April 2020

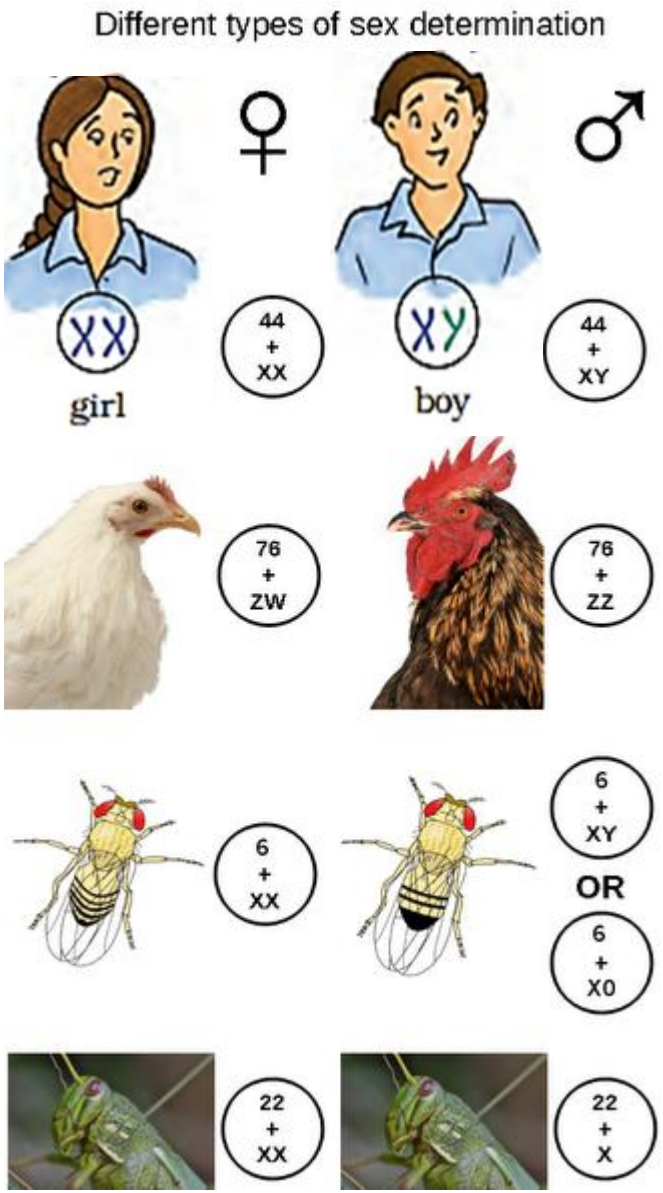
ZOB 603: Genetic and Cell Biology

Topic
3.0 Genetic Determination of Sex
3.1 *Drosophila*
3.2 Human

Developed By –
Dr. Ganesh Kumar Maurya
Assistant Professor (Zoology)
MMV, BHU

Sex & its Determination

- The word SEX is derived from the Latin word ***sexus*** meaning separation.
- Sex is the morphological ,physiological & behavioural difference observed between egg and sperm producing organisms.



WHAT IS SEX DETERMINATION?

- It refers to the hormonal, environmental and genetical especially molecular mechanism that make an organism either male or female.

- It **determines** the development of **sexual** characteristics in an organism.

Sex Determination in *Drosophila*

- In *Drosophila,* sex determination is achieved by Genic balance mechanism (given by Calvin Bridges , 1926)i.e. a balance of female determinants on the X chromosome and male determinants on the autosomes.
- Ratio of X chromosomes: haploid sets of autosomes (X:A) determine the sex.

X chromosome = Female producing effects

Autosomes = Male producing effects

Y Chromosome= Fertility factor in male required for sperm formation but not in sex determination.

X:A ratio
Female = 1.0 (2X:2A)
Male = 0.5 (1x:2A)
0.5 < X:A < 1.0 = intersex

XO *Drosophila* are sterile males.

TABLE 5.2

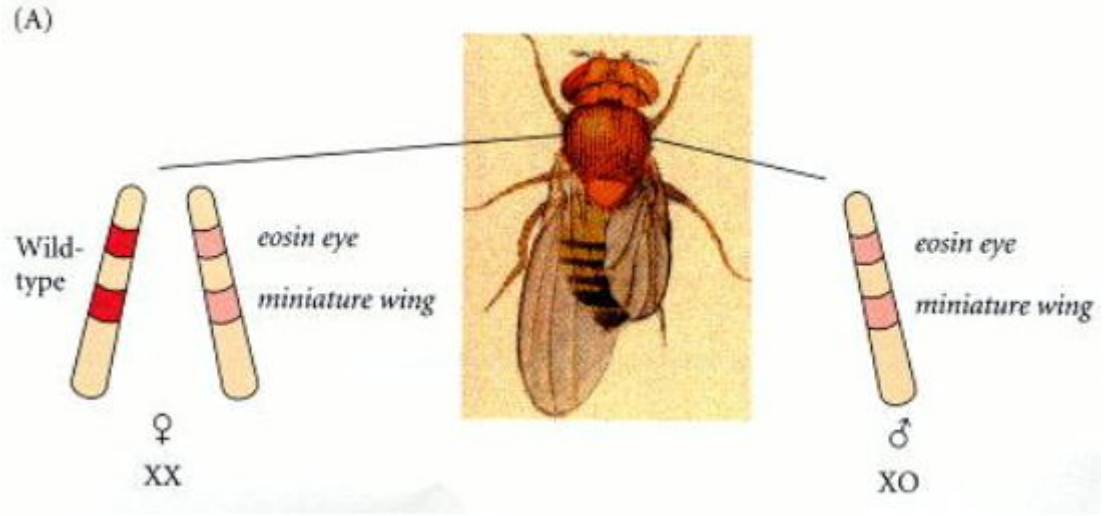
Ratio of X Chromosomes to Autosomes and the Corresponding Phenotype in *Drosophila*

X Chromosomes (X) and Sets of Autosomes (A)	X:A Ratio	Phenotype
1X 2A	0.5	Male
2X 2A	1.0	Female
3X 2A	1.5	Metafemale
4X 3A	1.33	Metafemale
4X 4A	1.0	Tetraploid female
3X 3A	1.0	Triploid female
3X 4A	0.75	Intersex
2X 3A	0.67	Intersex
2X 4A	0.5	Tetraploid male
1X 3A	0.33	Metamale

Gynandromorphs

Gynandromorphs are animals in which certain regions of the body are male and other regions are female.
Examples - *Drosophila* and certain other insects
- This can happen when an X chromosome is lost from one embryonic nucleus. The cells descended from that cell, instead of being XX (female), are XO (male)

- In *Insects,* each cell makes its own sexual "decision" because they lack sex hormones to modulate such events.
- So, the XO cells display male characteristics, whereas the XX cells display female traits.



3 types
- **Bilateral Gynandromorph**
- **Anterior-Posterior Gynandromorph**
- **Sex Piebalds**.

MOLECULAR MECHANISM OF SEX DETERMINATION IN *Drosophila*

Sex Determination in *Drosophila i*nvolves the following events:-

1.ESTABLISHMENT OF X:A RATIO

2.CONVERSION OF X:A RATIO INTO A MOLECULAR SIGNAL

3.DIFFERENTIAL ACTIVATION OF SxI GENE

4.DIFFERENTIAL REGULATION OF TRANSFORMER (Tra)GENE

5.ACTION OF Dsx- THE SWITCH GENE OF SEX DETERMINATION

6.PRODUCTION OF MALE OR FEMALE

1. ESTABLISHMENT OF X:A RATIO

• Establishment of X:A ratio involves interaction between proteins that are encoded by several X-linked genes and proteins encoded by several autosomal genes.
• X-linked genes are also called numerator genes. e.g. (sisterless; sis) sis-a ,sis-b ,sis-c, runt. Numerator proteins like Sis form homodimer, which is their active form.
• Autosomal genes are also called denominator genes. e.g. daedpan(dpn), extramacrochaetae (emc).
• The denominator genes encode denominator proteins which antagonize numerator proteins and block their activity by forming heterodimers with the numerator proteins.

• In XX embryo – Denominator proteins < Numerator protein
(because two X chromosomes provide more numerator protein than denominator from autosome).
-
Output -> Embryo Follow Female Pathway

• In XY embryo – Denominator proteins > Numerator protein
(Because only single X chromosome produces numerator proteins less than denominator proteins from autosome).

Output →Embryo Follow Male Pathway

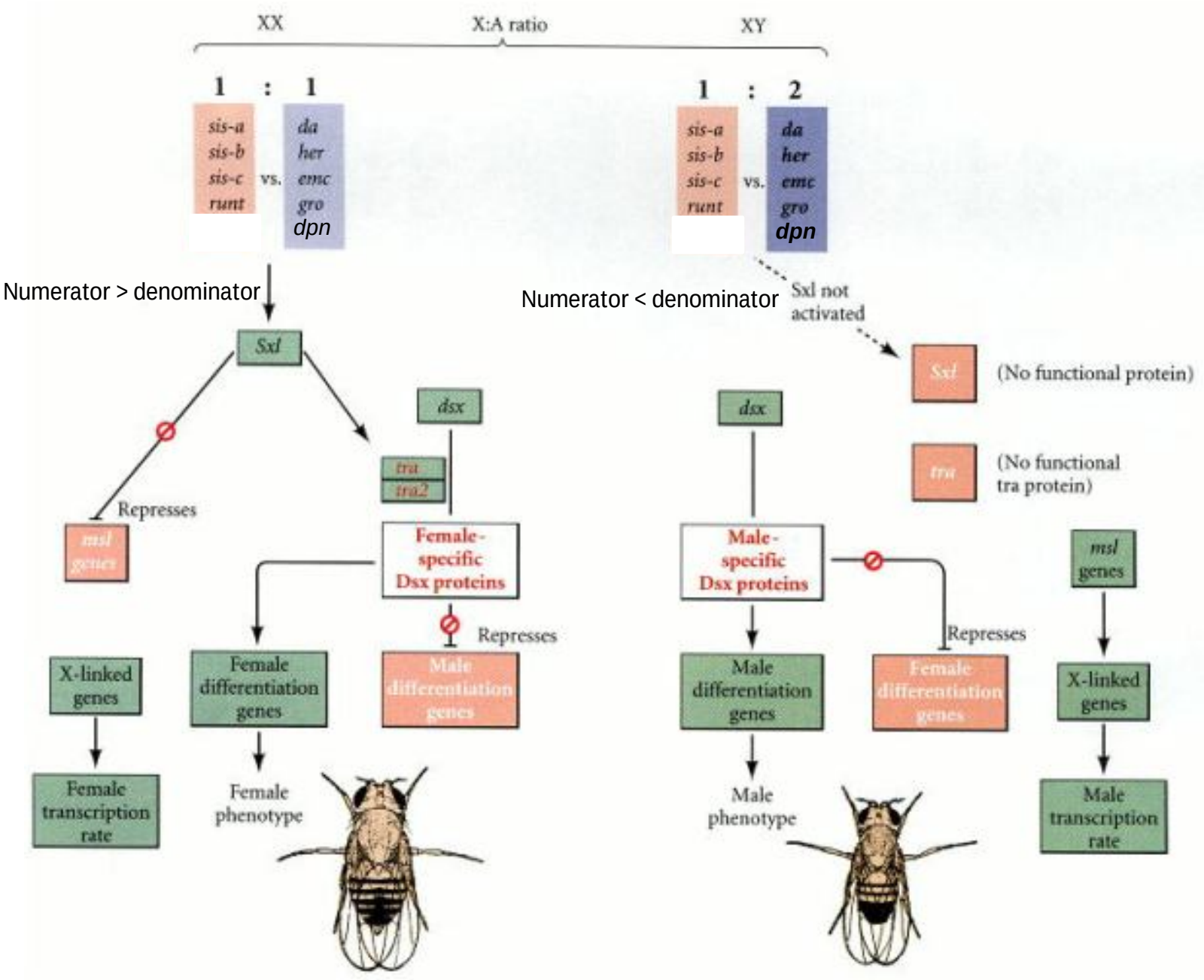
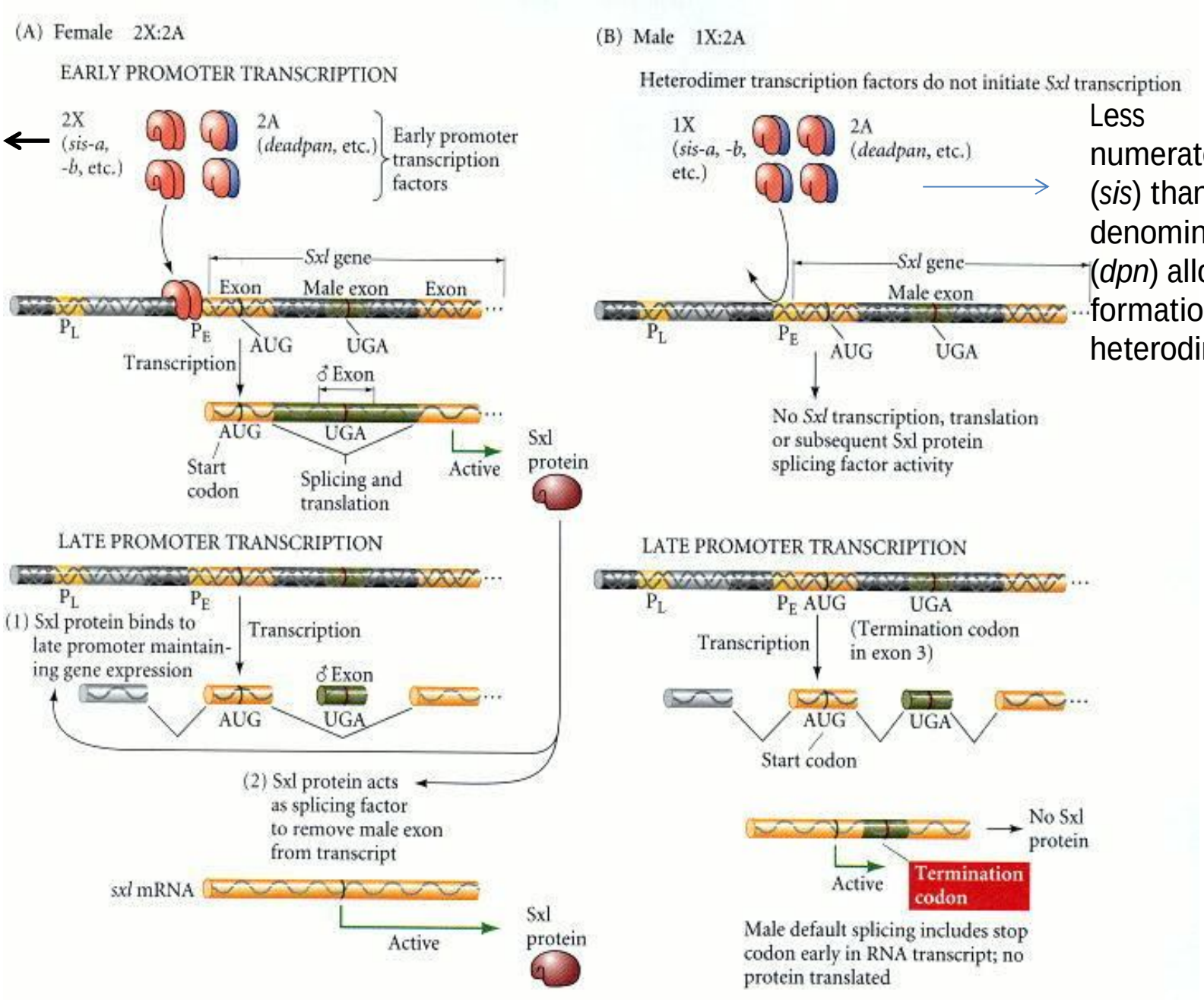


Fig. Regulation cascade for *Drosophila* somatic sex determination.

2. CONVERSION OF X:A RATIO INTO A MOLECULAR SIGNAL

More numerator (*sis*) than denominator (*dpn*) allows formation of more homodimer of *sis* than its heterodimer with *dpn*



Less numerator (*sis*) than denominator (*dpn*) allows formation of heterodimer

CONVERSION OF X:A RATIO INTO A MOLECULAR SIGNAL

1. Sis protein homodimer formed only in XX female, that binds to the early promoter (PE) of sex-lethal (*sxl)* gene and transcribe early Sxl m-RNA.
2. Sxl is the master regulator of the sex determination pathway in *Drosophila*.
3. Sxl protein is synthesised from early Sxl m-RNA. In XX Drosophila, Sxl is active during the first 2 hours of after fertilization.
4. After a few divisions , transcription starts from PM promoter instead of PE.
5. Transcription from PM promoter is also initiated in XY embryos .

3. DIFFERENTIAL ACTIVATION OF Sxl GENE

1.If SXL protein is already available (i.e., from early translation) the sxl premRNA is spliced to form the functional female specific message.

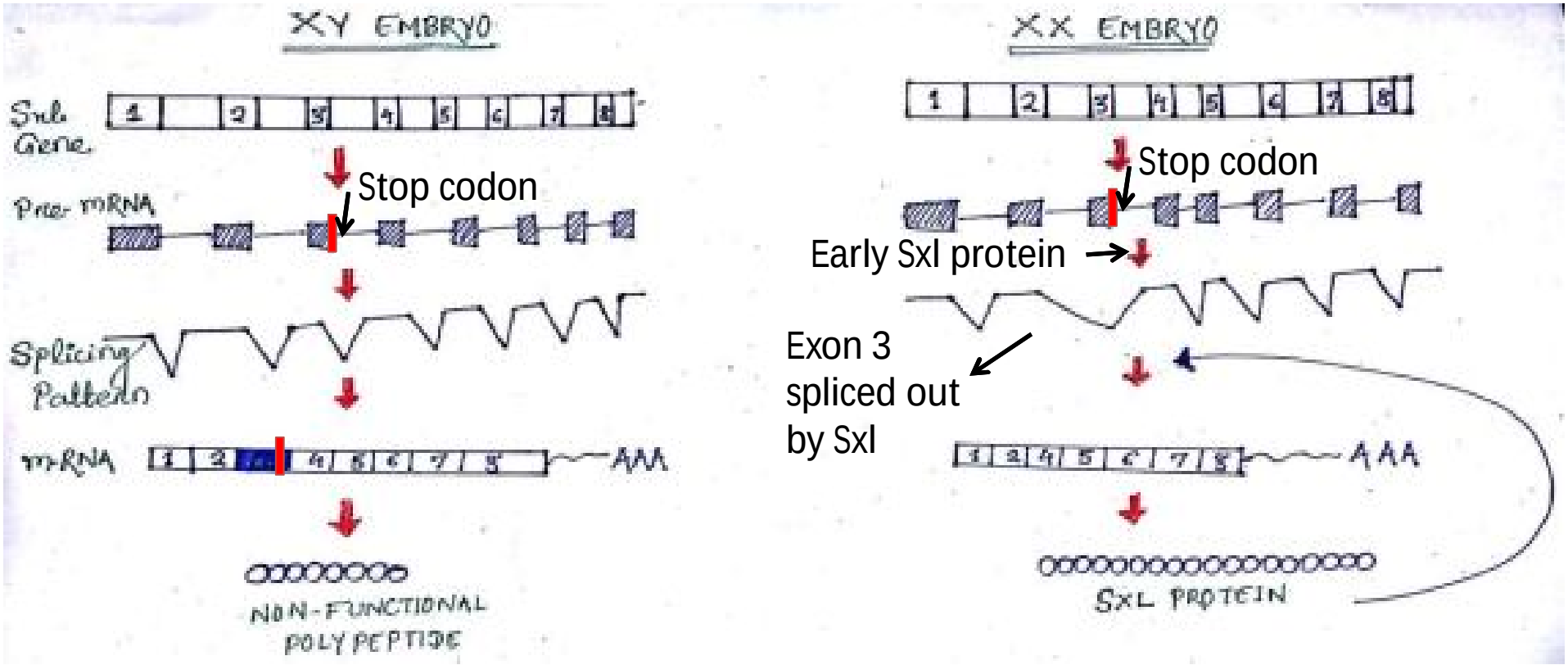
2. This encodes a functional protein of 314 amino acids.

3.In wild type Drosophila with one X chromosome & 2 sets of autosomes, Sxl gene is transcribed from the late promoter. In absence of early SXL protein, the RNA splicing does not exclude the male specific exon in the m-RNA.

4. The resulting RNA encodes a non-functional peptide as the male specific exon contains a translation termination codon (UGA) after amino acid 48.

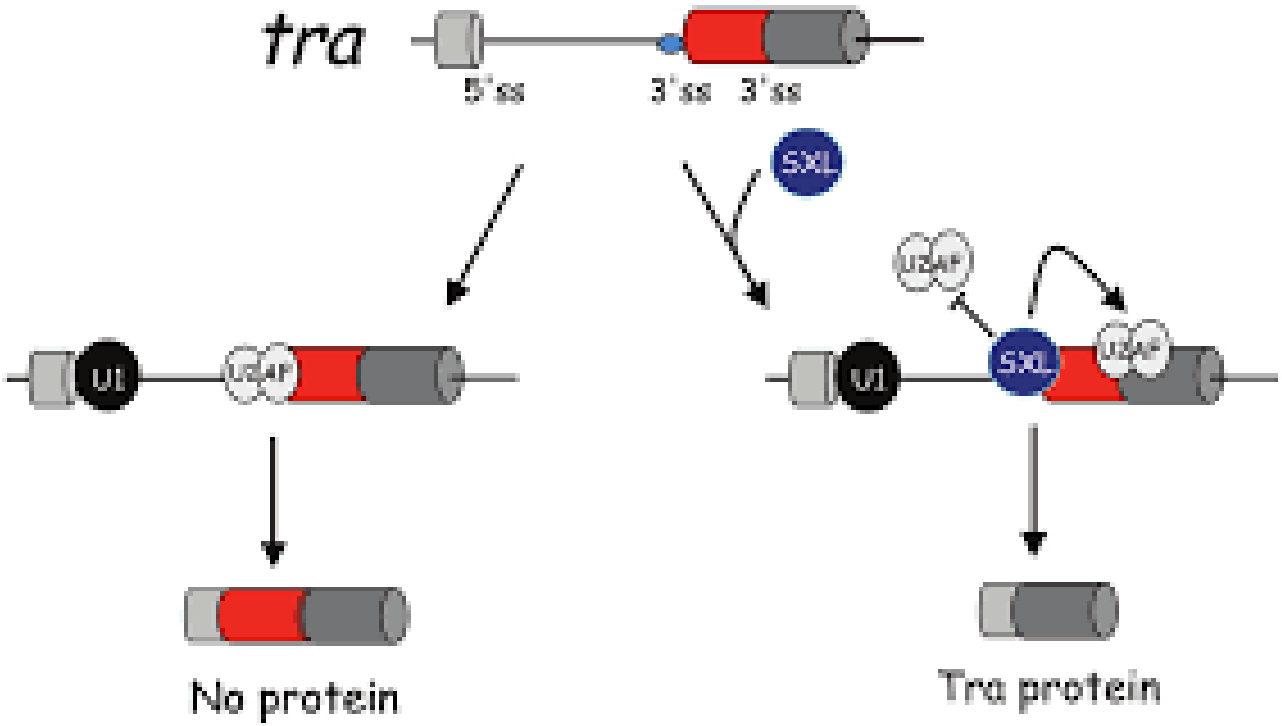
Mechanism of sxl Splicing

1. In male the m-RNA is spliced in a manner that yields 8 exons and the termination codon is within exon no 3.
2. In female, RNA processing yields only 7 exons and the male specific exon 3 is spliced out as a large intron.



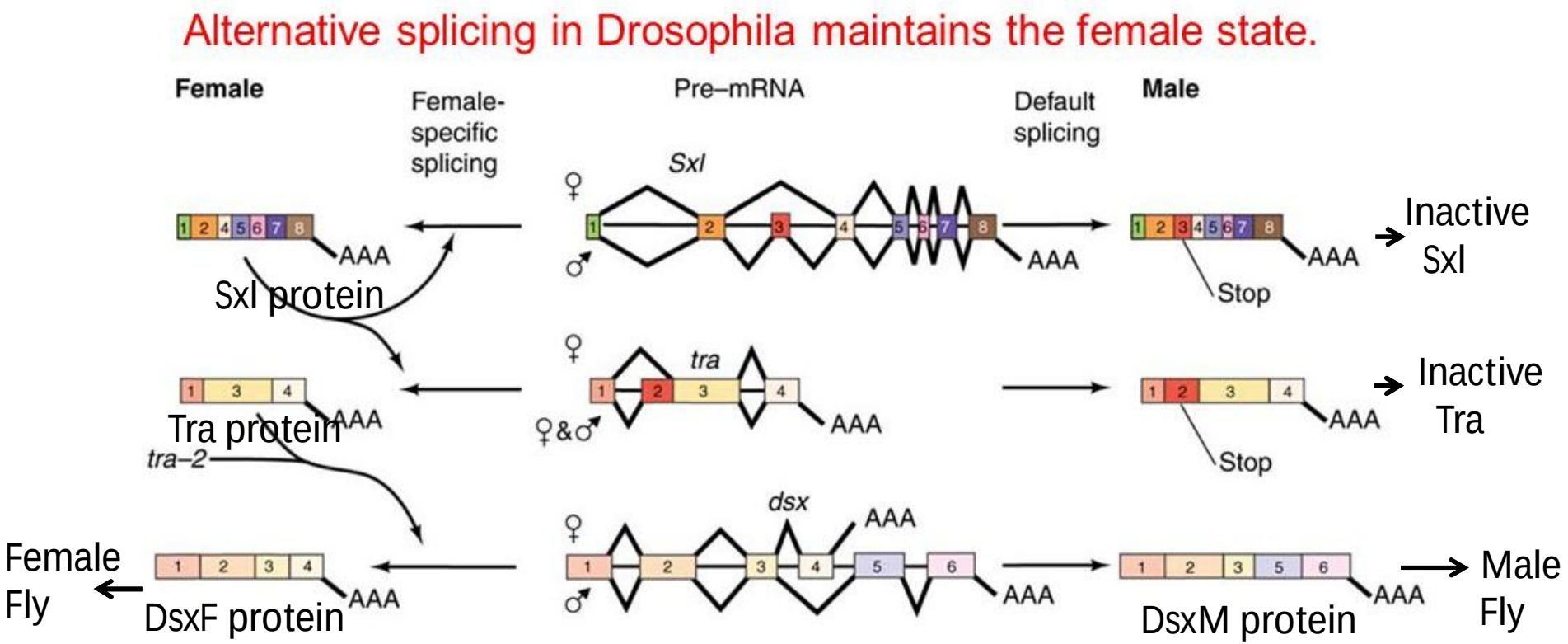
4. DIFFERENTIAL REGULATION OF TRANSFORMER GENE (*tra*)

1.The expression of *tra* gene is controlled by SXL protein.
2. The *tra* gene is transcribed into pre-tra mRNA.
3. The *tra* pre-mRNA is spliced alternatively to create a female specific mRNA and a non specific mRNA. The Sxl protein control the alternative splicing pf *tra* pre-mRNA.
4. Male specific *tra* mRNA contains a termination codon in the second exon due to lac of Sxl.
5. This exon is spliced out in XX female to form female specific m-RNA.
6. Female specific *tra* RNA is translated into a functional TRA protein.
7. But in male TRA protein is not synthesized due to stop codon in exon 2.



5. Dsx- THE SWITCH GENE OF SEX DETERMINATION

1.Doublesex (*dsx*)gene is an autosomal gene that can produce 2 different proteins through alternate splicing of its m-RNA.
2. A gene named *tra* 2 produce TRA 2 protein in both male and female.
3. The *dsx* gene produce pre *dsx* mRNA.
4. If the TRA & TRA 2 both are present, the *dsx* transcript is processed in a female specific manner and produces DSXF protein.
5.In absence of TRA, a male specific transcript is made which encodes DSXM protein.



6. PRODUCTION OF MALE OR FEMALE

1.DSXF represses the genes required for male development and activates female specific genes. Ultimately the XX Drosophila develops into a female.

2.DSXM inhibits female traits and promote male traits. Ultimately the XY *Drosophila* develops into a Male.

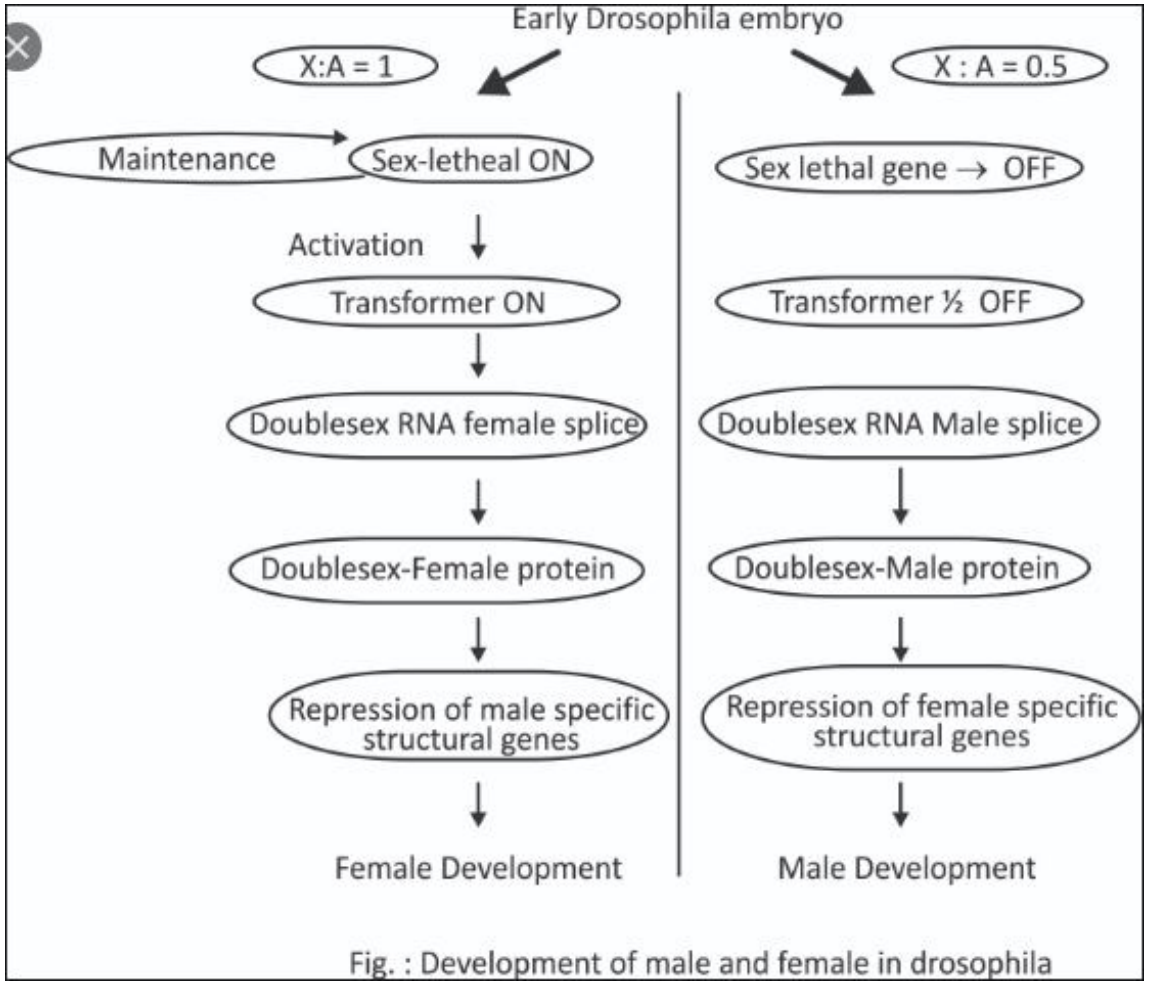
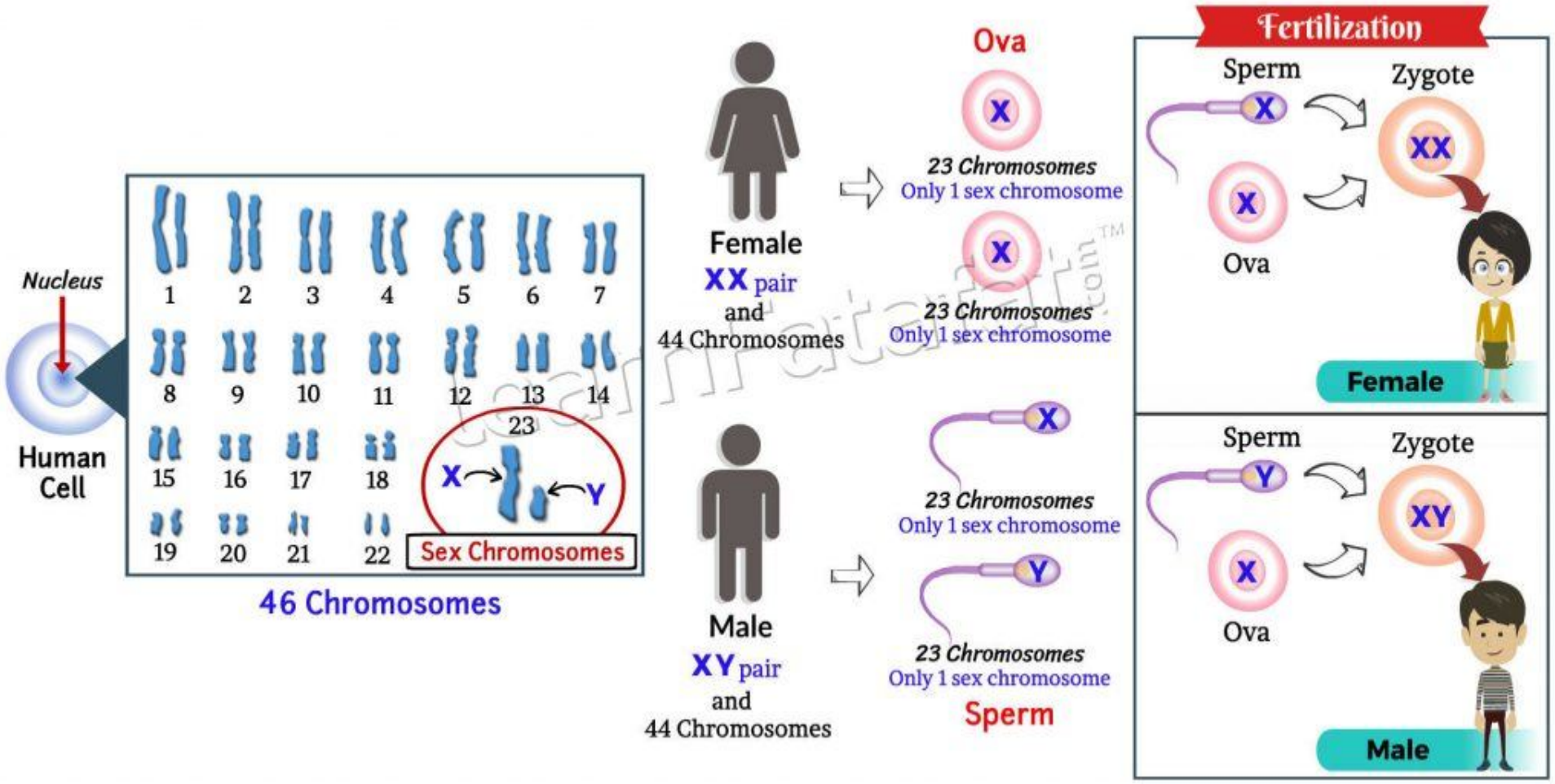


Fig. : Development of male and female in drosophila

SEX DETERMINATION IN HUMAN



The development of human reproductive systems

Sry = ***S***ex determining
region of the ***Y*** chromosome

↓

Major factor for
sex determination

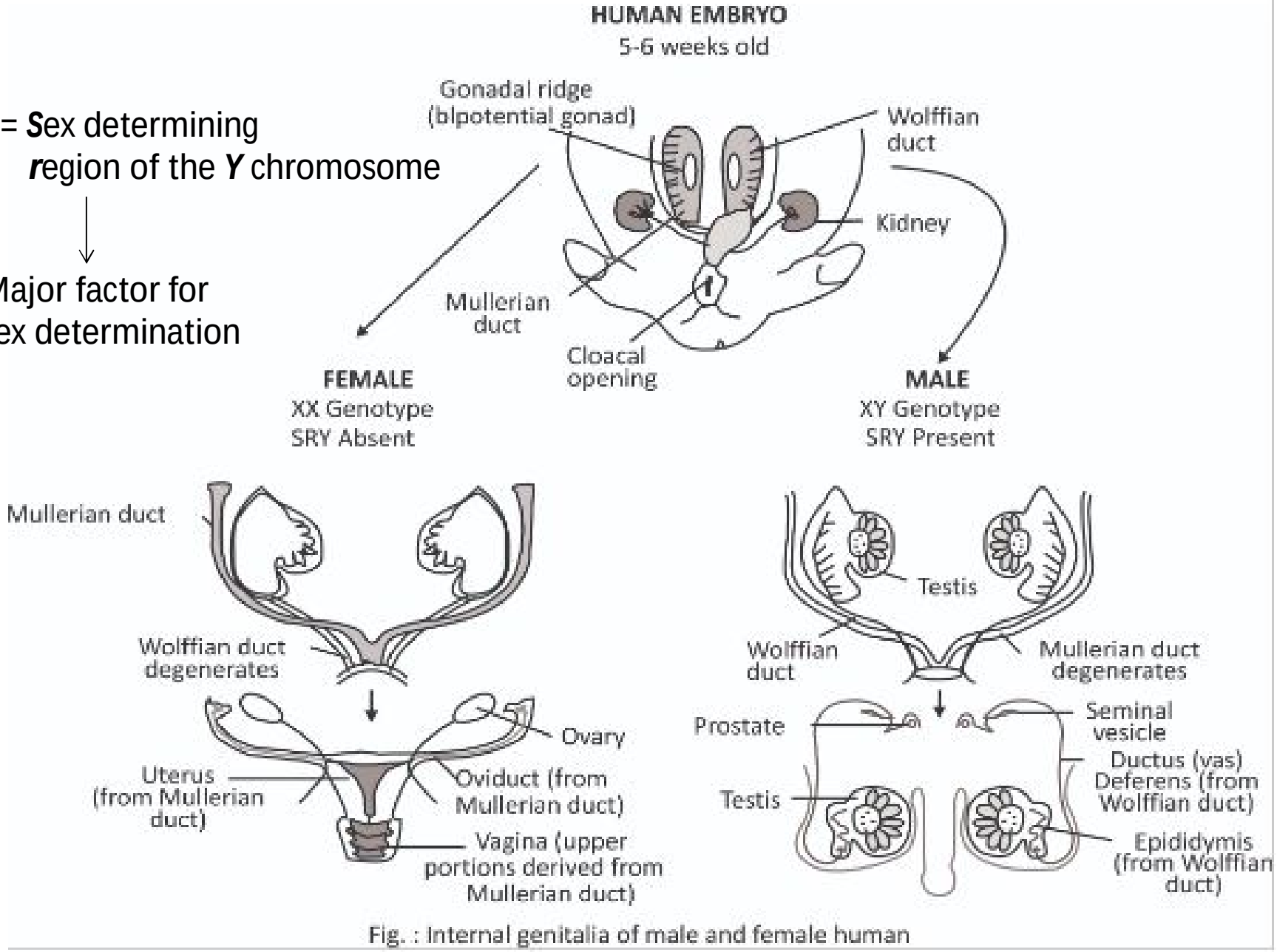
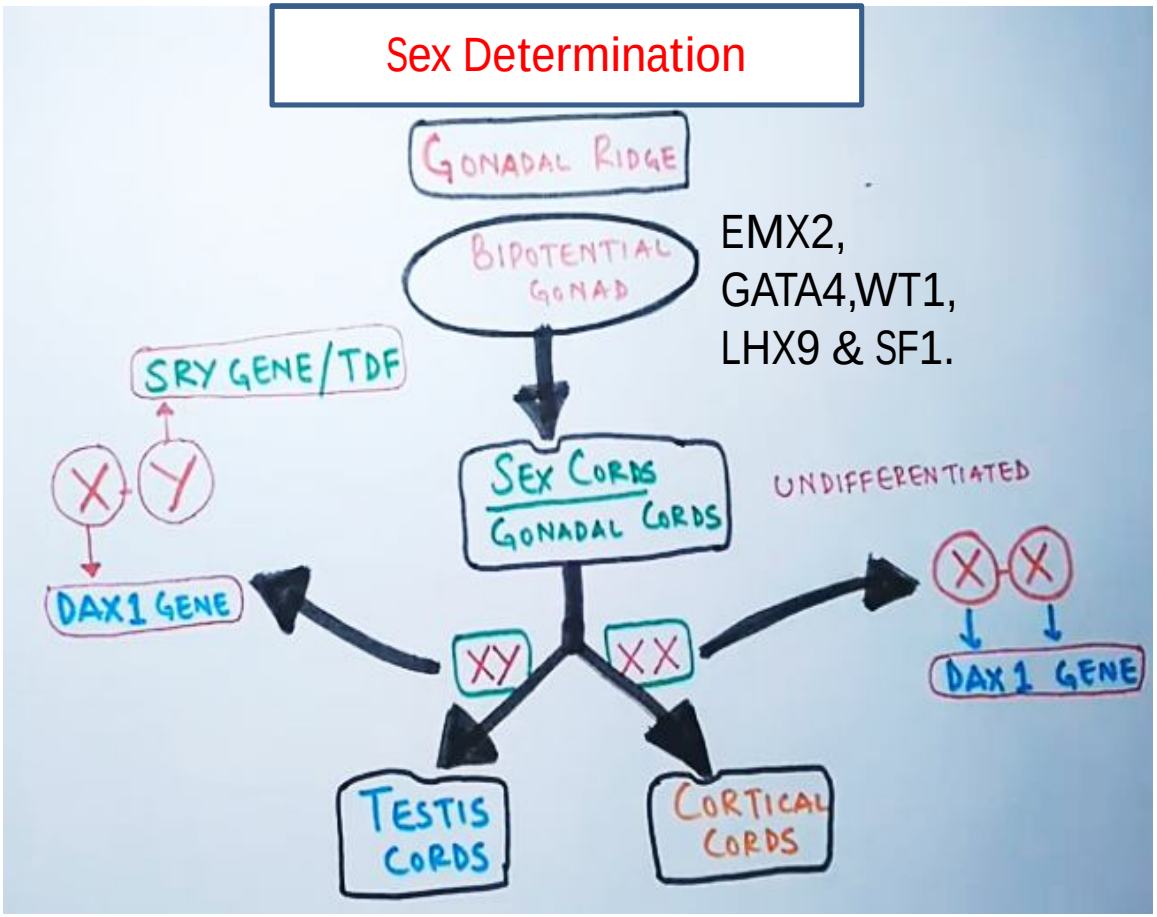


Fig. : Internal genitalia of male and female human

MOLECULARE MECHANISM

- Embryos are developed completely neutral for about 2 weeks. Then it follow either male or female pathway.
- Early embryo has a bipotential gonad.
- The bipotential gonad later can follow one of the 2 alternative pathway and develop into testes or ovary, depending on whether cell carries XY or XX chromosomes, respectively.
- Normal growth & maintenance of bipotential gonad is regulated by few genes including EMX2, GATA4,WT1,LHX9 & SF1.

- Male phenotype in human is determined by *sry* gene present on the short arm of the Y-chromosome.
- Dax1 (Dosage sensitive sex reversal) present on X-chromosome is responsible for female phenotype., but the female phenotype is due to lack of Sry region in XX.
- Loss of function of *sry* results in complete male to female sex reversal.



Sex-determining region of the Y Chromosome (SRY) :-

- It is region of sex determination factors that resides on the short arm of the Y-chromosome. This region has a male specific DNA sequence that encodes a peptide of 223 aminoacids and acts as a transcription factor. The *sry* encodes the human testis determining factor (TDF).

DAX1 :-

- It's a potential ovary-determining gene on the X-chromosome, If an organism has tow copies of DAX1 on an active X chromosome, the SRY signal would be reversed. It encodes a protein that competes with SRY factor and significant for the ovary development. It antagonizes the function of SRY, and down-regulates SF1 expression to assist the ovarian development.

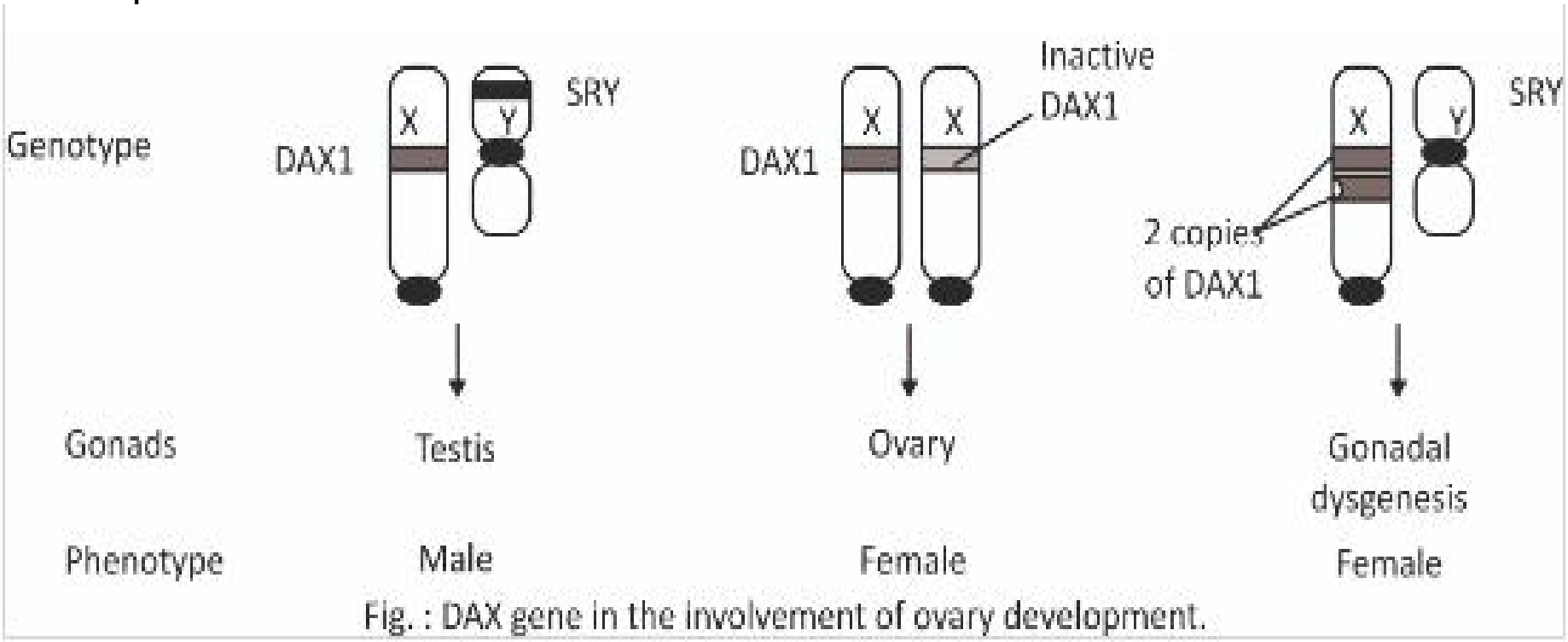
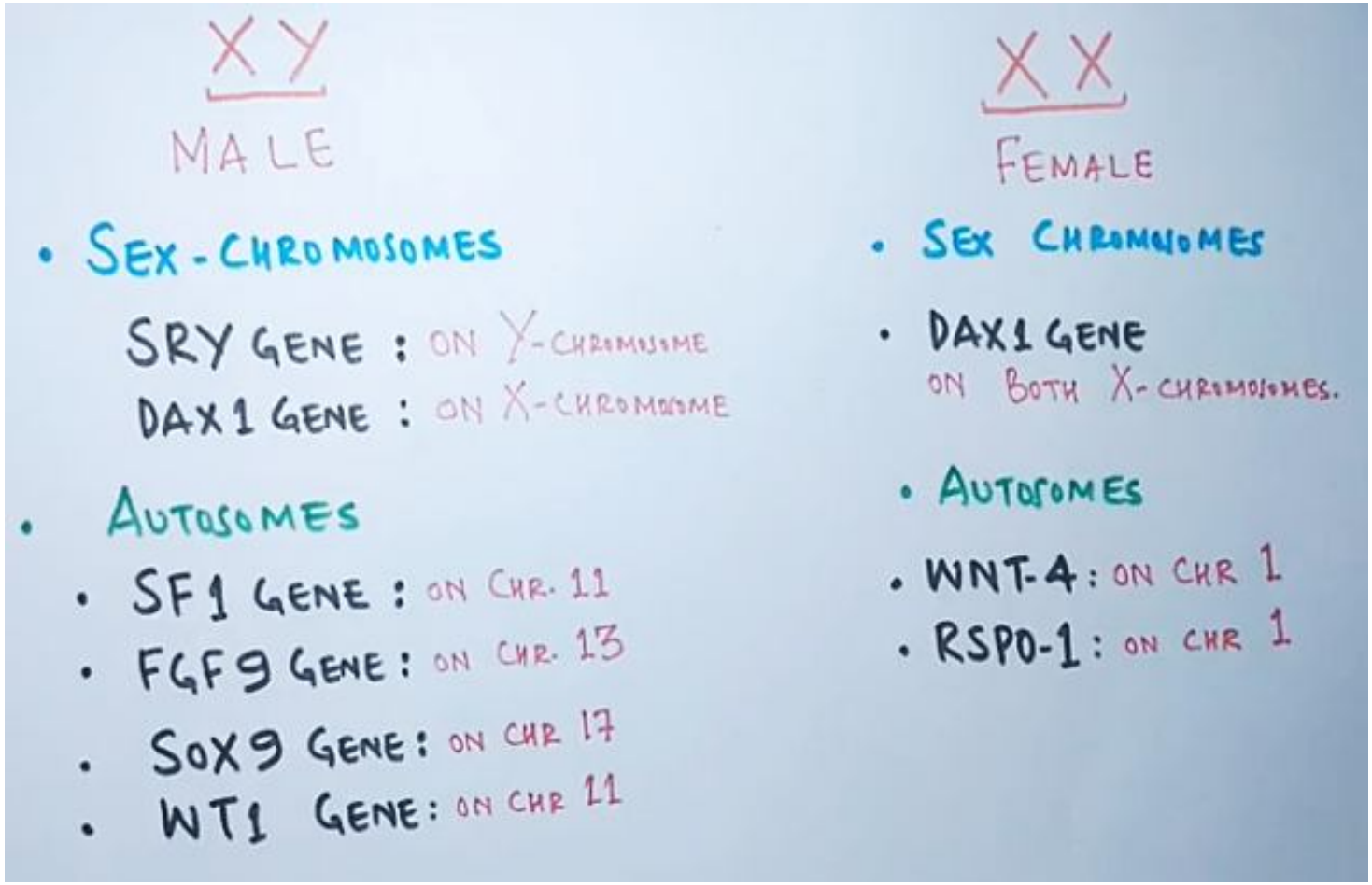


Fig. : DAX gene in the involvement of ovary development.

In addition to Sex chromosome, autosomes also play roles in sex determination.
- There are certain genes on autosomes which work in XX or XY specific manner.

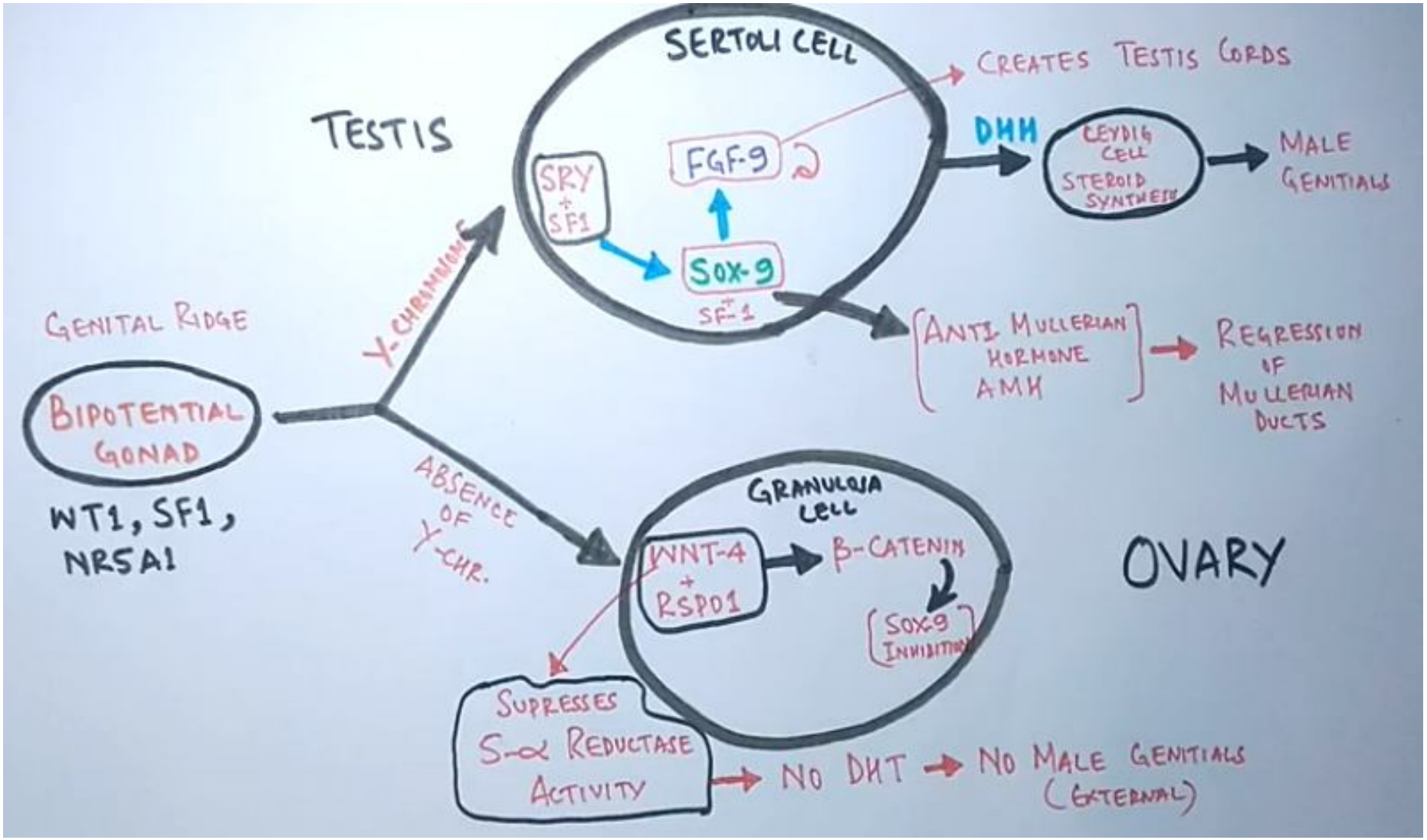
SF1 – Steroidogenic Factor 1
FGF9 – Fibroblast growth factor 9
Sox 9 – SRY related HMG Box
WT1 = Wilms Tumor Protein

WNT4 – Wingless related integration site
RSPO1 – R-Spondin 1



Pathways of testicular and ovarian differentiation

- The genital ridge gives bipotential gonad which undergo either Testis Pathway in XY embryo or ovary pathway in XX embryo to give male or female, respectively.



TESTIS PATHWAY

- Sry is expressed only in some somatic cells of the bipotential gonad.
- Sry gene encodes SRY or TDF protein (Testis determining factor).
- SRY with SF1 form an active transcription factor and activate transcription of *sox*9 gene.
- Sox9 produce SOX9 protein and induce testis development.
- SOX9 activates FGF9 protein. FGF9 works in positive feedback loop with Sox9 to maintain own level.
- FGF9 cause some somatic cells of the gonad to differentiate into sertoli cells.
- FGF9 also antagonise the WNT4 which is responsible for female differentiation.
- Sertoli cell produce anti-mullerian hormone by joint action of Sox9 and SF1, which supress the development of female reproductive tract (Mullerian duct).
- Sertoli cells also induce other somatic cells of the gonad to become leydig cells
- Sertoli cells secretes DHH (Desert Hedge Hog) which binds to patched receptor of leyding cells and induces steroidogenesis by them.
- Leydig cell secrete male sex hormone testosterone.
- This hormone completes the development of testis.

OVARIAN PATHWAY

- In absence of *sry* expression gonad develops into ovary.
- In absence of SRY, FGF9 is not synthesised. So, WNT4 is expressed in bipotential gonad.
- WNT4 with RSPO1 activates β-catenin.
- β-catenin inhibits the SOX9 which is required for synthesis of anti-mulerian hormone (AMH).
- So, in absence of AMH, Wolfian ducts develop into female reproductive organs.
- WNT4 with RSPO1 suppresses the 5-α-Reductase activity which is required for conversion of Testosterone in to DHT (Di-Hydroxy Testosterone).
- DHT is essential for maturation of male genitals.
- Some somatic cell become follicle cell.
- Other somatic cell become theca cell and secrete the female sex hormone estrogen.
- Estrogen completes the development of ovary and contribute in development of female secondary sexual characters.

References for further reading……..

1. **Developmental Biology**, 6th Edition,
   Book by Scott F, Gilbert
2. Snustad,P. & Simpsons,M.J.(2003)Principles of genetics 3rd ed.John Wiley & Sons,Inc.:USA