid near bubble starts to flow
- Produces enough stress to disrupt cell membranes

Hyperthermia is proven teratogen

- Biologic tissue exposed to us can produce heat and temp rise
- General threshold is 1.5-2°C above maternal core before teratogenicity
- An increase of 2.5-5° can occur within an hour
- With modern US machine we never see a rise more that 1°C
- No evidence of effect below 39°C
- "diagnostic exposure that produces an in situ rise of no more than 1.5° above normal levels may be used without reservation on thermal grounds"
- "a diagnostic exposure that elevates embryonic and fetal in situ temperature above 41°C for 5 minutes should be considered potentially hazardous."

- Soft tissue first to be produced embryologically
- Temp rise can be predicted
- Skeleton produced later – no boney effects in first weeks of gestation
- Routine B-mode never causes rise of more than 1.5°C
- In first trimester however there may be greater exposure because of lack of bone protection

Prospective studies - animal only or tissue culture

- No difference in malformations, abortions, stillbirth
- Possible reduction in growth
- Growth gap gone after 3 months

- Few studies dealing with chromosome anomalies and U/S
- Little or no change with one exceptional study
- No epidemiologic data
- Unethical not to perform U/S on a pregnancy
- No difference in childhood malignancies

Microwaves

Effects studied with no effects

- Fish tail tissue
- Mouse testes
- Mouse hepatocytes
- Human erythrocytes
- Firefly light organ
- Drosophila larvae
- Amphibian embryos
- Chick embryos
- Mouse ovaries
- Rat thymus
- Mouse CNS
- Human lymphocytes

Electromagnetic Fields

- increasing generation of electric power during this century is not associated with a concomitant rise in the incidence of birth defects
- Over 70 EMF research projects dealing with animal and in vitro studies that are concerned with some aspect of reproduction and growth
- Large proportion of the embryology studies utilized the chick embryo and evaluated the presence or absence of teratogenesis after 48 to 52 hours of development
- Results of chick embryo data are inconsistent
- Embryo culture or cell culture studies are of little assistance in determining the human risk of EMF
- In vitro or in vivo studies in nonhuman species can be used to study only mechanisms and the effects that have been suggested by human investigations

Video Display Terminals

No evidence of harm in humans due to VLF radiation

FUNDAMENTAL QUESTIONS

1. What teratogenic effects are known to result from hyperthermia?
2. What does the temperature/effect curve look like?
3. Is there a clinical temperature above which a pregnant woman should not be permitted to reach?
4. Describe the tissue effects of ultrasound energy?
5. At what energy levels can one begin to see effects?
6. Are there any demonstrated clinical effects of diagnostic ultrasound?
7. Are there any risks in living near microwave towers?
8. Describe the possible mechanism of action of microwave teratogenesis

PRENATAL GENETIC DIAGNOSIS

Indications for prenatal diagnosis

- Advanced maternal age
- Positive maternal serum alpha fetal protein
- Balanced maternal or paternal translocation
- Risk for detectable Mendelian disorder
- Family history of neural tube defects
- Abnormal fetal ultrasonography
- History of fetal wastage
- Parental concern

Alpha Fetal Protein

- Glycoprotein MW 70,000 Dalton
- Produced by yolk sac and liver at 4-8 weeks
- Liver later becomes dominant source
- Most MSAFP gets to mother by diffusion
- Transmembranous transport from AF is 6%
- Any increase in production (twins) or increase in AF (NTD) leads to increase
- Expressed as multiples of the median (MOM) for given GA
- Maternal race, weight, multiple pregnancy, IDDM
- Each lab must establish norms and risks
  - Diabetics have overall increased risk for anomalies
  - AA have higher AFP at any given GA
  - FH impacts risk as well
  - Concentrated in very thin, diluted in very obese
- Elevated for α-fetal protein & acetylcholinesterase
- MSAFP is also expressed as MoM for normal pregnancies of same gestational age
- Using 2.5 MoM we can detect 93-96% of open spina-bifida and 100% of anencephalics
- False positives are high (contamination of AF by fetal blood – especially if placenta is crossed by needle)
- AChE is so large that it is not in fetal urine. It will detect 99% of open spina-bifida

Other causes of elevated AFP

- Abdominal wall defects
- Renal agenesis
- Fetal demise or impending demise
- Teratoma
- Congenital nephrosis
- Congenital diaphragmatic hernia
- Some maternal tumors
- IBD in mother
- Feto-maternal hemorrhage
- Oligohydramnios
- Fetal growth restriction

Incidence of chromosomal abnormalities by age

Figure removed due to copyright restrictions.

Sonography

- All women had been encouraged to have amniocentesis if at risk in past years
- Modern sonography will detect virtually all lesions
- Gestational age errors, multiple gestation, fetal demise are all detectable
- Normal U/S allows 90% reduction in risk for NTD based on α-fetal protein
- Early genetic sonography is highly sensitive and statistically superior to later ultrasonography for Down syndrome detection.
- Early midtrimester sonography achieves a diagnostic accuracy similar to that currently reported for first-trimester nuchal translucency.

Triple Screen

- A Fetal Protein (AFP)
- Human chorionic Gonadotropin (hCG)
- Unconjugated estriol (μE3)
- Estimate risk of fetus with trisomy 18 and trisomy 21
- Low AFP, hCG and μE3 → trisomy 18
- Low AFP, μE3 and high hCG → trisomy 21
- Biologic basis for this unknown
- Three specific adjustments
    - Maternal weight
        - Obese women have lower MASAFP – dilution
    - Diabetes Mellitus
        - IDDM have AFP that is 2/3 that of non-diabetic
    - Race
        - AA women have AFP 9-15% higher
    - Smoking ????
        - Have lower incidence of trisomy 21 !!!!!

First Trimester Screening

- Now an option for pregnant women if certain criteria are met
- Nuchal translucency (NT), have allowed for earlier, noninvasive screening for chromosomal abnormalities and, when combined with serum screening in the first trimester, have comparable detection rates as standard second-trimester screening
- Low serum AFP (31% of trisomies)
- Free beta-hCG reduced in aneupoidy
- Schwangerschafts protein 1 (SP1) also known as pregnancy specific beta-1 glycoprotein - median for abnormal group (trisomy 18 and 21) is ½ that of normal group

Quadruple Screen

- AFP, estriol, Hcg, Inhibin

Inhibin in combination with alpha fetal protein

- The best three-analyte combination was maternal serum -fetoprotein, free -human chorionic gonadotropin, and dimeric inhibin A
- 97% of Down syndrome cases were detected at a false-positive rate of 16%.
- At a slightly higher false-positive rate (18%) maternal serum -fetoprotein, estriol, and intact human chorionic gonadotropin detected only 79% of cases.
- 67% (37/55) detection was obtained with use of the 2-analyte combination of a-fetoprotein and dimeric inhibin A

Nuchal Translucency

- Higher rates of nuchal translucency screening were associated with lower rates of chorionic villus sampling and invasive testing.
- The addition of first-trimester screening may lead to reduced rates of invasive testing and fewer losses of normal pregnancies.
- The use of nuchal translucency adds to the sensitivity of detection but can add as much as $300,000 in cost for each detected Down's syndrome baby.
- Increased nuchal skin alone, in the absence of other ultrasonographic dysmorphologic features, does not generally help to identify fetuses with other abnormal karyotypes.
- The nuchal thickness/humerus length ratio and maternal age had a 79.8% detection rate at a 22.1% false-positive rate, compared with maternal age plus humerus length (sensitivity, 55.1%) or maternal age plus nuchal thickness (sensitivity, 66.7%) at the same false-positive rate. For women >35 years old the values were 80% and 22.0%, respectively.
- Nuchal thickness, humerus length, and maternal urine β-core fragment levels are another sensitive assay from Down's syndrome
- Normal nuchal thickness in the midtrimester indicates reduced risk of Down syndrome in pregnancies with abnormal triple-screen results
- Midtrimester nuchal thickness measurement significantly detected postnatally confirmed CHD in chromosomally normal fetuses.

PAPP

- First-trimester free -human chorionic gonadotropin and pregnancy-associated plasma protein A screening for Down syndrome can achieve detection rates as high as those associated with –alpha-fetoprotein and human chorionic gonadotropin or alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol screening in the second trimester.
- Liklihood ratios for detection of various abnormalities with given tests

TEST	DOWN SYNDROME	UNAFFECTED
No test	1.0	1.00
NT alone	4.9	0.28
PAPP-A, fbhCG	10.6	0.15
NT, PAPP-A, fbhCG	36.1	0.08
AFP, uE3, hCG (triple test)	8.4	0.13
AFP, uE3, hCG, INH-A (quad test)	14.0	0.08
All 7 tests	260.9	0.02

-

Screening Strategies

- Triple screen: maternal age and midtrimester serum alpha-fetoprotein, human chorionic gonadotropin (hCG), and unconjugated estriol
- Quad screen: triple screen plus serum dimeric inhibin A
- First trimester screen: maternal age, serum pregnancy-associated plasma protein A and free b-hCG and fetal nuchal translucency at 10 to 14 weeks' gestation
- Integrated screen: first-trimester screen plus quad screen, but first-trimester results are withheld until the quad screen is completed when a composite result is provided
- Sequential screen: first-trimester screen plus quad screen, but the first-trimester screen results are provided immediately and prenatal diagnosis offered if positive
- Combined first-trimester screening for fetal Down syndrome is more cost-effective than universal second-trimester triple serum screening.

Chorionic Villus Sampling (CVS)

- 1991 – 5/289 pregnancies who had transabdominal CVS at 8 to 9.5 weeks
- Background incidence 5.42/10,000
- In a study of over 80,000 CVS the rate was 6.0/10,000
- Decreased perfusion, embolization
- Should be done at 9-12 weeks
- At least 200-250 yield "*experience*"
- Reserved for women "at risk "
- Fetal loss had been 1:200 (.5%)
- Age of 35 chosen
- Loss now considered to be 1:400 or even lower
- Maternal screening and u/s are so good that only those with abnormal findings need have amniocentesis
- Costs
- Pregnancy loss
- Transvaginal fluid leakage
- Bleeding
- Fetal trauma
- Difficulty in obtaining fluid
- Uterine contraction
- Membrane tenting
- Fetal movement and pocket obliteration
- Bloody fluid
- Mislabeling

Other Technologies

- PUBS
- Fetal tissue sampling
    - Liver
    - Muscle
    - Skin
- Fetal Cells in Maternal Blood
- Fetal Cells Vaginal Fluid

FUNDAMENTAL QUESTIONS

1. What is AFP and from where does it arise?
2. How does it get into the maternal circulation?
3. In what conditions is it elevated and why?
4. In what conditions is it reduced?
5. Describe the rate of rise of chromosomal trisomies with advancing gestational age?
6. How is an amniocentesis performed?
7. When and how do neural tube defects occur?
8. What conditions are commonly diagnosed by amniocentesis?
9. What are the risks of amniocentesis?
10. What methods are available for first trimester prenatal diagnosis?
11. What is PAPP?
12. What is the role of inhibin?
13. What ultrasonographic findings are seen in trisomy 21 in the first and second trimester?
14. What are the risk of amniocentesis? CVS?
15. What is CVS, how is it performed, what can be diagnosed?
16. What are the risks of CVS?
17. List five screening strategies and the benefits of each?

FETAL SURVEILLANCE

An Example of a Fetal Heart Rate Tracing

Figure removed due to copyright restrictions.

Variability

- Beat to be rate changes reflect CNS activity
- Vagal tone is modulated by CNS activity
- Variability is a measure of fetal (or adult) arousal state
- REM sleep produces considerable variability
- REM sleep associated with fetal breathing
- Variability is reduced or eliminated by
  - Drugs (barbiturates, narcotics, $MgSO_4$, diazepam)
  - Infection
  - Hypoxia
  - Prematurity
- Long term variability reflects continuous changes in the sympathetic-parasympathetic balance
- Sinus rhythm exhibits fluctuations around the mean heart rate
- Frequent small adjustments in heart rate are made by cardiovascular control mechanisms (the details of this are not well worked out)
- Results in periodic fluctuations in heart rate
- The main periodic fluctuations are
  - Respiratory
  - Sinus arrhythmia
  - Baroreflex-related
  - Thermoregulation-related

•Inspiratory inhibition of the vagal tone: heart rate shows fluctuations with a frequency equal to the respiratory rate

•This inspiratory inhibition is evoked primarily by central production of impulses from the medullary respiratory to the cardiovascular center

•Peripheral reflexes due to hemodynamic changes and thoracic stretch receptors contribute to respiratory sinus arrhythmia

•Respiratory sinus arrhythmia can be abolished by atropine or vagotomy - parasympathetically mediated (esp. in fetus)

•10-second rhythm in heart rate originates from self-oscillation in the vasomotor part of the baroreflex loop

- –This results from negative feedback in the baroreflex and are accompanied by synchronous fluctuations in blood pressure
- –The frequency of the fluctuations is determined by the time delay of the system
- –Augmented when sympathetic tone is increased decrease with sympathetic or parasympathetic blockade

- Peripheral vascular resistance also exhibits intrinsic oscillations of low frequency
- These oscillations can be influenced by thermal changes in the skin
- Thought to arise from thermoregulatory peripheral blood flow adjustments
- Fluctuations in peripheral vascular resistance accompanied by fluctuations with the same frequency in blood pressure and heart rate
- Mediated by the sympathetic nervous system

Sleep State

- Investigations in the fetus and newborn have revealed that during rapid eye movement (REM) sleep LTV is increased and STV is decreased compared to during non-REM sleep
- These differences between REM and non-REM sleep are due mainly to a shift in the vagal-sympathetic balance from a higher sympathetic
- Vagal tone during REM sleep shifts to higher vagal tone during non-REM sleep
- In addition, the slower and more regular breathing in non-REM sleep (more respiratory sinus arrhythmia, thus more STV) contributes to the differences found

Adults

- Adult heart rate variability has been investigated primarily in awake adults
- Enables investigators to instruct the participants to breath at fixed frequencies
- Heart rate variability studies in adults have revealed that ***body posture influences*** heart rate variability
- In the ***upright*** position ***baroreflex-related*** heart rate variability is ***enhanced*** due to an increased sympathetic tone.
- ***Respiratory*** sinus arrhythmia is augmented in the ***supine position***

Fetal and Neonatal Heart Rate Variability

- In obstetrics it has been noticed that acute hypoxia resulted in an increase in heart rate variability
- Chronic hypoxemia was accompanied by low heart rate variability
- Low heart rate variability is associated with low Apgar scores and pH at birth
- Attributed to depression of the central nervous system
- Persistent fixed fetal heart rate pattern was also described in **anencephaly and fetal decerebration**
- Reduction in heart rate variability appears to be a rather **late sign of fetal compromise**

Fetal and Neonatal Heart Rate Variability

- In asphyxiated ***newborns***, diminished heart rate variability is also found
- **Transient** loss of heart rate variability indicates a good prognosis
  - –Due to cerebral edema,
- **Sustained** loss of heart rate variability
  - –predicts neurologic sequelae
  - –neonatal death
  - –probably due to irreversible damage to the brain or brain stem

Fetal and Neonatal Heart Rate Variability

- Severe neonatal respiratory distress syndrome is accompanied by a reduction in low-frequency heart rate variability
  - –transient depression of the medulla oblongata due to elevated pCO_2 levels and acidosis
- If the respiratory distress improves --> heart rate variability increases
- Reduction in LTV in newborns with clinically significant patent ductus arteriosus
  - –ascribed to a marginal oxygen supply of the myocardium that limits fluctuations in heart rate
- Loss of heart rate variability also has been found in infants with periventricular hemorrhage
  - –damage of vasomotor areas in the medulla oblongata
  - –due to increased intracranial pressure
- In infants who subsequently died of the sudden infant death syndrome
  - –higher heart rate
  - –lower heart rate variability

Time Domain Analysis

- Two types of heart rate variability indices
- Beat-to-beat or short-term variability (STV)
  - Represent fast changes in heart rate.
- Long-term variability (LTV) indices
  - Slower fluctuations (fewer than 6 per minute)
- Calculated from the R-R intervals occurring in a chosen time window (usually between 0.5 and 5 minutes)
- Example of a simple STV
  - Standard deviation (SD) of beat-to-beat R-R interval differences within the time window
- Examples of LTV indices
  - SD of all the R-R intervals
  - difference between the maximum and minimum R-R interval length, within the window

Fourier Analysis

- Respiratory sinus arrhythmia gives a spectral peak around the respiratory frequency
- Baroreflex-related heart rate fluctuations are found as a spectral peak around 0.1 Hz in adults
- Thermoregulation-related fluctuations are found as a peak below 0.05 Hz
- CNS (cortical) contribution seen as higher frequency components

- Heart rate variability can be assessed in two ways
  - statistical operations on R-R intervals (time domain analysis)
  - by spectral (frequency domain) analysis of an array of R-R intervals
- Both methods require ***accurate timing of R waves***
- Analysis can be performed on
  - Short electrocardiogram (ECG) segments (lasting from 0.5 to 5 minutes)
  - 24-hour ECG recordings.

- Spectral analysis introduced as a method to study heart rate variability
- Increasing number of investigators prefer method to that of calculation of heart rate variability indices
- Main advantage of spectral analysis of signals
  - Possibility to study their frequency-specific oscillations (not only the amount of variability)

  - The oscillation frequency
- Decomposing the series of sequential R-R intervals into a ***sum of sinusoidal functions*** of different amplitudes and frequencies

- Fourier transform algorithm
- Displays as a power spectrum with the magnitude of variability as a function of frequency
- Power spectrum reflects the amplitude of the heart rate fluctuations present at different oscillation frequencies

Such mathematical transformations may be used to analyze drug effects on CNS

FUNDAMENTAL QUESTIONS

1. What is the difference between beat-to-beat rate and average rate?
2. Why do changes in BTB rate occur?
3. What is the significance of reduced BTB variability?
4. What is the effect of hypoxia, narcotics, barbiturates and benzodiazepines on BTB variability?
5. Describe three ways of quantifying BTB variability?
6. What is the effect of placental insufficiency on fetal heart rate in labor?
7. What is the effect of umbilical cord compression? What is the mechanism?
8. What happens to fetal pH during normal labor?
9. What is the long term impact of intrapartum asphyxia?

PREGNANCY TERMINATION TECHNOLOGY

Introduction

- *1973: Roe v. Wade; Trimester Approach*
- *Number of Abortions Stable or Decreasing*
- *~ 1/3 of Women 15-44 Undergo Abortion*
- *Abortion 10-11x Safer than Continuing Pregnancy, 2x Safer than PCN Injection*
- *91% 1st TM, 9% 2nd TM, .01% 3rd TM*
- *1965: Illegal Ab = 17% of Maternal Deaths*

Termination of Pregnancy

- *Practiced since antiquity*
- *Many societies accept this practice,*
- *Some reject it,*
- *Even considered it a crime.*
- *Most widely used method early in the first trimester is surgical*
  - *Vacuum aspiration*
  - *Safer and less painful than dilation and curettage*
- *Estimated 26 million pregnancies are terminated legally each year throughout the world*
- *20 million are terminated illegally*
  - *More than 78,000 deaths*
- *United States, legal - performed by trained personnel rate of death from surgical termination of pregnancy is 0.6 per 100,000 women*
- *Serious morbidity less than 1 percent*
- *Abortion*
  - *Lack of information on contraception*
  - *Fear the side effects of contraceptive methods*
  - *Often considered when contraception fails*
  - *Countries where contraceptives not widely available.*
- *Abortion services are not always readily available.*
- *United States has one of the highest abortion rates among developed countries*
- *1995 approximately 86 percent of U.S. counties no abortion providers or facilities*

Incidence of First Trimester Ab

- *Most common surgical procedure*
- *Maximum increase occurred between 1972 and 1980*
- *During 1980's rate remained stable*
- *1990 – 1.4 million legal abortions reported*
- *Since 1990 they have decreased by 2-4% per year*
- *1994 – under 1.3 million*
- *Probably an underestimate*
- *CDC figures about 15% less than private sources*
- *Most women are young white and unmarried*
- *Half performed before the eighth week*
- *Five of six in the first trimester*
- *Young women obtain Ab later than older women*
- *90% obtain Ab in their own states*
- *Diagnosis and recognition of pregnancy*
  - *Delays start of prenatal care*
  - *Increases risk of complications*
  - *Limits options of abortion methods*

Methods of Abortion

Techniques

- *Pharmacologic*
  - *Saline*
  - *Other hypertonic agents*
  - *Prostagalndins*
  - *Phospholipids*
  - *Sertotonin and MAO inhibitors*
  - *Pastes*
  - *Systemic toxins*
- *Mechanical*
  - *Extra amniotic fluids*
  - *Bougies & metreurynter*
  - *Supercoils*
  - *Suction D&E*
- *Hysterotomy*

Primitive Attempts

- *Horseback riding & violent excercise*
- *Sitz baths*
- *Coitus*
- *Boxing with blows to abdomen*
- *Electrical stimulation*
- *Potassium permanganate*
- *Air insufflation*
  - *Mouth*
  - *pump*

Foreign Body Method

- *Catheter – soft – rigid (stylet)*
- *Knitting needles*
- *Coat hanger*
- *Screw driver*
- *Curtain rod*
- *Umbrella ribs*
- *Wires*
- *Paint brushes*
- *Chopsticks*
- *Toothbrushes*
- *Goosequills*
- *“abortion machine”*

Douches

- *Nozzle flush with the vagina*
- *Nozzle into the cervix*
- *Nozzle into the posterior fornix*
- *Soap*
  - *Peritonitis*
  - *Emboli*
  - *Hemolysis*
- *Turpentine*
- *Pine oil*
- *Hydrogen peroxide*

Curettage Techniques

- *Use of aspiration dates back to Russian physician first reported in 1927*
- *Chinese 1958*
- *Widespread in europe in 1960's*
- *U.S. since 1970*

Mechanical Techniques

- *Metreurynter*
  - *Described by Manabe in 1969*
  - *Dilate cervix to 12 to 16 Hegar*
  - *Rubber balloon tipped device similar to Foley*
  - *Inflate with 200-300 cc saline*
  - *Weights of 300-800 grams attached and hung from end of bed*
  - *Oxytocin given*
  - *Antibiotics given*
  - *Laminaria tents speed up process*

Mechanical Techniques

- *Bougies – elastic GU rods .5 to 1 cm diameter, 30-40 cm long*
- *One or two inserted extra-ovularly*
- *Ends cut off*
- *Oxytocin infusion given*
- *Removed when labor established*
- *#8-10 Hegar dilator needed to insert*
- *31 hours mean time to abort*
- *Live fetus*

Supercoils

- *First appeared in USA May 1972 in Philadelphia health dept report*
- *Originated by L.A. psychologist*
- *Reported only in lay press*
- *20 women transported to Philadelphia from Midwest hotel*
- *Coils inserted and women shipped back to hotel to abort*

Pastes

- *Developed in Germany in 1930's*
- *Interruptin*
  - *Composite of various ethereal oils such as crocus, aloe, eucalyptus, camphor, iodine, thmol*
  - *Mixed with soaps, olive oil, cocoa butter*
  - *Given through undilated cervix*
  - *If no labor in 24 hours then oxytocin 15-50 units given IM or buccal*

  - *By 1932 there were 25 fatalities*

Urea Instillation

- *80 grams in 150 cc 5% Dextrose*
- *100-250 cc fluid removed*
- *166 or 332 mu/min oxytocin*
- *Diazepam or meperidine*
- *71/74 aborted*
- *Mean time 18.2 hours*
- *BUN rose to 33*
- *WBC increased, fibrinogen drop, FDP's rise, platelets drop*

Extra-amaniotic Solutions

- *Widely used in japan*
- *Extra-ovular catheter inserted and various fluids instilled*
- *30-50 cc Rivanol (disinfectant)*
  - *96% abortion rate - Manabe*
  - *95% abortion rate – Nabriski*
- *Japanese reported on toxic effects of saline and found no advantage*
- *Glucose used as well*

Mechanism of Saline Abortion

- *Spontaneous increase in contractions in 1-2 hours*
- *Increased oxytocin sensitivity*
- *E_2, E_3, P and HCG all fall within 3 hours of injection*
- *Intrauterine volume increases 26% in 3 hours*
- *Na concentration reaches 900-3400 meq/l*
- *Osmolarity increases from 270 mOsm/l to 1980-3960*
- *Mothers Hct falls 10%*
- *Serum N_a peaks at 2-4 hours*
- *Coagulation changes occur rapidly*
  - *Platelets fall*
  - *Fibrinogen falls below 100 mg%*
  - *FDP's in urine in 98%*

Saline Instillation

- *Most widely used until early 80's for 12-28 weeks*
- *Also used for dead fetus evacuation*
- *Difficult before 14 weeks*
  - *Pelvic location of uterus*
  - *Membranes fuse to wall after 16 weeks*
  - *No ultrasound available*
- *100-200 cc 20% NaCl*
- *Times*
  - *Average to abort 34.5 hours*
  - *>72 hours in 11-14%*
  - *>1 week in 5%*

Saline Instillation

- *Time to abort*
  - *Unrelated to gravidity*
  - *Unrelated to age of patient*
  - *Unrelated to gestational age*
  - *Unrelated to amount of saline*

Complications of Saline AB

- *Fever and infection – 2 to 16%*
  - *Pyrogens ?*
  - *Staph, coliforms, diphtheroids. Strep*
- *Hemorrhage (w or w/o DIC) – 4%*
- *Coagulopathy*
- *Siezures*
  - *Headaches, thirst, water intoxication*
- *Peritoneal spillage – peritonitis*
- *Bladder injection*
- *Intramyometrial injection – necrosis*
- *Rh isoimmunization (transplacental hemorrhage)*

Surgical Techniques

- *≤ 14 Weeks:*
  - *Suction Curettage*
  - *Medical Abortion (≤ 56 days LMP)*
- *14-24 Weeks and Beyond:*
  - *Dilatation and Evacuation (D+E)*
  - *Intact D+E ("D+X")* {evacuation/extraction*}*
  - *Labor Induction Methods (Prostaglandins)*
  - *Amnioinfusion (HS, Urea, Prostaglandins)*

Cervical Dilatation

- *Mechanical:*
  - *Done at Time of D+E*
  - *Convenient for Patient*
  - *May be Uncomfortable*
  - *Increased Risk of Perforation (Compared with Osmotic Dilators)*
- *Osmotic Dilators (e.g. Laminaria)*
  - *Increased Time, Inconvenience*
  - *Less Pain, Decreases Perforation Risk*
- *Examples:*
  - *Laminaria japonicum, L. digitatum*
  - *Dilapan*
  - *Lamicel*

Laminaria

- *Hydroscopic seaweeds*
  - Laminaria digitata
  - Laminaria japonicum
- *Gamma radiation*
  - *Does not kill spores*
- *Various sizes*
  - *Strings*
  - *Collar*

Laminaria

- *Inserted 3-6 hours prior to surgery*
- *May be up to one day*
- *Past internal os*
- *Usually results in at least 8 mm dilation*
- *Direct dehydrating effect on cervix*
- *Provoke release of prostaglandins*
- *12 fold decrease in cervical lacerations*

Suction Curettage

- *Office, Clinic or Hospital Setting*
- *Local (Paracervical Block) or IV Sedation*
- *General Anaesthesia Increases Risk*
- *Prophylactic Doxycycline Decreases Endometriitis Risk*
- *Rigid or Osmotic Dilators Used*
- *"No-Touch" Technique*

Dilatation and Evacuation

- *Avoid Mechanical Dilatation if Feasible*
- *Requires Additional Experience and Training*
- *Safer than Amnioinfusion in Most Cases when Performed by Experienced Operator*
- *Less Emotionally Traumatic for Most Patients (Compared With Labor Induction)*

Complications

- *Bleeding*
- *Infection*
- *Retained POC*
- *"Missed Abortion"*
- *Perforation – low risk, high risk variants*
- *Hematometra ("postabortal," or "re-do" syndrome)*
- *Undiagnosed Ectopic Pregnancy*

Menstrual Regulation

- *Aspiration up to 50 days LMP*
  - *Menstrual extraction*
  - *Menstrual aspiration*
  - *Menstrual induction*
  - *Minisuction*
- *Extremely safe*
  - *4-6 mm Karman cannula*

- *Foot or hand pumps*
- *Syringes*

Menstrual Regulation

3.9 % major complication rate

- *Hypotension*
- *Fever*
- *Cervical lacerations*
- *Acute infection*
- *Anesthesia reactions*
- *Uterine perforation*
- *Excessive blood loss*

- *Immediate about 0.85%*
- *Most delayed*
  - *Failed procedures*
  - *Infections*
  - *Ectopic pregnancy (undiagnosed)*
- *Often performed w/o documentation of pregnancy*
- *Prior to 1979 pregnancy test was positive only > 6 weeks LMP*
- *No need to know*
  - *In a study of 500 cases only 65% pregnant*
- *Paracervical or no anesthesia*
- *Rotates and scrapes*
  - *1-10 minutes*
  - *Sensation of bare endometrium, bubbles*

Possible Inhibitors of Myometrial Contractility

- *Progesterone*
- *Prostacyclin*
- *Relaxin*
- *Nitric oxide*
- *Parathyroid hormone-related peptide*
- *Corticotropin-releasing hormone*
- *Human placental lactogen*
- *Calcitonin gene-related peptide*
- *Adrenomedullin*
- *Vasoactive intestinal peptide*

Stimulators of Contractility

- *Increased of contraction-associated proteins*
  - *Myometrial receptors for prostaglandins*
  - *Myometrial receptors for oxytocin),*
- *Activation of certain ion channels*
  - *Increase in connexin 43*
- *Increase in gap junctions*
  - *Electrical synchrony*
  - *Allows effective* coordination *of contractions.*
- *Stimulated to contract by the actions of*
  - *Oxytocin*
  - *Stimulatory prostaglandins E(2)) and F(2)(alpha)).*

Initiation of Parturition

- *Fetus is in control in most viviparous animals*
- *Sheep and cow fetus triggers labor*
- *Human placenta lacks glucocorticoid-inducible enzyme 17(alpha)-hydroxylase-17,20-lyase*
- *Regardless final pathway for labor* ends in the uterus
- *Characterized by the development of regular phasic uterine contractions*

Initiation of Parturition

- *Myometrial contractions mediated through the ATP-dependent binding of myosin to actin*
- *Myometrial cells are sparsely innervated*
  - *become even less so during pregnancy.*
- *Regulation of the contractile mechanism largely humoral*
- *Parturition cascade at term*
- *Removes the mechanisms maintaining uterine quiescence*
- *Recruits factors promoting uterine activity*
- *Multiple positive-feedback loops*

Initiation of Parturition

- *Series of changes within the myometrium, decidua, and cervix*
- *Occurs over a period of* days to weeks
- *Synthesis and release of prostaglandins within the uterus*
- *Formation of myometrial gap junctions*
- *Activation of myometrial oxytocin receptors*
- *Endocrine, paracrine, and autocrine factors*
  - *switch in the pattern of myometrial activity from irregular contractures to regular contractions*

Physiologic Actions of Drugs Inducing Abortion

- *Implantation of a fertilized ovum (embryo)*
- *Complex interactions with the endometrium.*
- *Embryo becomes* attached *to the endometrial epithelium*
- Invades *the endometrial stroma on day 6 to 10 after ovulation.*
- *Depends on progesterone which*
  - *Modifies the transcription of many genes involved implantation process*
  - *Inhibits myometrial contractions*
- *Drugs used to terminate pregnancy*
  - *Inhibiting synthesis of progesterone,*
  - *Inducing myometrial contractions,*
  - *Antagonizing the action of progesterone*
  - *Inhibiting the development of the trophoblast.*

Role of Progesterone

- *Progesterone binds to its receptor*
- *Complex forms a dimer and binds to a segment of the promoter region of different target genes*
- *This genomic effect leads to changes in the structure of epithelial-cell membranes*
- *Synthesis of implantation proteins*
- *Progesterone decreases uterine contraction, probably by a genomic effect.*
- *In contrast, during labor, oxytocin and prostaglandins induce uterine contraction.*
- *Prostaglandins and oxytocin bind to their respective receptors*
  - *Increased phospholipase C activity*
  - *Increased intracellular inositol triphosphate (IP(3))*
  - *Increased calcium*
- *The released calcium interacts with myosin light-chain kinase (MLCK) on the contractile filaments to cause uterine contraction.*
- *Progesterone also exhibits nongenomic action by binding to oxytocin receptor and inhibiting the action of oxytocin*
- *During a normal pregnancy blastocyst attaches to the receptive endometrium, or decidua, on day 6 or 7 after ovulation.*
- *The trophoblast then traverses adjacent cells and invades the endometrial stroma.*
- *The agents used to terminate pregnancy are*
  - *Methotrexate - which inhibits trophoblast division*
  - *Prostaglandins -which increase muscle contraction*
  - *Epostane - decreases progesterone synthesis*
- *Mifepristone - progesterone antagonist*
  - *blocks the binding of progesterone to its receptor*
  - *amplifies the action of prostaglandins on the myometrium*
  - *induces cervical softening*

Oral Agents

- *Ergot*
- *Quinine*
- *Strychnine*
- *Whiskey*
- *Turpentine*
- *Phosphorus*
- *Castor oil*
- *Rosemary, nutmeg, aloe, cloves, thyme*
- *Spanish fly*
- *Arsenic, copper, lead, mercury*
- *Folate antagonists*

Prostaglandins

- *Mifepristone*
- *Misoprostol*
- *Gemeprost*

METHOTREXATE

- *50 mg/sq meter body surface IM*
- *800 ug misoprostol vaginally 3 to 7 days later*
- *Tylenol and codeine for cramps*
- *Return 1 week after misoprotol*
- *If beta hCG not 50% less then offer D&C*
- *Cytotoxic drug used to Rx ectopic and moles*
- *Lethal to trophoblast by blocking folic acid in fetal cells so they cannot divide*
- *Used with misoprostol it is 95% effective*
- *Several protocols are in use*

Inhibition of Progesterone Synthesis

- *Modified steroidal molecules*
  - *(2(alpha),4(alpha),5(alpha),17(beta))-4,5-epoxy-17-hydroxy-4,17-dimethyl-3-oxoandrostane-2-carbonitrile (**epostane**)*
  - *Block at receptor*
- *Inhibitors of ovarian and placental 3(beta)-hydroxysteroid dehydrogenase,*
  - *(**trilostane**)*
  - *Inhibit synthesis of progesterone from its precursor, pregnenolone.*
- *Action of epostane in reducing progesterone synthesis and terminating pregnancy is prevented by the administration of progesterone.*

Anti-Progesterones

- *First progesterone antagonist (antiprogestin) to be developed was **mifepristone***
- *Known as RU 486 or RU 38486*
  - *binds to the progesterone receptor with an affinity five times as great as that of progesterone*
- *Also inhibits transcription resulting in the down-regulation of progesterone-dependent genes*
  - *Decidual necrosis and detachment of the products of conception.*
  - *Endometrial blood vessels, causing damage that further compromises the embryo.*
- *Directly promote uterine contractions*
  - *Increasing myometrial-cell excitability*
  - *Cause cervical dilation.*

Epostane

- *Epostane given alone or in combination with prostaglandin E(2))*
- *Terminate pregnancies of less than 56 days' duration*
- *A dose of 200 mg must be given every six or eight hours for seven days*
- *Epostane caused nausea in 86 percent of women*
- *Success rate of only 84 percent*
- *Currently **not** used for this purpose.*

Prostaglandins

- *Natural prostaglandins - unstable,*
- *Lack specificity, and are poorly tolerated*
- *parenteral prostaglandin analogue sulprostone discontinued - associated with cardiovascular complications - acute myocardial infarction and severe hypotension*
- *Synthetic prostaglandin E(1)) compounds currently used are misoprostol and gemeprost*
- *Misoprostol is inexpensive, can be stored at room temperature, and is available in many countries for the treatment and prevention of peptic ulcer caused by nonsteroidal anti-*

inflammatory drugs.

Prostaglandin Side Effects

- *Dose related*
- *Fever*
- *Chills*
- *Gastrointestinal*
- *Lactation*
- *Bronchospasm*
- *Pre-treatment with Lomotil/Compazine*

Efficacy of Prostaglandins

- *Oral doses of misoprostol ranging from 400 to 3200 micrograms induce abortion in only 4 to 11 percent of women with pregnancies of 56 days' duration or less.*
- *Bioavailability is greater when the drug is administered vaginally and higher success rates have been reported with vaginal administration.*
- *Results with doses ranging from 800 to 2400 micrograms vary considerably*
  - *Rates of complete abortion of 22, 47, 61, and 94 percent have been reported.*
  - *Differences not related to the dose of misoprostol or the duration of gestation*

Side Effects of Prostaglandins

- *High incidence of side effects*
  - *Pain, dizziness, nausea, vomiting, diarrhea, chills, and rashes.*
  - *Fifty-three percent of women given 5 mg of gemeprost required opiate analgesia, as compared with 16 percent given 3 mg*
  - *Women receiving more than 3 mg of gemeprost frequently had to remain in the hospital overnight*
- *Misoprostol failures*
  - *Scalp or skull defects, cranial-nerve palsies, and limb defects such as talipes equinovarus*
- *The increase in uterine pressure related to uterine contractions or vascular spasm may be the cause*

MIFEPRISTONE

- *19-norsteroid*
- *AFFINITY*
  - *progesterone receptor - strong*
  - *glucocorticoid receptors - strong*
  - *androgen receptors – less*
- *Stimulates synthesis of PG by decidua*
- *Available*
  - *France*
  - *United kingdom*
  - *Sweden*
  - *China*
- *1980 compound synthesized at Roussel-Uclaf (hence **RU**-486)*
- *Became available in France soon thereafter*
- *Teutch (1975) studied how small chemical alterations in steroid molecules affected ability to bind*
  - *Developed a method of synthesizing versions of steroids that did not exist in nature*
  - *Alain Belanger (post-doc) the produced the molecules*
- *Initial effort was to produce a gluco-corticoid antagonist to aid wound healing*

- *Most potent was RU-38486 which was also found to block progesterone*
- *Teutsch*
- *Belanger – postdoctoral fellow*
- *Deraedt – progesterone binder as well*
- *Sakiz – corp. exec. Created formal project*
- *Barton – Nobel Laureate chemist*
- *Philibert – supvr. Of RU-486 project*
- *Hodgden – East Va med Sc. – TAB in monkey*
- *Bailieu & Hermann (Geneva) – TAB in humans*

Timetable

- *1950 – Aminopterin (folate antagonist used to produce medically indicated abortions*
- *1972 – PGE2 and PGF2α induced abortion (intolerable side effects)*
- *1975 – Selective prostaglandin analogs (still had side effects)*
- *1980 – More stable analogs (gemeprost {PGE1 methyl ester}, sulprostone {16- phenoxyl-tetranor-PGE2})*
- *1982 – Etienne-Emile Baulieu investigated glucocorticoid blockers and discovered RU-486 (mifepristone)*
- *1985 – Addition of prostaglandin aided in expulsion*
- *1988 – Licensed in France*
- *1993 - Methotrexate*

Rationale for Use of Mifepristone

- *Progesterone needed to sustain early pregnancy*
- *W/o progesterone uterus expels pregnancy*
- *Through prostaglandin mediated mechanism*
- *Epostane (3β-hydroxysteroid dehydrogenase inhibitor) prevents synthesis of progesterone {dosing every 6 hours for many days)*
- *Mifepristone binds the receptor with equal affinity as progesterone without activation*
- *Alters endometrium by affecting the capillary endothelium of the decidua (trophoblast separates and bleeding ensues)*
- *Also affects the tissues of the cervix*

MIFEPRISTONE

- *Most effective in early pregnancy*
- *7 weeks or less LMP have 95% rate*
- *9 weeks have 80%rate*
- *No good studies above 9 weeks*
- *Similar to miscarriage*
- *Use narcotics rather than NSAIDS*
- *1% need curretage*
- *.1% need transfusion*

MIFEPRISTONE

- *Three visits*
  - *600 mg mifepristone (5% expel)*
  - *400 ug cytotec orally 48 hours later*
  - *Return in 2 weeks for checkup*
  - *Dose not yet established (200 to 600 ug)*
- *600 mg orally*

- *36-48 hours later give a prostaglandin analog*
  - *Gemeprost transvaginally*
  - *Sulprostone IM*
  - *Misoprostol PO (400 ug)*
- *Earlier PG analogs unstable at room temp*
- *Misoprotol (Cytotec) used for treatment of ulcers*
- *Second dose may be given if no abortion*
- *Currently people are using 200 mg mifepristone and 800 ug misoprotol for 56 days with complete abortion rates of 97%*

Methotrexate and Prostaglandins

- *Methotrexate and misoprostol very effective in terminating pregnancy*
- *Dose of 50 mg per square meter of body surface*
- *Intramuscular injection*
- *Oral administration (25 or 50 mg) is also effective.*
- *Three to seven days after the methotrexate has been administered, misoprostol (800 microg) is administered by the vaginal route.*
- *Success <56 days: ranges from 84 to 97 percent.*
- *Efficacy*
  - *Immediate success (before misoprostol)*
  - *During the 24 hours after its administration*
  - *Delayed success (>24 hours after misoprotol)*
- *Abortion is often delayed;*
- *12 to 35 percent of women, it occurs approximately 20 to 30 days after the administration of misoprostol*

FUNDAMENTAL QUESTIONS

1. What is the safety of first trimester abortion?
2. What early methods were tried to terminate pregnancies?
3. What is a D&E and a D&C?
4. How are second trimester terminations accomplished today? How about 15 years ago?
5. What is a “saline abortion”?
6. How are prostaglandins employed to terminate pregnancies?
7. What are some of the complications of D&E?
8. What is methotrexate and how does it work?
9. What is the safest method of first trimester abortion?
10. What are some of the theories of the initiation of parturition?
11. Describe the sides effects of prostaglandin therapy for termination.