Embryological Development of the Urogenital System

“Urogenital tract development involves a complex interplay of multiple cell types, and it occurs during a relatively narrow time window. The temporal pattern of gene expression and the spatial relationships of the developing tissues is vitally important for normal development.”

“The close association between the müllerian and mesonephric ducts has clinical relevance, because damage to either duct system will most often be associated with damage to both – uterine horn, kidney and ureter”.

Oocyte Development

DNA Synthesis, Genetic recombination, Meiotic arrest in diplotene of prophase I (lampbrush chromosomes), Maturation signal, Meiosis II arrest in metaphase II, Ovulation signal, Fertilization, Release from meiosis II arrest, Re-establishment of diploid state, Mitosis
Ovulation and Oocyte Capture by Oviduct Fimbriae

Fimbriae move to cover the ovary surface and the oviduct initiates rhythmic contractions to move the oocyte toward the uterus.

Fertilization Occurs in the Upper Region of the Oviduct

Total number of sperm/ejaculation range between 280-500 million
Total number of sperm reaching upper oviduct for fertilization range from 1500-3000

Steps in Fertilization
Fertilization Releases the Oocyte From Meiosis II Block

- Activation of a receptor tyrosine kinase pathway
- Activation of PLC → PIP₃
- Increased Ca²⁺ concentration
- pH shift (6.8 → 7.2) (H⁺ ↔ Na⁺)
- Release from meiosis II block
- Nucleus becomes reprogrammed to initiate mitotic cell cycle

Pronuclei Development, Fusion and Formation of First Mitotic Division Figure

- DNA synthesis
- Chromosome alignment
- First Cleavage - 24h

First Mitotic Division Figure

- Compaction – tight junction formation – E-cadherin appears
- Zona pellucida “hatching” at day 5 post fertilization

Early Cell Division and Formation of the Blastocyst

- Cell Division
- Compaction
- Hatching
Overview of Early Development Leading to Blastocyst Implantation

*Secretes progesterone, estradiol, inhibin A

Abnormal Implantation Sites – Associated with Early Embryo Hatching From the Zona Pellucida

Normal implantation usually occurs along either the posterior or anterior wall of the uterine body

Abnormal Implantation Sites

<table>
<thead>
<tr>
<th>Location</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal or recto-uterine pouch</td>
<td>1.2%</td>
</tr>
<tr>
<td>Ampullary</td>
<td>54%</td>
</tr>
<tr>
<td>Inferior</td>
<td>25%</td>
</tr>
<tr>
<td>Isthmus</td>
<td>17%</td>
</tr>
<tr>
<td>Interstitial</td>
<td>2%</td>
</tr>
<tr>
<td>Ovarian</td>
<td>0.5%</td>
</tr>
<tr>
<td>Cervical</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

Representative Stages of Blastocyst Implantation into the Endometrium

The villous syncytiotrophoblast cells secrete human placental lactogen (HPL), also called human chorionic somatomammotropin (HCS), estrogen, progesterone and HGC during the course of pregnancy.
Formation of the Trilaminar Germ Disc and Primitive Streak Occurs during Week Two of Development

Cross sectional Representation Showing Cell Movement Through the Primitive Streak Forming the Mesoderm and Endoderm

Schematic representation of cell movement through the primitive streak. Internalizing cells moving through the primitive node give rise to the embryonic axis proper (notochord, axial mesoderm). All others give rise to endoderm, intermediate and lateral plate mesoderm and extraembryonic tissues.

Computer-generated image from a Micro-MRI, actual length 1.5 mm. Image represents a side view of an embryo during its third week of development. The white line is the early embryo.

Embryogenesis of the Female Reproductive Tract

• Origin of reproductive structures is closely tied to early renal development because both are derived from the intermediate mesoderm tissue, a region between the somites and lateral plate mesoderm.
• Forms the longitudinal urogenital ridge that divides into the nephrogenic ridge and the genital ridge.
  - By gestational weeks 3-4 the mesonephric ducts have extended down to merge with the cloaca.
  - By approximately week 5 the uterine buds form from the mesonephric (Wolffian) duct near the cloaca and migrate cranially into the metanephric mesenchyme to form the ureter and induce the metanephric kidney.
Gonad development is dependent on the presence of primordial germ cells (PGCs).

- PGCs form in the posterior region of the human embryo at the angle between the allantois and the wall of the yolk sac and split into two migratory cell populations.
- These move caudally from the yolk sac wall through the hindgut endoderm (diapedesis) and along and finally up the dorsal mesentery into their respective genital ridges.

Genital ridges are paired regions of intermediate mesodermal mesenchyme medial to the developing kidney.

- Induced by actions of the mesonephric tubule cells.
- Appear during week 4 and remain as indifferent gonads until week 7.
- Epithelial cell proliferation cause the region to bulge out into the coelom.
- Mesenchyme cell migration disrupts the sex cords and establishes the ovarian stroma.

- Upon entering the genital ridges, the PGCs associate with the secondary sex cords and cease motility.
- Within sex cords PGCs proliferate giving rise to oogonial nests (germ cell cysts) connected by cytoplasmic bridges. All mitosis ceases by the 3rd trimester of gestation
- First primary follicles detected at mid-gestation (week 16) and increase to approximately 7 million (week 20).
- Upon cessation of mitosis, oogonia enter meiosis I as primary oocytes and become arrested in prophase I in the diplotene I substage (crossover).
- By puberty the number of primary follicles has decreased (via apoptosis) to a little more than 400,000.
The Indifferent gonad

- In the early embryo at the indifferent gonad stage both male and female ducts are observed.
- In females in the absence of the SRY genes, testosterone and AMH (MIS) the mesonephric ducts degenerate and the gonad develops into an ovary.
- The mesonephric (Wolffian) ducts persist only in vestigial form and are detected either adjacent to the uterus as Gartner’s cysts or as the tubular remnants epoöphoron and paroöphoron of the mesoovarium.
- In the developing ovary the primitive sex cords degenerate and the genital ridge mesothelium forms the secondary sex cords which will become the granulosa and follicle cells that the surround the oocytes.
- At 4.5 weeks primordial germ cells enter the gonad and in developing ovaries, remain in the cortical region.

Embryogenesis of the Female Reproductive Tract

- Normal development of the female reproductive tract entails a series of highly orchestrated, complex interactions that direct differentiation of the Müllerian ducts and urogenital sinus (UGS) to form the internal female reproductive tract. Although they originate from different germ layers, the developmental fate of the müllerian ducts (intermediate mesoderm) and UGS (endoderm) are interconnected.
- Abnormalities of the female reproductive tract are usually the result of fusion defects and are often, but not always, associated with various multiple malformation syndromes such as Antley-Bixler, Bardet-Biedl, Johanson-Blizzard or Fraser Syndromes.

Hox genes confer regional specificity along the anterior/posterior axis of animals

Hox genes in mouse and human with their phylogenetic counterparts in Drosophila
Hox gene expression in the developing female reproductive tract


Origin of the Müllerian (Paramesonephric) Ducts

- Formed by invagination of the coelomic epithelium of the mesonephros, at about week 6, in a cranial → caudal direction, coursing lateral to the mesonephric (Wolffian) ducts toward the midline.
- Cranially the duct opens into the coelom as a funnel-like structure (ostium tubae).

Origin of the Müllerian (Paramesonephric) Ducts

As the two ducts descend caudally and medially they merge at about 10 weeks gestation at the midline in a caudal → cranial direction to form the uterovaginal canal, fusing behind the cloaca, near the mesonephric ducts to terminate at the sinovaginal bulbs adjacent to what will become the urogenital sinus. Mesenchyme surrounding the uterus condenses to form the myometrium. Dissolution of the midline septum is usually completed by 20 weeks.
Abnormalities of Uterine Formation

- Anomalies may be congenital or acquired.
- Present with abnormalities of the menstrual cycle, pelvic pain, infertility or pregnancy wastage.
- True incidence of anomalies is unknown because in the absence of symptoms most go undiagnosed.
- 57% of women with uterine defects have successful fertility and pregnancy.
- The mean incidence of uterine malformations was reported as 4.3% for the general population and/or for fertile women. (Grimbizis et al., Hum Reprod Update. 2001 Mar-Apr. 7(2):161-74.)
- Müllerian defects are associated with renal anomalies in 30-50% of cases, ranging from renal agensis and severe hypoplasia to ectopic or duplicate ureters (Shara FI, J. Reprod. Med. 34:29, 1998).
- Some form of müllerian hypoplasia or agenesis affects one in every 4,000 - 5,000 females.

Abnormalities of Uterine Formation

Table 1. ASRM classification of müllerian anomalies

<table>
<thead>
<tr>
<th>Classification</th>
<th>Anomaly</th>
</tr>
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</table>
| Class I (agenesis/hypoplasia) | a. Vaginal  
b. Cervical  
c. Fundal  
d. Tubal  
e. Combined anomalies |
| Class II (unicornate) | a. Communicating  
b. Noncommunicating  
c. No cavity  
d. No horn |
| Class III (didelphys) | Didelphys |
| Class IV (bicornuate) | a. Complete  
b. Partial |
| Class V (septate) | a. Complete  
b. Partial |
| Class VI (Anomalies) | Anomalies |
| Class VII (DIE treated) | DIE related |

ASRM, American Society of Reproductive Medicine; DIE, dyshormonogenesis.

Anatomical Abnormalities in Uterine Development
Abnormalities of Uterine Formation Without Obstruction

- Most commonly formed by defects in Müllerian duct fusion and/or septal reabsorption.
- Various genetic abnormalities of the uterus that can be detected by hysteroscopy:

Abnormalities of a Didelphys Uterus with the Presence of an Obstruction – OHVIRA syndrome

- In some, though rare cases, the formation of a didelphys uterus is accompanied by either a complete or incomplete unilateral vaginal obstruction, and ipsilateral renal agenesis (Obstructed Hemivagina and ipsilateral Renal Anomaly). A. Complete vaginal obstruction. B. Incomplete vaginal obstruction. C. Complete vaginal obstruction with a lateral communicating double uterus. D. Magnetic resonance image (Axial T2-W) OHVIRA syndrome showing uterine didelphys, obstructed hemivagina, and ectopic ureter on MR imaging in a 17-year-old girl. large arrows - two widely separate uterine horns; asterisk - obstructed left hemivagina distended with fluid; arrowhead - nondilated right hemivagina. Modified from (From: Rock JA, Jones HW Jr: Am J Obstet Gynecol 138:340, 1980).

Mayer-Rokitansky-Küster-Hauser syndrome

Uterine agenesis – a condition whereby the vagina and uterus are underdeveloped or absent due to a failure of Müllerian duct formation. Frequency = 1:4000-10,000 female births (Amer Coll OB-GYN, May, 2013)

Third most common cause of primary amenorrhea after pregnancy and gonadal failure (such as from Turner syndrome).

The first noticeable sign of MRKH syndrome is that menstruation does not begin by age 16 (primary amenorrhea).

Females with MRKH have a normal chromosome pattern (46, XX) and normally functioning ovaries.

They also have normal female external genitalia and normal breast and pubic hair development.

Inheritance pattern is unclear - signs and symptoms of the condition frequently vary among affected individuals in the same family. However, in some families, there is an indication for an autosomal dominant pattern of inheritance.
MRKH syndrome Classifications

Classifications:
- Typical MRKH – Isolated uterovaginal aplasia/hypoplasia. Prevalence – 64%
- Atypical MRKH – Uterovaginal aplasia/hypoplasia with renal malformation or uterovaginal aplasia/hypoplasia with ovarian dysfunction. Prevalence – 24%
- MURCS syndrome – (Mullerian, renal, cervicothoracic, somite) uterovaginal aplasia/hypoplasia with renal malformation, skeletal malformation, and cardiac malformation. Prevalence – 12%

Development of the Urogenital Sinus

- By the 7th week the cloaca is divided by an upper urorectal septum (Tourneux fold) that meets the medial Rathke’s folds forming a separate rectum and urogenital sinus.
- The urogenital sinus can be considered as having three parts:
  - Cephalad (cranial; vesicle) portion which gives rise to the urinary bladder.
  - Pelvis (middle) portion that gives rise to the female urethra.
  - Caudal (phallic) portion giving rise to distal vagina, greater vestibular (Bartholin), urethral and paraurethral (Skene) glands (also known as the lesser vestibular glands).

Development of the Vagina

- Vaginal development is not well understood.
- Earlier understanding was that the upper part of the vagina derived from Müllerian ducts and the lower part from the sinovaginal bulbs (formed by fusion to form the vaginal plate) all derived from the urogenital sinus.
- Current molecular studies show the whole vagina may be derived from the Müllerian duct with bone morphogenetic protein 4 (BMP4) reshaping the intermediate mesoderm-derived Müllerian duct into the vaginal primordium.
- Whatever the precise case, the most inferior portion of the uterovaginal canal becomes occluded by a cellular mass derived from the sinovaginal bulbs that forms the vaginal plate.
Development of the Vagina

- Epithelial cells of the vaginal plate desquamate during the second trimester causing the canalization of the vaginal lumen.
- The hymen is the partition that remains between the dilated canalized fused sinovaginal bulbs and the urogenital sinus, becoming perforated either shortly before or after birth.

Vaginal Abnormalities

- In vaginal atresia the urogenital sinus fails to contribute the caudal portion of the vagina. The lower fifth to third of the vagina is replaced by 2–3 cm of fibrous tissue, above which lie a well-differentiated upper vagina, cervix, uterine corpus, and fallopian tubes.
- Transverse vaginal septa occur at several locations and may be complete or incomplete. These septa are usually about 1 cm thick (but can be thicker) and located near the junction of the upper third and lower two-thirds of the vagina, however, septa may be present in the middle or lower third of the vagina. Occurrences have been reported as 46%, 35%, and 19% in the upper, middle, and lower portion of the vagina (Rock, et al., Obstet. Gynecol. 59:448, 1982). Perforations are usually central but may be eccentric in location.
- Vaginal septa may also be longitudinal (sagittal or coronal). Longitudinal septa, which rarely produce clinical problems, probably result from abnormal mesodermal proliferation or persisting epithelium.

Phenotypic differentiation of the external genitalia in female embryos

- Default development of the female external genitalia occurs due to the lack of the SRY region of the Y-chromosome and the lack of TDF.
- During week 3 mesenchyme cells from the primitive streak migrate around the cloacal membrane forming a pair of slightly elevated cloacal folds.
- Cloacal folds unite anteriorly forming the genital tubercle.
- In week 6 the cloacal membrane subdivides into the urogenital and anal membranes, subdividing the cloacal folds into the urethral and anal folds. Another pair of elevated swellings, the genital swellings appear on each side of the urethral folds. At this point the indeterminate stage of external genitalia development has been reached.
- From this point the following changes occur:
  - The genital tubercle develops into the clitoris
  - The genital swellings become the labia majora
  - Urethral folds develop into labia minora
  - The introitus (vaginal orifice) develops between the urethral folds.
21-hydroxylase Congenital Adrenal Hyperplasia (CAH). Is the most common form (90-95% of cases) of this genetic condition causing the adrenal glands to make excess male hormones (androgens). In this case, ovaries, the uterus, fallopian tubes, upper vagina, and other müllerian structures are normally formed.

Prenatal exposure to substances with male hormone activity. Certain drugs, including progesterone (taken in the early stages of pregnancy to stop bleeding) and anabolic steroids, can cause developing female genitals to become more masculinized.

Depending on the severity of hyperandrogenism, a female infant can be mildly affected, obviously ambiguous, or so severely virilized as to appear to be a male. (see Prader scale)

Molar Pregnancy - The Hydatidiform Mole

- An abnormal form of pregnancy in which a non-viable fertilized egg implants in the uterus, which fails to come to term.
- Develops when an egg that is missing its nucleus is fertilized. These may or may not contain fetal tissue.
- Considered a gestational trophoblastic disease that grows into a mass in the uterus with swollen chorionic villi that grow into clusters resembling grapes due to their fluid accumulation/dissection.
- A complete mole is caused by a single (50%) or two (10%) sperm combining with an egg which has lost its DNA. The sperm then undergoes reduplication forming a "complete" 46 chromosome set. The genotype is typically 46 XX (diploid), but can also be 46 XY (diploid). These have a higher risk of developing into a choriocarcinoma — a malignant tumor of trophoblast cells than do partial moles.
- A partial mole occurs when an egg is fertilized by two sperm or by one sperm which reduplicates itself yielding trisomal genotypes 69 XXXY or 69 XXXXY.
- Some of these moles occur in women who carry mutations in the gene NLRPT, located on the long (q) arm of chromosome 1), predisposing them towards molar pregnancy.

Molar Pregnancy – The Hydatidiform Mole (continued)

- A common complication of pregnancy (1/1000) in the US, with much higher rates in Asia.
- Etiology of the condition from the oocyte perspective is not well understood.
- In rare cases a hydatidiform mole co-exists within the uterus with a normal viable fetus, due to two conceptuses.
- The diploid set of sperm-only DNA means that all chromosomes have sperm-patterned methylation suppression of genes (imprinting). This leads to overgrowth of the syncytiotrophoblast (androgenic development) with an underdeveloped embryo.
- Experimentally derived dual egg-patterned methylation leads to a channeling of resources to the embryo, with the underdevelopment of the syncytiotrophoblast (gynogenic development).
- Both conditions reflect the requirement that male and female derived haploid genomes are needed for proper fetal development due to specific parental dependent genomic imprinting.
Prader-Willi Syndrome – Disorder Due in Part to Imprinting on the Long Arm of Chromosome 15

- Prader-Willi Syndrome (PWS) is a genetic disorder and the most common syndromic cause of obesity (1:10,000 – 30,000 worldwide). Occurs equally in both sexes and all races.
- The genetic defect is lack of expression of the paternally inherited region of chromosome 15 (15q11-q13).
- Normally this region on chromosome 15 is expressed from the paternal allele, while the maternal allele is hypermethylated, thus “silencing” the transcripion of genes on the maternal chromosome.
- Clinical manifestations involve primary neuropsychiatric and endocrine defects with secondary involvement in many different systems including hypogonadism: Incomplete, delayed or abnormal pubertal development.
- Men are thought to be infertile; in women there are two known case reports of a PWS patient reproducing.

Human Twinning

“i wish I had a twin, so I could know what I’d look like without plastic surgery”. – Joan Rivers

Incidences of Twinning*

Increases as the population ages (delayed child bearing).
Use of ovulation-enhancing drugs (such as clomiphene).
Increased use of assisted reproductive technology (ART).
Although the dizygotic (DZ) twinning rate varies widely under different circumstances, the monozygotic (MZ) twinning rate has been remarkably constant, between 3.5 and 4 per 1000 pregnancies.

Murphy and Hey found the rate to have slightly increased in the 1990s. In recent national statistics, 3.3% of 4 million births in the United States were multiples, or 1 in 30 gestations. (Murphy M. and Hey K.: Twinning rates. Lancet 349:349, 1997).


* From: Creasy, Robert K., MD: Resnik, Robert, MD; Iams, Jay D., MD; Lockwood, Charles J., MD; Moore, Thomas R., MD; Greene, Michael F., MD, Creasy and Resnik's Maternal-Fetal Medicine: Principles and Practice, Seventh Edition. 2014
Origins of Dizygotic Twinning

Non-identical DZ twins occur when two fertilized eggs implant in the uterine wall that were independently fertilized by two sperm.

Dizygotic embryos implanting close to one another may fuse portions of their chorionic membranes and placentas.

The frequency of monozygotic twinning is 0.3% of all pregnancies.

Monozygotic Twins

- Dichorial/Diamniotic (33%)
- Monochorial/Diamniotic (66%)
- Monochorial/Monoamniotic (rare)

Monozygotic Twinning Formed as a Result of Cell Splitting Prior to Compaction (8-16 Cell Stage) Leads to the Formation of Two Morulas

(Dichorial/diamniotic twinning)
Monozygotic Twinning in Early Development After Compaction
(≈ 16 cell stage) Results in Separate Inner Cell Masses - Placental Vessels May Form Anastomosis With Each Other

Twin-twin transfusion syndrome (TTTS) in MZ pregnancies

- A serious condition that can occur in identical twins that share a placenta. (3/10,000 births; 15-20% of monochorionic twins)
- Abnormal blood vessel connections form in the placenta and allow blood to flow unevenly between fetuses.
- Causation not due to an arteriovenous shunt but rather when a cotyledon is fed by an artery from one twin and the blood is then drained by a vein into the other twin.
- May exist singly or can be multiple, and blood may shunt in opposing directions.
- The donor fetus continuously shunts blood into the recipient fetus.
- Leads to hypervolemia (hypertension) of the recipient and hypovolemia (hypotension) of the donor. Cardiac compensation (hypertrophy in the recipient) ensues first and can be seen in abortuses afflicted by TTTS. This is followed by a wide spectrum of bodily growth differences. In early pregnancy cardiac failure/death may result in recipient fetus.
- A common symptom is rapid uterine expansion resulting from polyhydramnios of the recipient, presumed to be secondary to excessive fetal urination. It usually manifests between 20 and 30 weeks of pregnancy, can attain enormous proportions, and is considered a frequent cause of preterm labor.

Development of Conjoined Twins

Actual cause of this event is unknown. It's believed that when the embryo splits later than 12 days post-fertilization — usually between 13 and 15 days — separation stops before the process is complete, and the resulting twins are conjoined.

Conjoined twins account for every 1 out of 200,000 births across the world. The University of Maryland reported that 40 to 60 percent of conjoined twins don't survive, while 35 percent live for about 24 hours. Generally, the likelihood of survival for conjoined twins can be as low as 5 percent, and up to 25 percent. About 70% of cases are females.
An alternative model of monozygotic twinning. In this model, splitting occurs at the postzygotic 2 cell stage, with each cell forming a distinct individual. If twin blastocysts hatch from the zona pellucida together, dichorionic diamniotic twins will result. If the 2 trophoectoderms fuse before hatching and the inner cell masses are separated within the shared trophoectoderm, monochorionic diamniotic twins will result. If the inner cell masses are fused and separated later, (after amnion formation) monochorionic monoamniotic twins will result. McNamara et al., A review of the mechanisms and evidence for typical and atypical twinning. Am J Obstet Gynecol 214(2):171-192, 2016.

Alternative Hypothetical Models of Twinning Thought to Arise Due to Alternative Reproductive Technologies (ART)

Hypothesis 1 (not shown) accords with the traditional model of monochorionic diamniotic twinning in which placental anastomoses may result in intertwin transfer of blood cells with subsequent blood cell chimerism. Hypothesis 2 follows the traditional model of dizygotic twinning up to the hatching stage. If 2 hatched blastocysts are in close proximity, as with the use of assisted reproductive technologies, trophoectoderm fusion may occur. Hypothesis 3 involves fertilization of a binovular follicle in which 2 oocytes exist within a single zona pellucida. In each hypothesis, fusion might also occur after implantation. In rare cases, cells from the inner cell mass may be transferred between twins, resulting in some degree of somatic chimerism (not shown). McNamara et al., A review of the mechanisms and evidence for typical and atypical twinning. Am J Obstet Gynecol 214 (2):172-192, 2016.

Other alternate hypothetical twinning processes due to ARTs