

(13)

Recommended reading - Larsen, Hurman
Embryology. 3rd
Edition.

(14)

- Developmental
Biology, by Gilbert,
5th Edition.

— X —

(13)

MOLECULAR MECHANISM OF SEX DIFFERENTIATION -

After formation of bipotential Gonad (mediated by WT1 and SF1), further differentiation depends on presence of Y chromosome

If Y present

If Y absent

- SRY present on Y chromo → In females Y is absent, therefore ~~WNT~~ WNT-4 along with RSPD-1 in granulosa cell suppresses activity of 5- α reductase, therefore no DHT and no male sex differentiation.
- This cascade lead to activation of Leydig cells to release testosterone, which is converted to DHT (mediated via 5- α reductase). Which further leads to development and maintenance of male reproductive system.
- At the same time Sertoli cell release AMH, leads to regression of Mullerian duct.
- ↓
- LEADS TO DEVELOPMENT OF MALE REPRODUCTIVE SYSTEM.
- X — X
- Further WNT-4 and RSPD-1 complex, ~~activate~~ activates β catenin leads to SOX-9 inhibition, therefore no AMH. Mullerian duct persist and become part of female reproductive system.
- ↓
- Development of Female reproductive system.

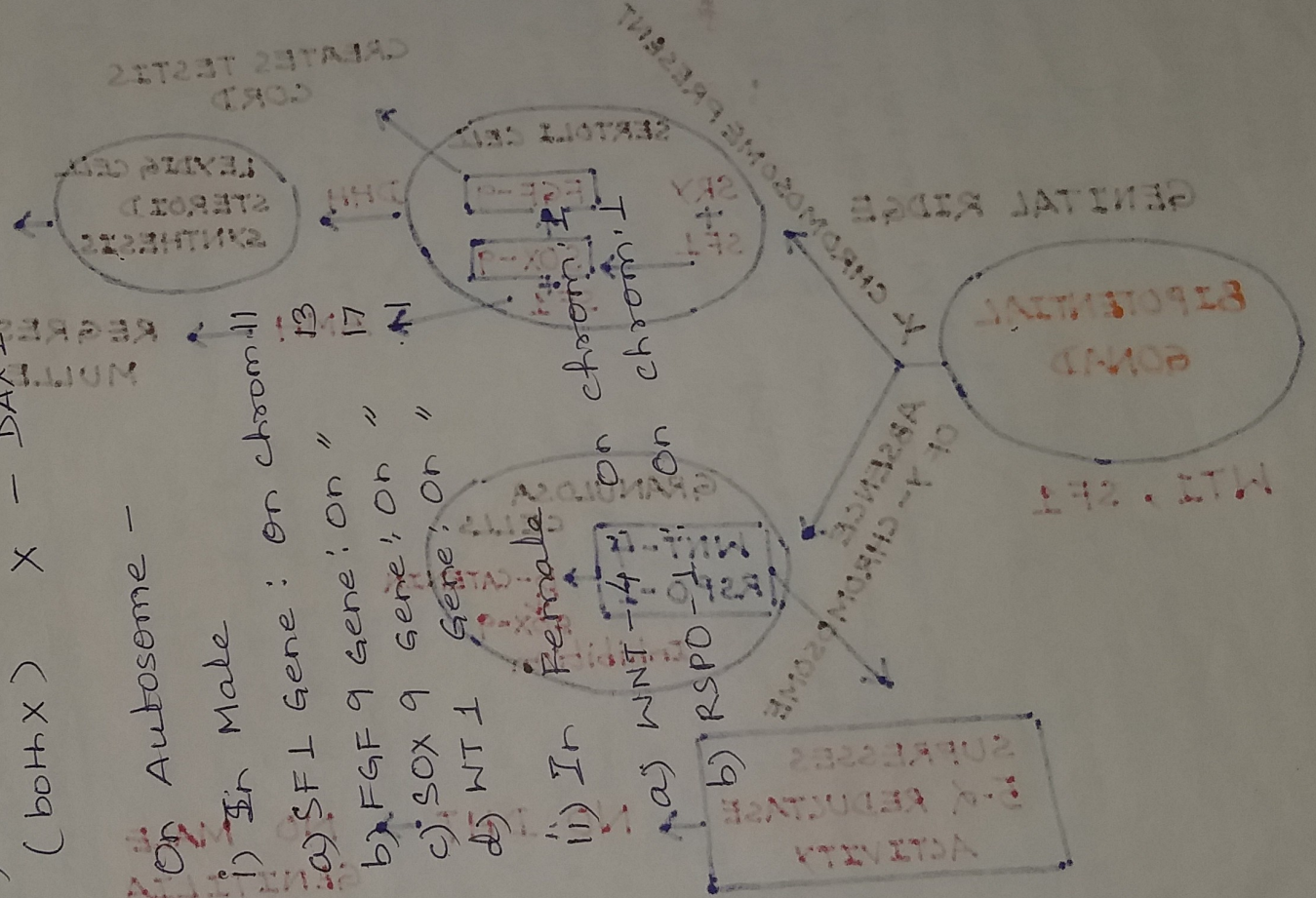
GENE FOR SEX DIFFERENTIATION:

I. On sex chromosome -

- i) In Male (One X and one Y)
 - X - DAX 1 Gene
 - Y - SRY Gene
- ii) In Female (both X)
 - X - DAX 1 Gene
 - X - DAX 1 Gene

II. On Autosome -

- i) In Male
 - a) SFI Gene: on chrom 11
 - b) FGF 9 Gene: on "
 - c) SOX 9 Gene: on "
 - d) WT 1 Gene: on "
- ii) In Female
 - a) WNT - 4 on chrom 1
 - b) RSPO - 1 on chrom 1



(9)

→ The germ cell deficient region of the medullary cord becomes the 'rete testis', it joins the 5 to 12 mesonephros tubules under influence of Testosterone to form the efferent ductules which drain into the mesonephric duct which develops into epididymis and Vas deferens.

→ The male duct system continuous with seminiferous tubules.

In females duct differentiation, is independent of ~~rete~~ presence or absence of ovaries, independent of gonadal secretion and is cell autonomous.

→ Paramesonephric distal tips adhere and contact the posterior wall of the pelvic urethra.

→ This wall forms a slight thickening, the sinusual tubercle, After contact with the sinusual tubercle the 'Mullerian duct' fuse with caudal to cranial, forming uterovaginal (genital) canal.

GENETIC BASIS OF TESTICULAR AND OVARIAN DIFFERENTIATION -

Once the bipotential gonad is formed further sex determination depends on the presence or absence of a gene located on the short arm of the Y chromosome c/a SRY (sex determination region) and it's interplay with 'SOX-9' and DAX-1 that determines if the fetus develops a testis or an ovary.

(10)

'SRY' Gene as Testicular Determiner

- In developing testis 'SRY' expressed in sexdoli cell, it decide whether or not a testis is formed
- 'SRY' is a high mobility group (HMG) transcription factor that regulates gene expression by bending DNA.
- One of SRY downstream target is SOX-9, another HMG type
- SOX-9, is originally expressed within the urogenital ridge in both sexes before appearance of SRY.
- SRY expression upregulates SOX-9 in the male while DAX-1 down regulates this gene in the female.
- SOX-9 is also expressed in sexdoli cells and with SRY, up-regulates AMH.

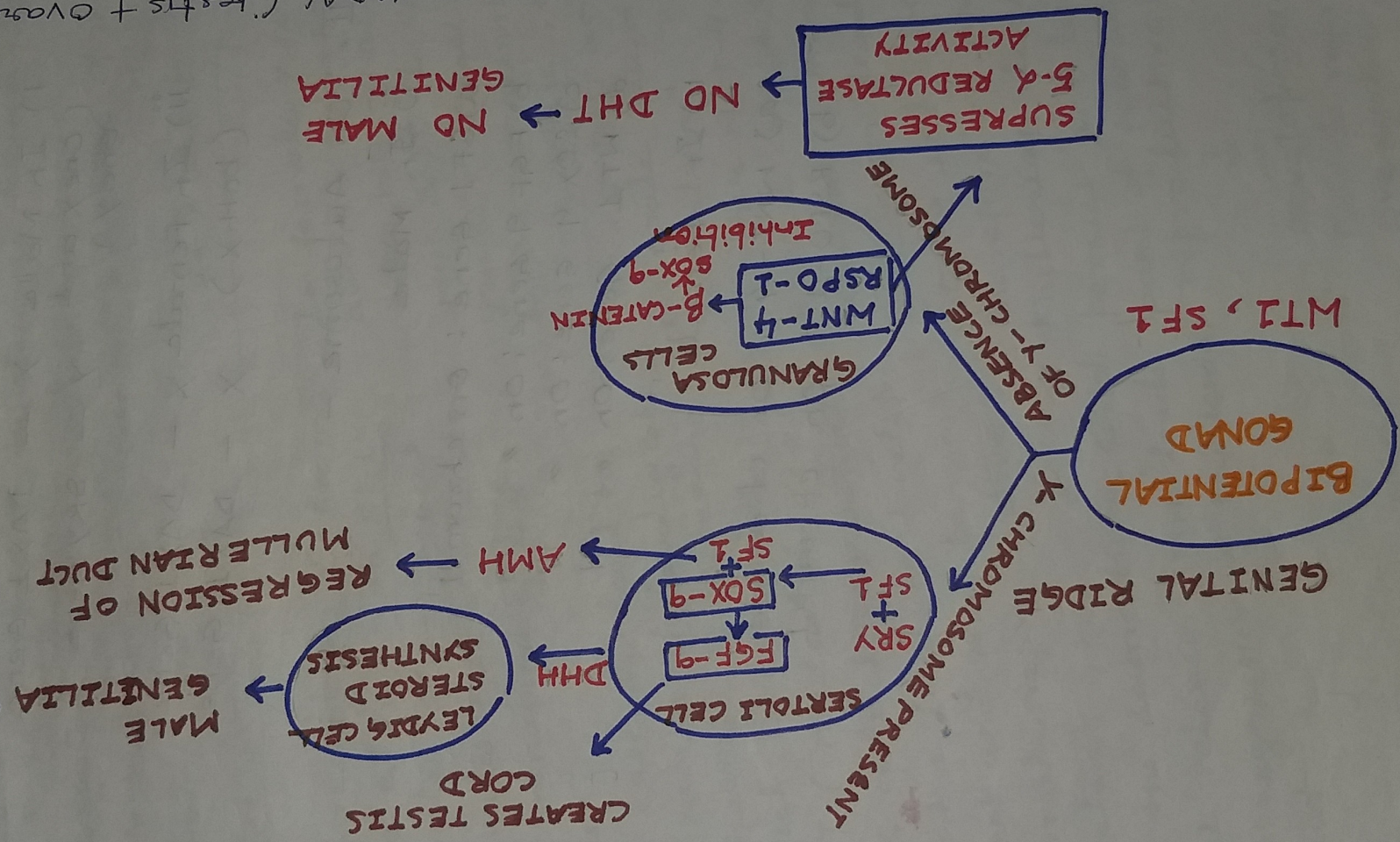
DAX-1, as Ovarian determination - independent

- The female phenotype is gonad independent (differentiation of external and internal genitalia), but not female for gonad development
- DAX-1, a member of the nuclear hormone receptor family, found on short arm of X-chromosome.
- The DAX-1 inhibits SRY directly or indirectly (by SRY's upregulation of SOX-9 inhibited)

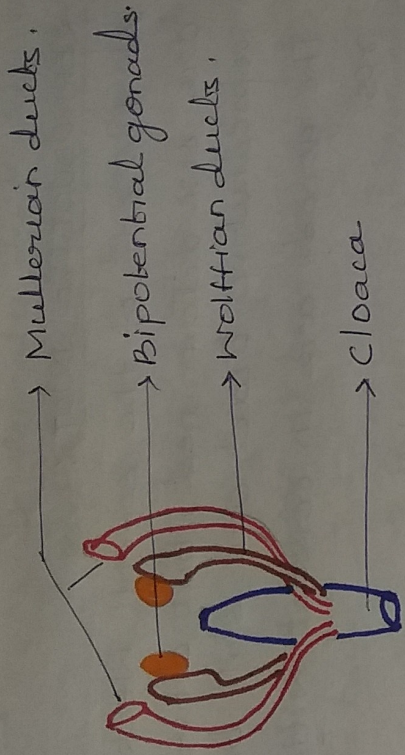
(11)

DAX-1 originally expressed in both male and female gonad but persists only in female gonad

FIG: FLOW DIAGRAM FOR GENETIC CASCADE OF GONADAL (TESTIS + Ovary) DIFFERENTIATION. (MOLECULAR MECHANISM).

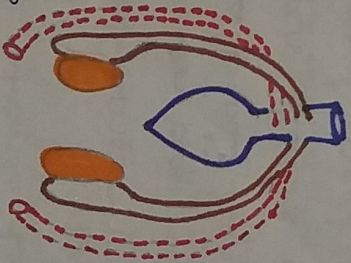


7

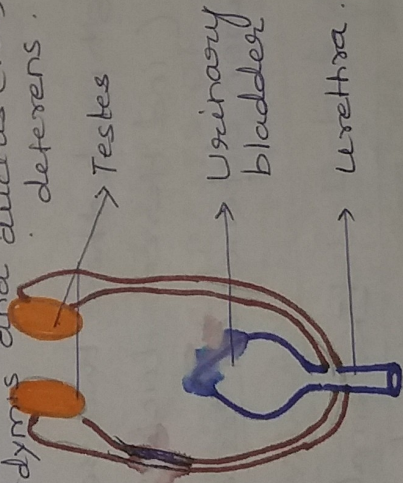


MASCULINIZATION

Mullerian ducts
degrade

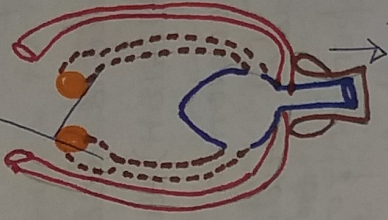


Wolffian ducts become
Epididymis and ductus (Vas)
deferens.

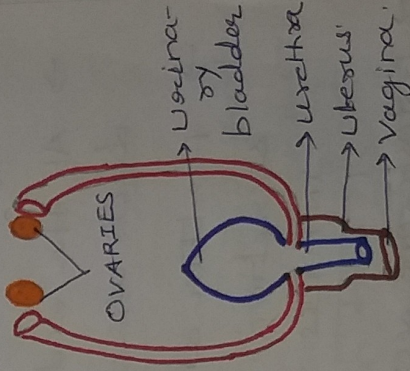


FEMINIZATION

Wolffian ducts
degrade



Mullerian ducts
become uterine
tubes and uterus



(6)

In presence of Sertoli cells, the germ cells remain in meiotic arrest and are inactive, 'Spermatogonia' till puberty. This is due to secretion of Sertoli cells (AMH) (Anti Mullerian hormone), also signals from mesenchymal cells (intermediate mesoderm) to differentiate in Leydig cells, which secrete testosterone.

⇒ FEMALE GONAD - Ovary develops slowly than testes. Primary sex cords enter the genital ridge at the same time ovary can't be distinguished histologically till 10-11th week.

The primary sex cords degenerate and the secondary sex cords (or cortical cords) extend from the surface epithelium (mesothelium). As these cords increase in size the PGC are incorporated into them.

At about 16 weeks these cords break up into isolated clusters called ova primordial follicles.

Each follicle consist of an oogenium (from PGC) and a single layer of flattened cells (follicular cells) derived from the cords

Oogenesis undergo a period of rapid proliferation

In the absence of SRY and AMH, PGC undergo the 1st prophase of meiosis.

8

REPRODUCTIVE DUCT :

- At the end of the seventh week the gonads are sex determined, but other part of the reproductive tract are still bipotential.
 - The gonads are in contact with the mesonephros
 - Although mesonephros regress, the mesonephric tubule grow into the gonadal ridge and these remain connected to mesonephric (Wolffian) duct
 - A second duct system, the paramesonephric (Mullerian) duct forms as an invagination of the coelomic epithelium on lateral aspect of gonadal ridge. The Mullerian ducts are independent of mesonephros and meet and fuse at the end of the eighth week contacting the Urogenital sinus.
 - At the end of the 8th week these two sets of ducts are present (indifferent duct stage).
 - Further differentiation depends on Testes secretion.
- IN MALES
- Testosterone secretion by Leydig cell acts locally and maintains mesonephric and Wolffian duct.
 - AMH (from Sertoli cells) acts via circulation of paramesonephric (Mullerian) duct system degeneration.
- IN FEMALES
- In females due to absence of testosterone - Mesonephric tubule and ducts degenerates
 - In absence of AMH paramesonephric duct forms fallopian tube, uterus & upper part of vagina

②

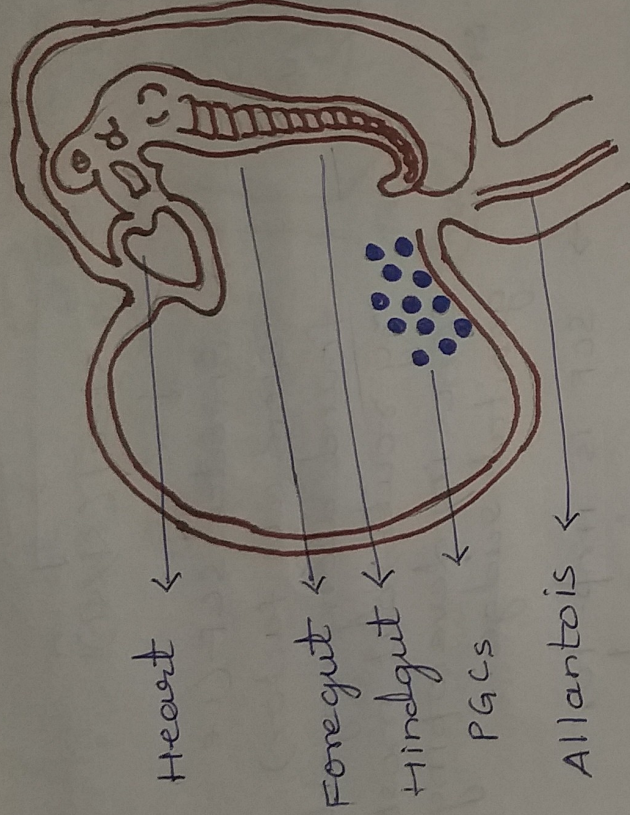
MIGRATION OF PGCS

PGCs migration guided by —

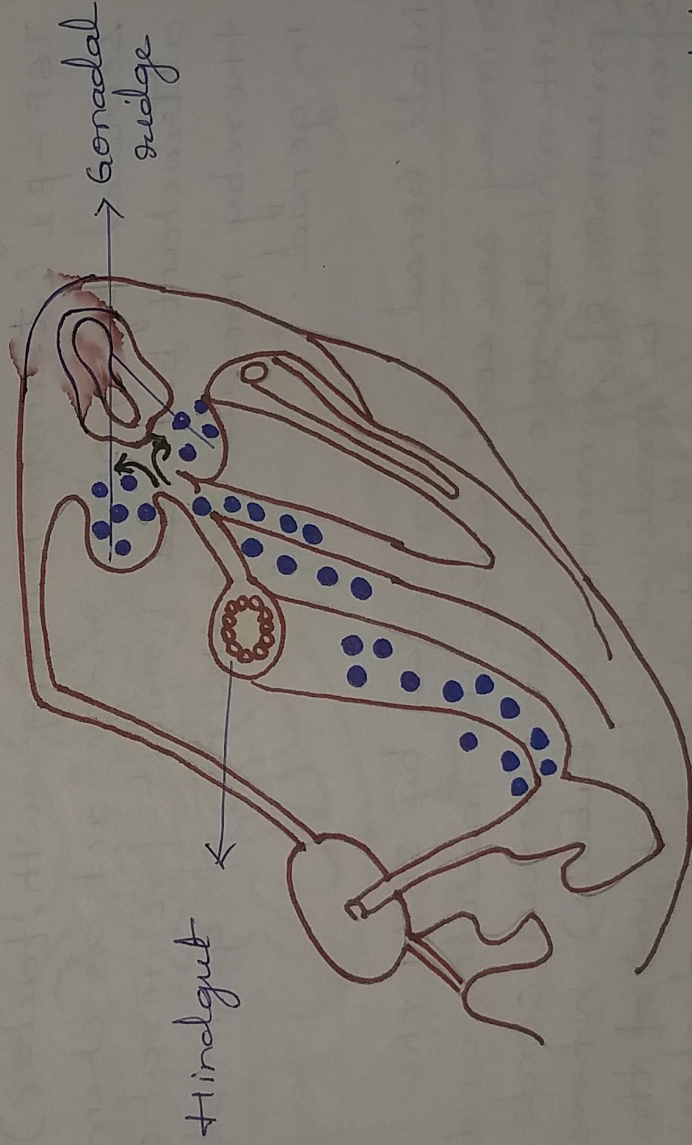
- i) C-Kit — is gene encoding (a encoding the receptor tyrosine kinase protein, present on PGCs. Encoded by W locus.
- ii) SCF (stem cell factor) encoded by 'SL' locus. found on migratory route of PGCs, but most concentrated in developing genital ridge. SCF is imp. for ~~scf~~ PGCs survival and migration
- iii) TGF- β 1 — (Transforming growth factor) also controls PGCs migration secreted by genital ridge, acts chemoattractant but inhibit proliferation of PGCs thereby modulating number of germ cells in gonad.

MALE GONAD DIFFERENTIATION — Regulated by SRY, the primary sex cord enter the medulla and differentiate into seminiferous cords (precursor of seminiferous tubule). The part of primary sex cord extend deepest in medulla forms 'rete testes'. During seminiferous tubule formation PGCs enters gonad and associate with tubule. PGC will give rise to sperm (after puberty), the cords give rise to the sustentacular cells of the tubules the 'SERTOLI CELLS'.

④



(A); Early Embryo (5th week)



(B); Migration of germ cells to gonadal ridge.

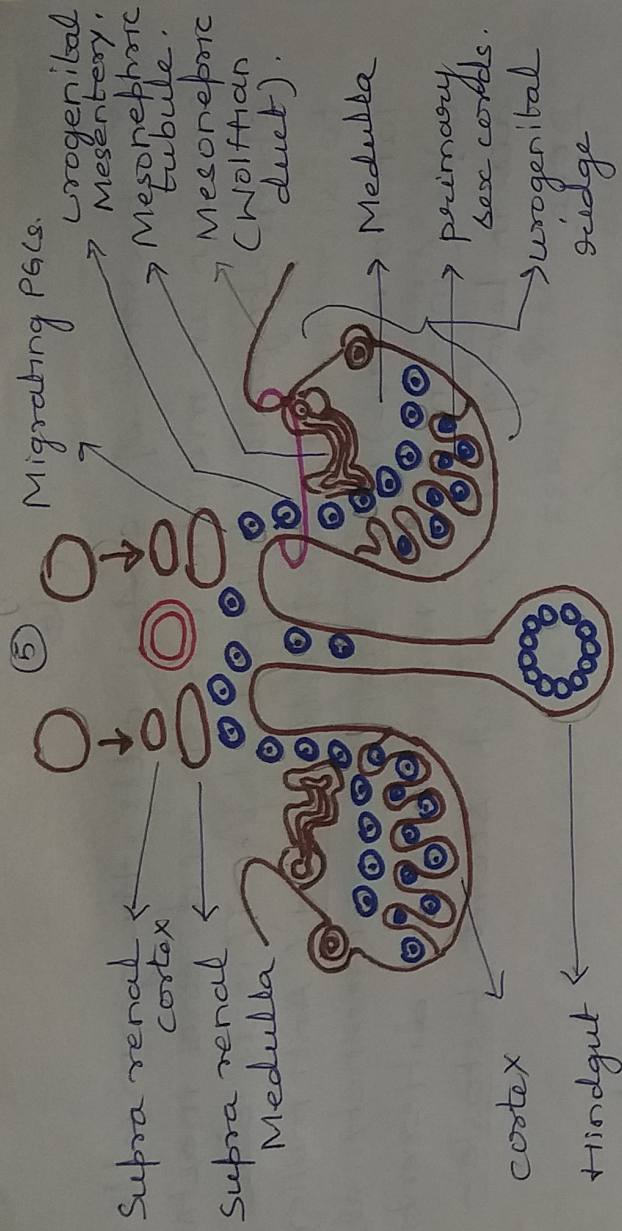


FIG: Indifferent or primordial Gonad.

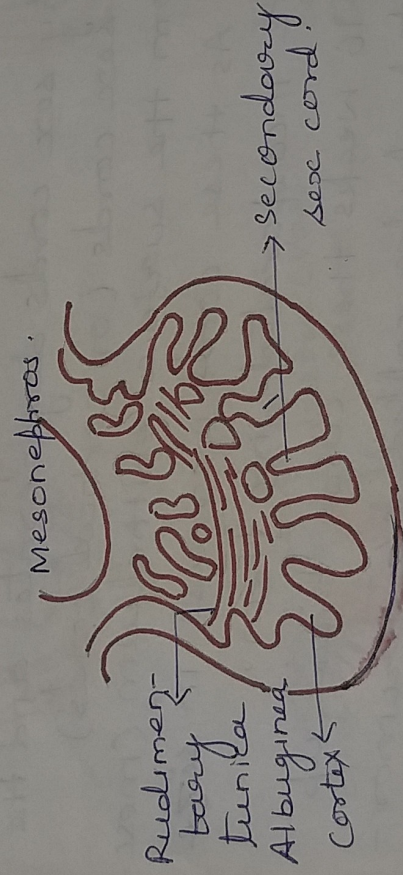


FIG: Developing ovary

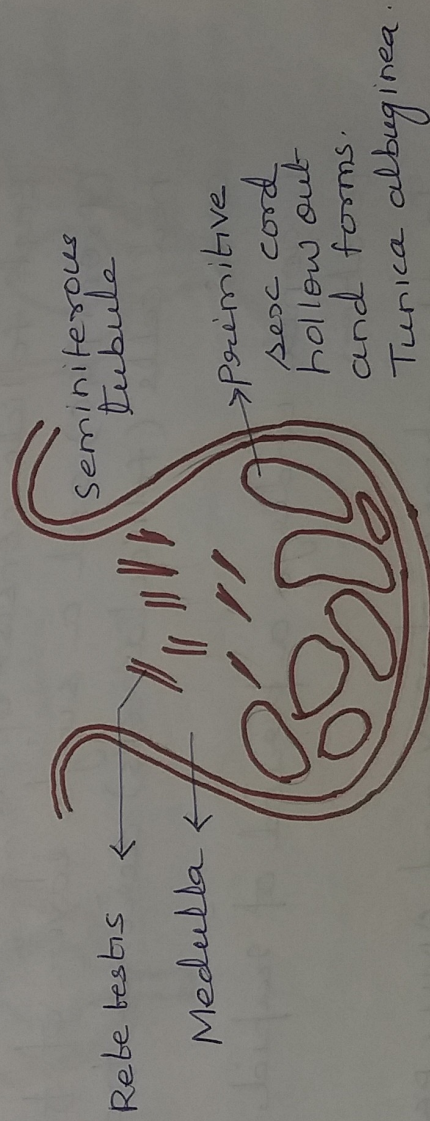
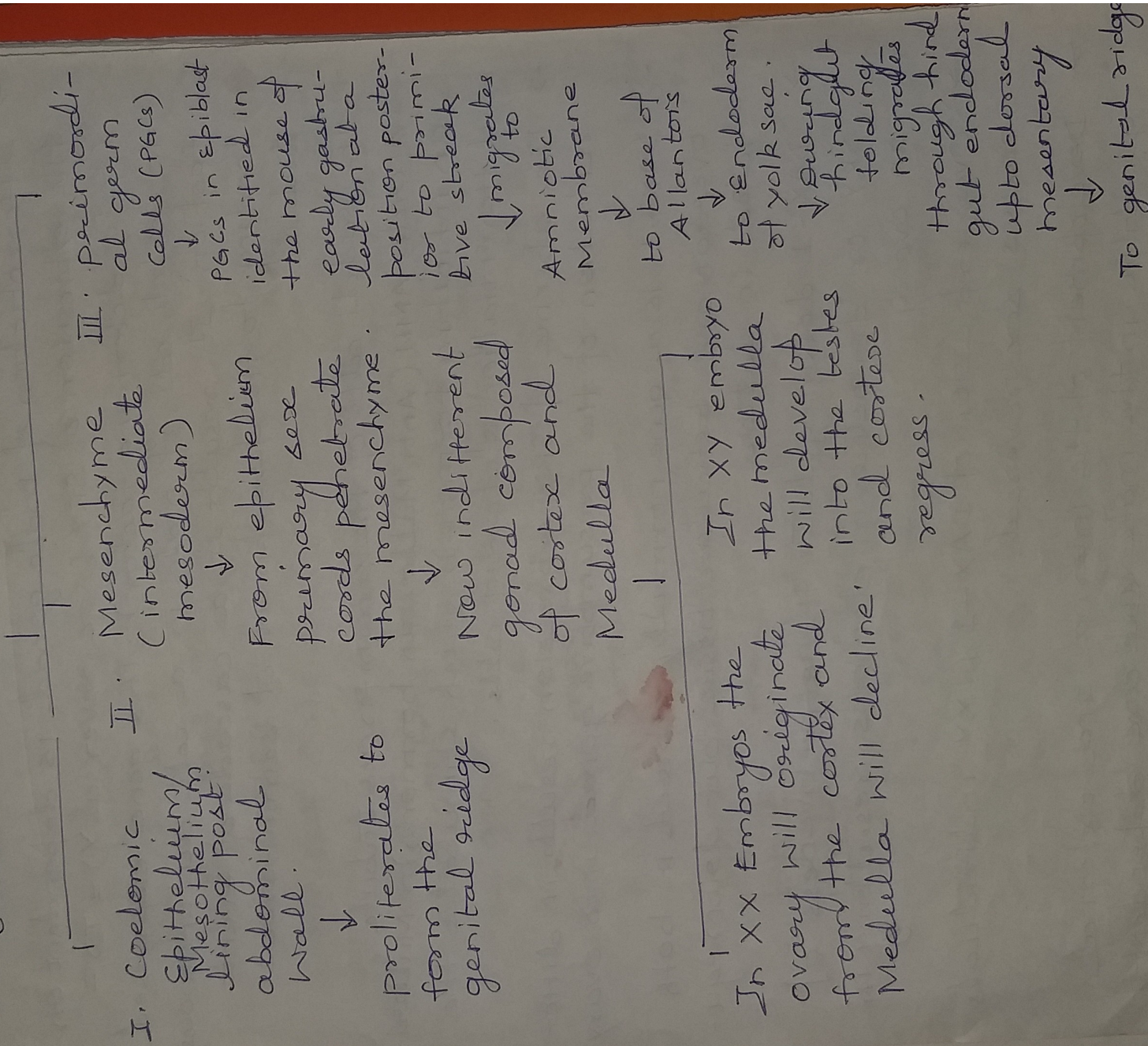


FIG: Developing testis.

②

→ Development of female internal and external structures are gonad independent

COMPONENTS OF GONADAL ORIGIN — (Gonad develop from 3 sources)



GONADAL DEVELOPMENT

- The male and female reproductive tracts are derived from the same embryonic fetal tissue. The gonads and internal and external genitalia begins as bipotential tissues.
- Differentiation of male gonad is dependent on the expression of SRY (sex reversal Y) = TDF (testes determining factor). This gene expressed in Sertoli cell of male in a cell autonomous fashion. It results in a cascade of events leading to the development of seminiferous tubule.
- The Sertoli cells of the seminiferous tubule secretes AMH (Anti Mullerian hormone) or Mullerian inhibiting hormone, which stimulates the differentiation of Leydig cells.
- The absence of SRY expression results in differentiation of the presumptive gonad into ovary.
- DAX-1 is a gene normally expressed in both ovarian and testicular tissue but downregulated in latter.
- DAX-1 downregulates the effectiveness of SRY or downstream elements resulting in an ovary.
- Over expression of DAX-1 in XY individuals causes sex reversal.
- Development of the internal and external genitalia in the male ~~and~~ are dependent on the gonad (testes).