School of Medical Sciences - Anatomy

Reproductive Embryology

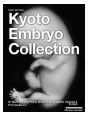
REI Trainees - Reproductive Medicine Seminar 2018
23rd March 2018

UNSW Embryology

https://embryology.med.unsw.edu.au

Background

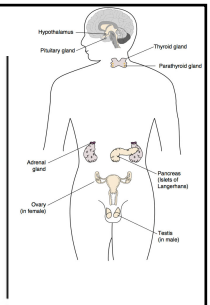
- Uterine Tube Biobank (Prof Ledger)
- Trophoblast differentiation
- Digital Embryology Consortium
- Kyoto Collection (eBook)
- Human SEM (eBook)
- UNSW Embryology



https://embryology.med.unsw.edu.au

Seminar Topics

1. Preimplantation
2. Implantation
3. Urogenital
4. Endocrine

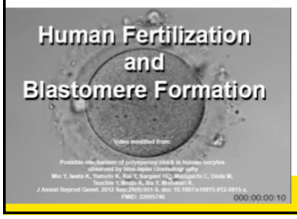


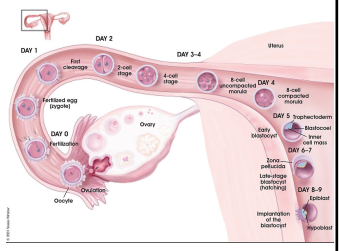
UNSW Embryology

http://tiny.cc/REI_Embryo_Seminar_2018

1. Preimplantation - Zygote

- Paternal - Genome
  - 2 pronuclei stage
  - Male pronucleus demethylated
    - epigenetics





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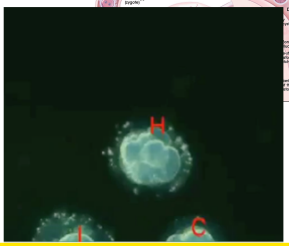
1. Preimplantation - Morula

- Mitosis
  - "embryonic" cell cycle S phases and M phases alternate without intervening G1 or G2 (MSMSMSMS, adult MG1SG2)
- day 4
  - solid ball 16-20 cells
  - peripheral cells flattened against zona pellucida

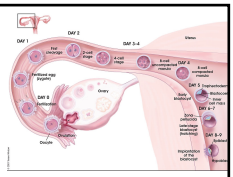
Day 3 to 6



UNSW SYDNEY

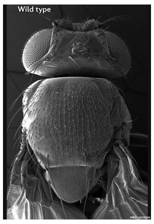
1. Preimplantation

- Blastocoel formation (day 5)
  - 2 cell types and fluid-filled cavity (blastoceol)
  - Trophectoderm (TE)
    - peripheral flattened cells
    - placenta and placental membranes
    - CDX2
  - Inner cell mass (ICM)
    - rounder cells located on one wall
    - entire embryo
    - OCT4, NANOG and SOX2

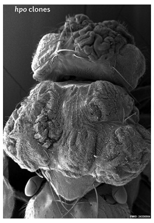


UNSW SYDNEY

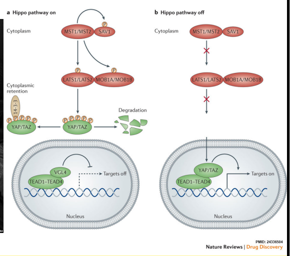
Hippo Pathway



Wild-type (WT)



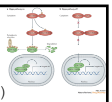
Hippo-type (hpo)



Johnson R & Halder G. (2014). PMID 24336504

https://embryology.med.unsw.edu.au/embryology/index.php/Developmental_Signals_-_Hippo

1. Preimplantation - Molecular



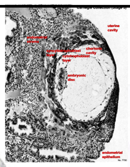
- **ROCK1 and ROCK2 signaling** (bovine)
  - RHO-associated coiled-coil kinases
  - promote formation of TE
  - inhibit formation of ICM
  - by polarizing outer cells to inactivate Hippo signaling
  - tension-dependent junctional localization
- **HIPPO signalling** (mouse)
  - ICM - active
  - TE – inactive
  - RHO/ROCK outside cells
    - apicobasal polarity
  - YAP/TAZ nuclear localization
    - transcriptional co-activators
    - downstream effectors of HIPPO

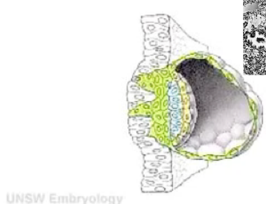
Adherens junctions -> ROCK -> Epithelial polarity -> Hippo inactive -> YAP and TAZ active (transcription factors)

ROCK - Mol Reprod Dev. 2018 Mar 15. PMID: 29542836

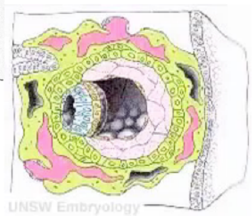
2. Implantation

GA Week 4





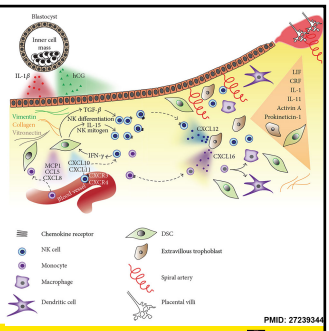
Uterine receptivity – about 6 days after the post-ovulatory progesterone (lasts about 2 to 4 days) decreased MUC1



Pro-invasive factors - IL-1β, IL-5, IL-6, IL-7, IL-8, IL-9, IL-13, IL-15, Eotaxin CCL11, IP-10 and RANTES.
Anti-invasive factors - IL-10, IL-12 and VEGF.

Implantation

- Species - Problem in literature
- Trophoblast and endometrial factors
- cytokines and other growth factors
- Drive cell recruitment, invasion, and spatial distribution and communication



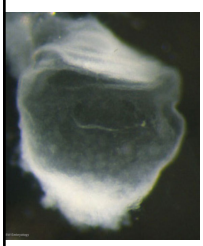
PMID: 27239344

Vinketova (2016), PMID: 27239344

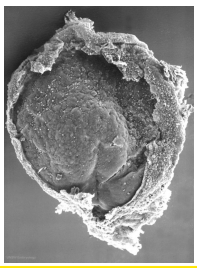
3. Urogenital

Gestational Age (weeks)	Event	Fertilisation Age (weeks)
5	primordial germ cells (PGCs) migrate during gastrulation	3
6	intermediate mesoderm, pronephros primordium	4
7	mesonephros and mesonephric duct (Wolffian duct)	5
8	ureteric bud, metanephros, genital ridge	6 (35 days)
9	cloacal divison, gonadal primordium - indifferent to first appearance of testis cords	7 (42 days)
10	paramesonephric duct (Müllerian duct), clear gonadal differentiation	8 (49 days)
11	paramesonephric duct fusion (female)	9 (56 days)
17	primary follicles (ovary)	15 (100 days)

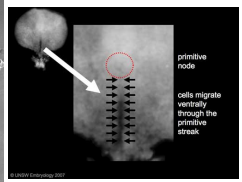
Week 5 – PGC Formation



Carnegie stage 7



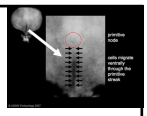
Gastrulation



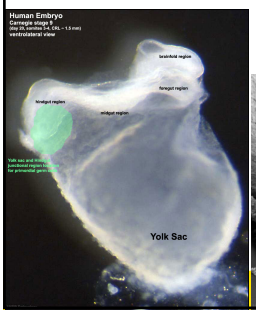
Ectoderm
Mesoderm
Endoderm

Remnant primitive streak cells - Sacrococcygeal teratomas
(most common solid tumor in newborn infants)

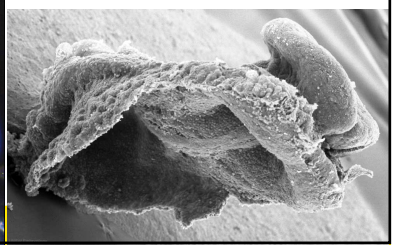
Week 5 – PGC Early Location



BMP4 - germ cell fate



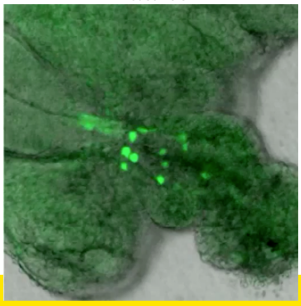
Carnegie stage 9



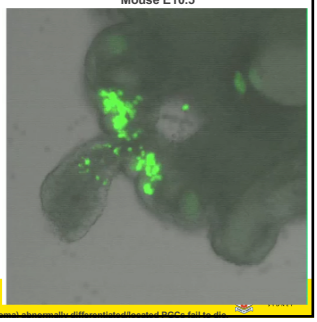
Week 6 – PGC Migration – 3 phases

PGC

Mouse E9.5



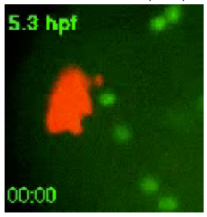
Mouse E10.5



Week 6 – PGC Migration

- Migration – Chemoattraction
  - **CXCR4** (C-X-C motif chemokine receptor 4)
    - expressed by PGCs
  - SDF-1 (Stromal cell-derived factor 1)
    - **CXCL12**, C-X-C motif chemokine ligand 12
    - expressed in the genital ridges and surrounding mesenchyme
  - Genome-wide DNA demethylation occurs during migration
  - Male - form gonocytes undergo fetal mitosis

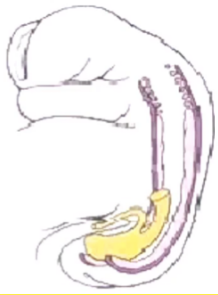
Chemotaxis of Zebrafish PGCs toward explant expressing SDF1a

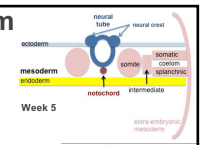


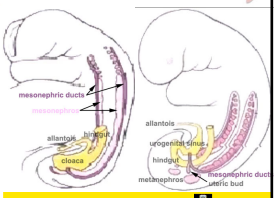
J Cell Sci. 2005 Sep 1;118(Pt 17):4027-38

OMIM - https://www.omim.org
HUGO Gene Nomenclature Committee - https://www.genenames.org

Week 6 – Intermediate Mesoderm







Carnegie stage 12

Week 6 to 7

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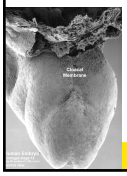
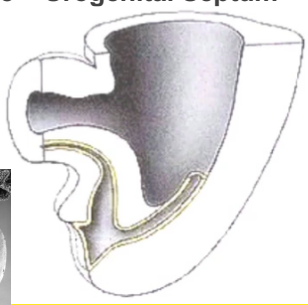
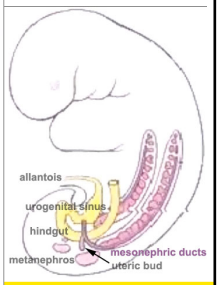
Week 8 – Urogenital Septum







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Genital Stages

1. Development of the indifferent gonad - (genital ridge) **early embryo**
2. Differentiation of gonad - (testis or ovary) **late embryo**, defining event in sexual differentiation
3. Differentiation of internal genital organs and ducts - **late embryo to fetal**
4. Differentiation of external genitalia – **fetal**
5. Development of secondary sexual characteristics - **puberty**

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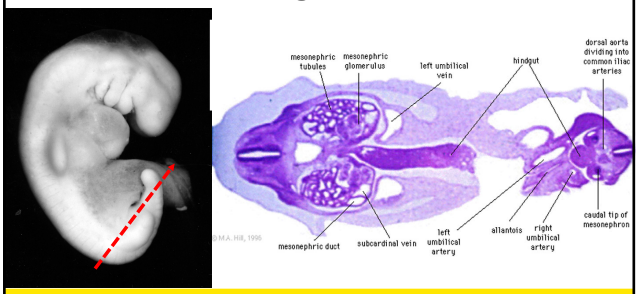
Week 7 – Genital Ridge



Carnegie stage 13

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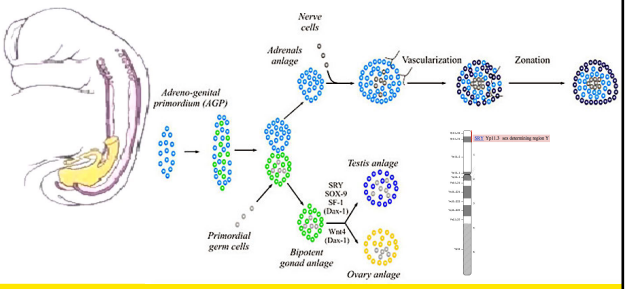
Week 8 – Indifferent Gonad



PGCs, Intermediate Mesoderm

Val etal., (2003) PubMed 14594453

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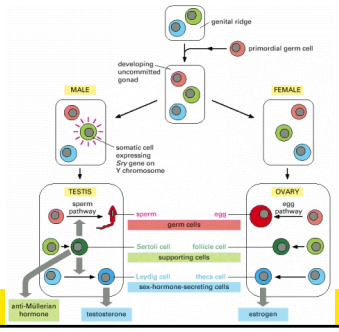
Sex Differentiation



Alberts etal (2002) MBoC

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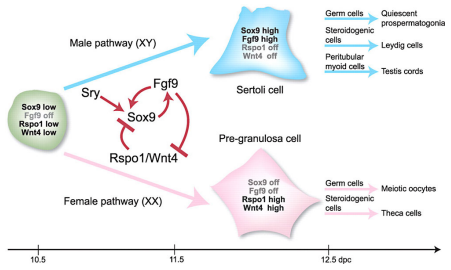
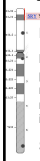
Sex Differentiation



Kocer teal., (2009) PMID: 19218284

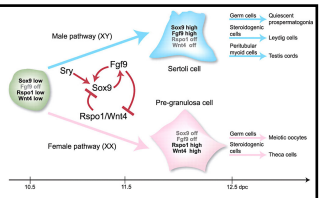
Male – Sertoli Cells



- Human SRY activation not known

  Mouse - Wilms tumor 1 (Wt1), GATA binding protein 4 (Gata4), zinc fnger protein, fog family member 2 (Zfpm2), chromobox homolog 2 (Cbx2), mitogen-activated protein kinase 4 (Map3k4), insulin receptors.

- SRY activates SOX9
  - SOX9 requires positive regulatory loop
  - Sox9 and Fgf9 feed-forward loop upregulates Fgf9 expression
  - repression of a WNT4
- Retinoic acid (RA) Sertoli to germ cells essential for adult mammalian spermatogenesis.



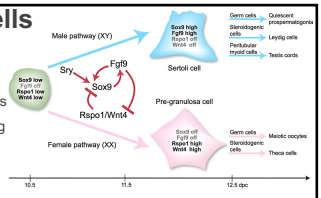
Sertoli cells - expression of SOX9, FGF9, and PTGDS

PTGDS - (lipocalin-type prostaglandin D2 synthase) catalyzes conversion of prostaglandin H2 to **prostaglandin D2**.

SOX9 - (17q24.3) transcription factor

Female - Granulosa Cells

- **Wnt4** secreted protein, inhibit testis-specific processes
  - mesonephros migration of endothelial cells
  - repress steroidogenesis by Sf1 or blocking recruitment of steroidogenic cell precursors
- **RSPO1** secreted protein
  - agonist WNT4 signalling
- **FOXL2** transcription factor
  - repress Sox9 by synergistic interaction with estrogen receptors α and -β (ER-α-β)
  - postnatal follicle development and female fertility maintenance

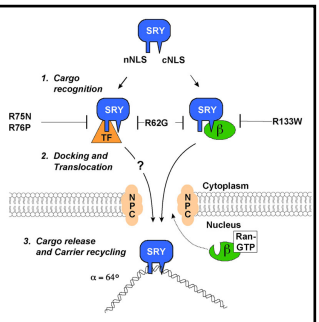


Granulosa cells - WNT4, R-spondin (RSPO1), β-catenin, FOXL2, follistatin (FST)

https://embryology.med.unsw.edu.au/index.php/Developmental_Signals_-_Wnt

XY Females

- Defective importin beta recognition
  - Lack nuclear import of SRY associated with XY sex-reversing
- Mutations
  - R75N and R76P (nNLS non-functional)
  - R133W (cNLS mutation)
  - R62G (nNLS mutation)



Harley VR, etal., (2003) PMID: 12764225

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Mammalian Sexual Development Genes

Gene (OMIM)	Protein Function	Gonad Phenotype of Null Mice	Human Syndrome
Bipotential gonad			
Wt1	Transcription factor	Blockage in genital ridge development	Denys-Drash, WAGR, Frasier syndrome
Sf1	Nuclear receptor	Blockage in genital ridge development	Embryonic testicular regression syndrome
Lhx9	Transcription factor	Blockage in genital ridge development	*
Emx2	Transcription factor	Blockage in genital ridge development	*
M33	Transcription factor	Gonadal dysgenesis	*
Testis-determining pathway			
Gata4/Fog2	Transcription/cofactor	Reduced *Sry* levels, XY sex reversal	*
Sry	Transcription factor	XY sex reversal	XY sex reversal (LOF); XX sex reversal (GOF)
Sox9	Transcription factor	XY sex reversal	Campomelic dysplasia, XX sex reversal (GOF)
Sox8	Transcription factor	XY sex reversal in combination with partial loss of *Sox9* function	*
Fgf9	Signaling molecule	XY sex reversal	*
Dax1	Nuclear receptor	Impaired testis cord formation and spermatogenesis	Hypogonadism
Pod1	Transcription factor	XY sex reversal	*
Dhh	Signaling molecule	Impaired differentiation of Leydig and PM cells	XY gonadal dysgenesis
Pgdfra	Receptor	Reduction in mesonephric cell migration	*
Pgds	Enzyme	No phenotype	*
Arx	Transcription factor	Abnormal testicular differentiation	X-linked lissencephaly with abnormal genitalia
Atrx	Helicase	ND	ATRX syndrome
Insl3	Signaling factor	Blockage of testicular descent	Cryptorchidism
Lgr8	Receptor	Blockage of testicular descent	Cryptorchidism
Hoxa10	Transcription factor	Blockage of testicular descent	Cryptorchidism
Hoxa11	Transcription factor	Blockage of testicular descent	Cryptorchidism
Amh	Hormone	No Müllerian duct degeneration	Persistent Müllerian duct syndrome
Misrll	Receptor	No Müllerian duct degeneration	Persistent Müllerian duct syndrome
Pax2	Transcription factor	Dysgenesis of mesonephric tubules	*
Lim1	Transcription factor	Agenesis of Wolffian and Müllerian ducts	*
Dmrt1	Transcription factor	Loss of Sertoli and germ cells	XY female[b]
Ovary-determining pathway			
Wnt4	Signaling molecule	Müllerian duct agenesis, testosterone synthesis, and coelomic vessel formation	XY female (GOF)
Foxl2	Transcription factor	Premature ovarian failure	BPES
Dax1	Nuclear receptor	XY sex reversal (GOF)	XY sex reversal (GOF)
RSPO1	Signaling molecule	XX sex reversal (LOF)	XX sex reversal (LOF)

Table Legend

* **BPES** - blepharophimosis-ptosis-epicanthus inversus syndrome

Modified from Wilhelm D, Iscl., (2007) PMID: 17237741

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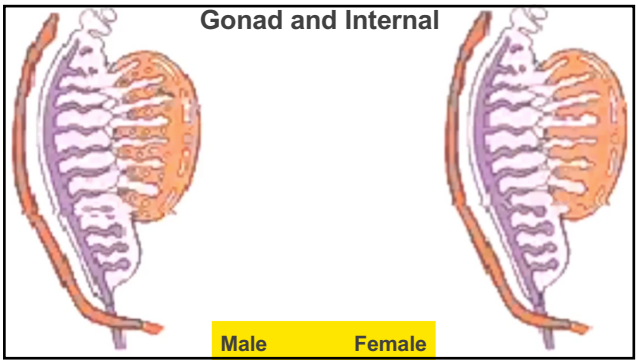
Gonad and Internal



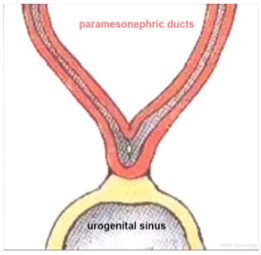
Male Female

Internal Genital

Wrong model of trigone development!

Male – mesonephric duct (Wolffian)

Vagina also generated from fused ducts (influence of BMP4) later estrogens



Female – paramesonephric duct (Mullerian)

See - Tanaka etal., 2010 J. Urol. PMID: 19914648

See - Robboy etal., (2017). PMID: 28918284

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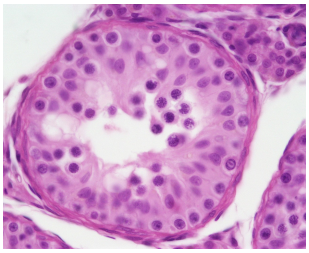
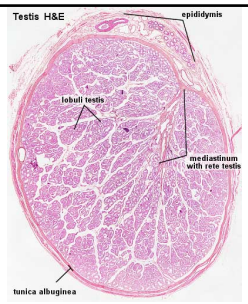
Testis Pre-Puberty

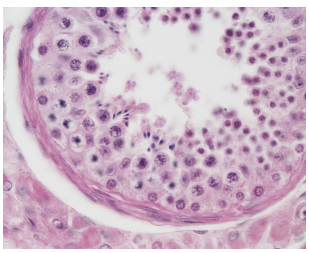


Seminiferous tubule



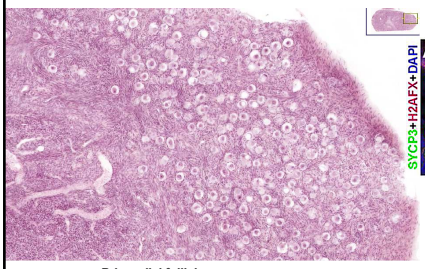
UNSW

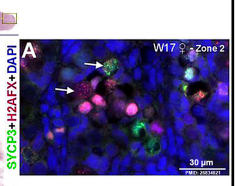
Testis Post-Puberty



Seminiferous tubule

Ovary Pre-Puberty





meiosis (second trimester)
H2AFX and SYCP3 meiotic markers

Primordial follicles

Heeren AM, etal., (2016) PMID: 26834021

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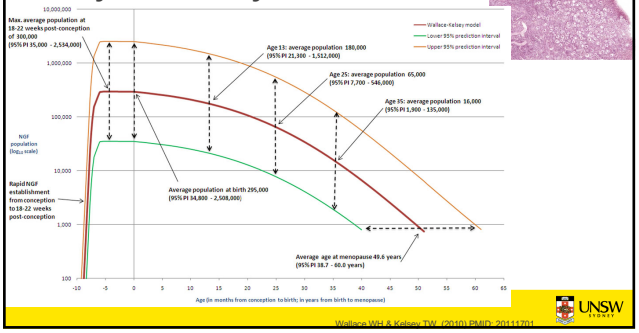
Ovary Pre-Puberty



Wallace WH & Kelsey TW. (2010) PMID: 20111701

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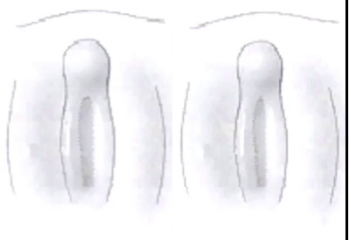
Male and Female External



Week 12 genital tubercle

Female **Male**

Historical - Spaulding MH. The development of the external genitalia in the human embryo. (1921)

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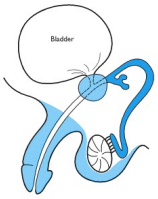
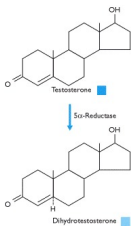
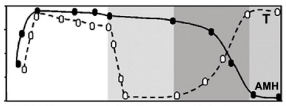
Male – Tract and External

- Reduction of T to DHT
- enzyme 5α-reductase expressed in the tissues
- There are two isoforms (type 1 and 2) that differ in tissue distribution and kinetics.



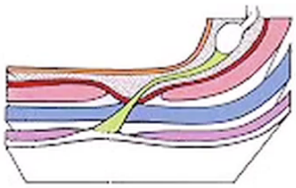


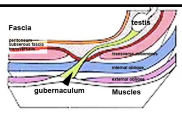
Human Serum Relative Levels



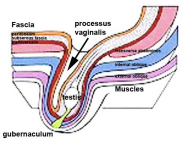
Nussey S, Whitehead S. (2001)

Male Testis Descent





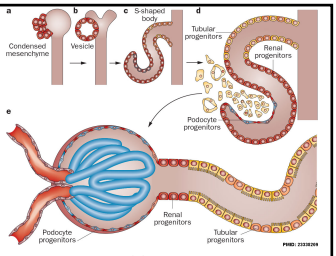
Gubernaculum - mesenchyme fold at or near the gonad



Insulin-like factor 3 (INSL3, relaxin-like factor) from fetal leydig cells acting through receptor (Rxfp2) and BMP and WNT pathways promote testis descent.

phase 1 - GA 10 - 15 weeks testes moves to inguinal region
phase 2 - GA 25 - 35 weeks inguino-scrotal (CGRP, EGF)

Renal - Nephron



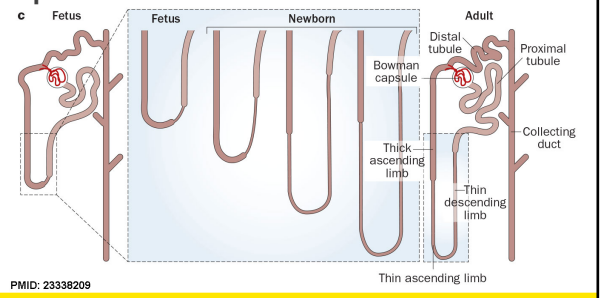
Adult human kidney 750,000 nephrons
(range 250,000 to 2,000,000)

13-19 weeks - vesicle (V) stage
20-24 weeks - S-shaped body (S) stage
25-29 weeks - capillary loop (C) stage
infants aged 1-6 months - maturation (M) stage

Ureteric Bud - developing ureter, pelvis, calyces, collecting ducts
Metanephric Blastema (intermediate mesoderm) - developing glomeruli, capsule, nephron tubules

Romagnani etal., (2013) PMID: 23338209

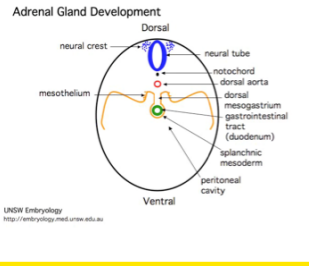
Nephron Maturation

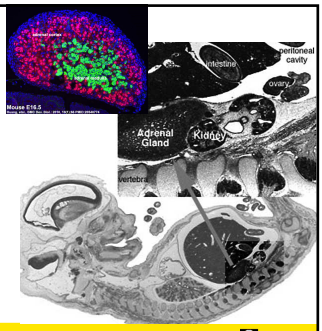


PMID: 23338209

Romagnani etal., (2013) PMID: 23338209

Adrenal Development



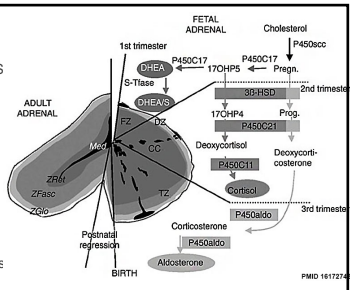


Adult Medulla - Adrenaline 80%, Noradrenaline 20%

Stage 22 GA week 10

Adrenal

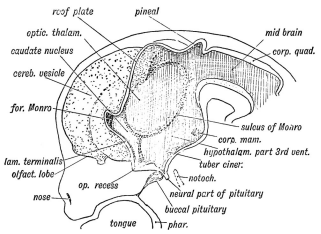
- **Fetal zone** – secrete DHEA and DHEA/S for placenta as estrogen precursors.
- **Second trimester** - neural crest chromaffin cells migrate through fetal cortex to future medulla
- **Third trimester** - new transitional zone allows production of fetal cortisol.
- **Near birth** - cells begin to secrete mineralocorticoids such as aldosterone.
- **Neonatal** - fetal adrenal cortex regresses
- **Adult** - zona glomerulosa, zona fasciculata and zona reticularis (3 years)



Chamoux E, etal. (2005) PMID: 16172742

Hypothalamus

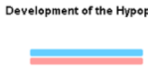
- Neuroectoderm - prosenecephalon then rostral diencephalon after induction by the underlying prechordal plate.
  - Prosomeric model - hypothalamus and telencephalon are part of secondary prosencephalon
- Sonic hedgehog (Shh) - initially expressed in prechordal plate, is essential for inductive process.
- ventro-lateral wall intermediate zone proliferation
- Mammillary bodies - form pea-sized swellings ventral wall of hypothalamus

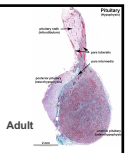


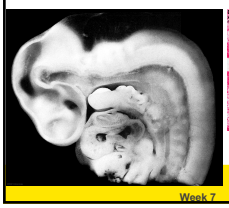
Neural tube - morphological model "primary and secondary vesicles" being replaced with gene expression model "prosomeres"

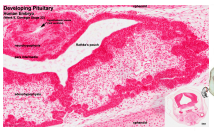
Pituitary

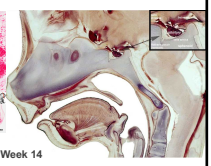
- **Neuroectoderm**
  - prosenecephalon, neurohypophysis
- **Ectoderm**
  - roof of stomodeum, Rathke's pouch, adenohypophysis





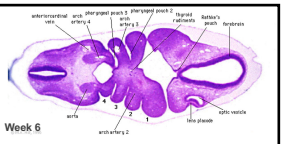

Week 7

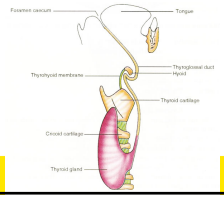

Week 10


Week 14

Thyroid

- **Week 6** - thyroid median endodermal thickening in the floor of pharynx (thyroid diverticulum)
- **Week 7-10** - thyroglossal duct - proximal end at the foramen cecum of tongue.
- thyroid diverticulum - hollow then solid, right and left lobes, central isthmus

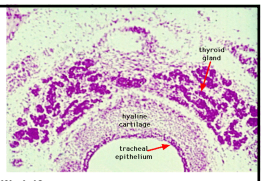

Week 6



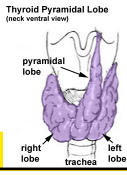

Week 10

Thyroid

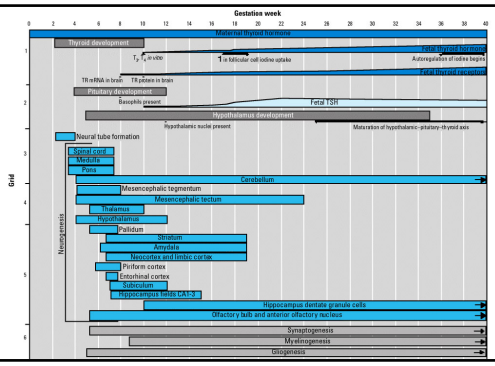
- **Week 13** - colloid appearance in thyroid follicles, iodine and thyroid hormone (TH) synthesis growth factors (insulin-like, epidermal) stimulates follicular growth
- Fetal TH - nitial secreted biologically inactivated by modification
- late fetal secretion develops brown fat
- Iodine deficiency - during this period, leads to neurological defects (cretinism)
- Birth - TSH levels increase, thyroxine (T3) and T4 levels increase to 24 h, then 5-7 days postnatal decline to normal levels



Week 10

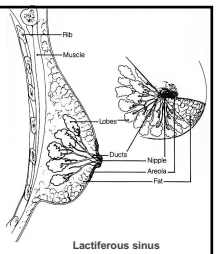


Pyramidal Lobe
50% anatomical dissections and more frequently in men than in women.



Breast

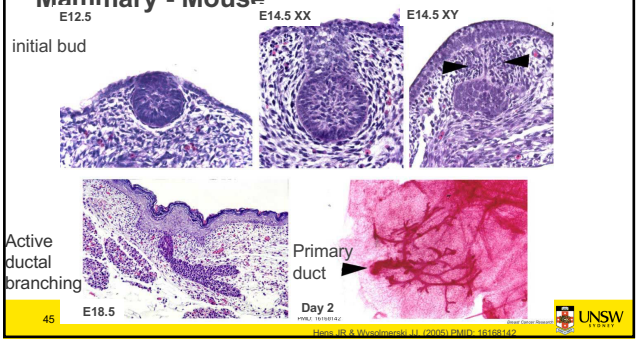
- Modified apocrine sweat glands
- *Key Molecular Factors - WNT10b, Gli3, Hh,*
- **GA Week 8**
  - epidermis (ectoderm) down-growth into dermis *(*mesoderm)
  - epithelia/mesenchyme inductive interaction
    - mesenchyme forms connective tissue and fat
  - **mammary ridges** - mammary bud formation, pair of ventral regions axilla to inguinal
  - buds branch to form lactiferous ducts
- **Birth** - only main duct formed at birth



Lactiferous sinus
interlobular (segmental) ducts
terminal ducts and lobules
(terminal ductal lobular unit TDLU)

apocrine

Mammary - Mouse



45

Hens JR & Wysolmerski JJ. (2005) PMID: 16168142

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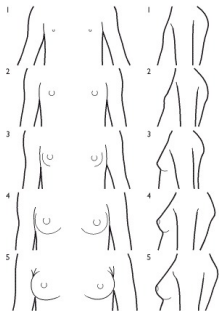
Breast - Tanner Stages

- prior to puberty male and female glands the same
- gland branching morphogenesis commences
- stimulated by - growth hormone, estrogen , insulin-like growth factor 1
- growth also influenced by other hormones - progesterone, prolactin, and corticoids



Tanner JM & Whitehouse RH. (1976) PMID: 952550

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Embryo Week 8-10 MRI