Biology of Sex

Phenotypic Sex

Genetic Sex

Brain Sex

Gonadal Sex

Legal Sex

PHENOTYPIC SEX

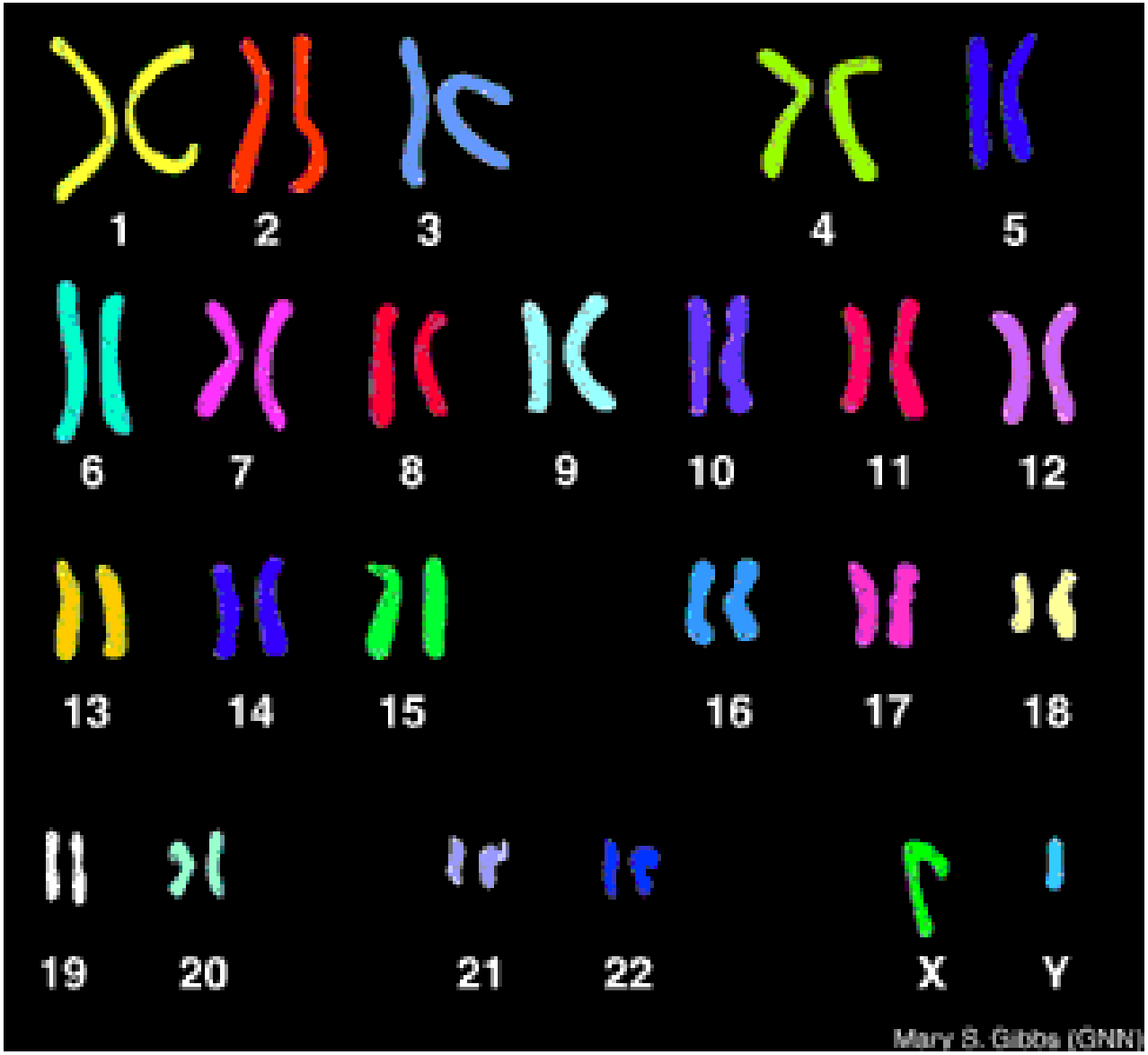
- Sum total of characteristics of each gender
  - Male or Female Reproductive Organs
  - Distribution of Hair
  - Mammary Development
  - Size – Males larger than females generally
  - Estrogens vs Androgens
  - Physique
    - Male – Narrow hips and broad shoulders
    - Female – Broad hips and narrow shoulders

PHENOTYPIC SEX

- True Hermaphrodyte: Intersex individual with both ovarian and testicular tissue – reproductive tract male or female or both – intersex pigs for example
- Pseudohermaphrodyte: Individual with only one type of gonad but other organs may be male or female – Testicular Feminization Syndrome (XY genotype; female phenotype)
- Transvestite – Dress like opposite sex
- Transexual – Surgical intervention and endocrine alterations to achieve desired phenotype.

GENETIC SEX

- Determined by sex chromosomes
  - Karyotyping
  - SRY gene – detect using molecular technique (PCR)
  - Mammals – XY Male; XX Female
  - Birds, Fish, Amphibians, Reptiles – ZW Female; ZZ Male
- X-Chromosome
  - About 300 genes: color vision, blood clotting, muscular dystrophy and so forth
- Y-Chromosome
  - Few unique genes – hairy ears
  - Unique genes for maleness and spermatogenesis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21
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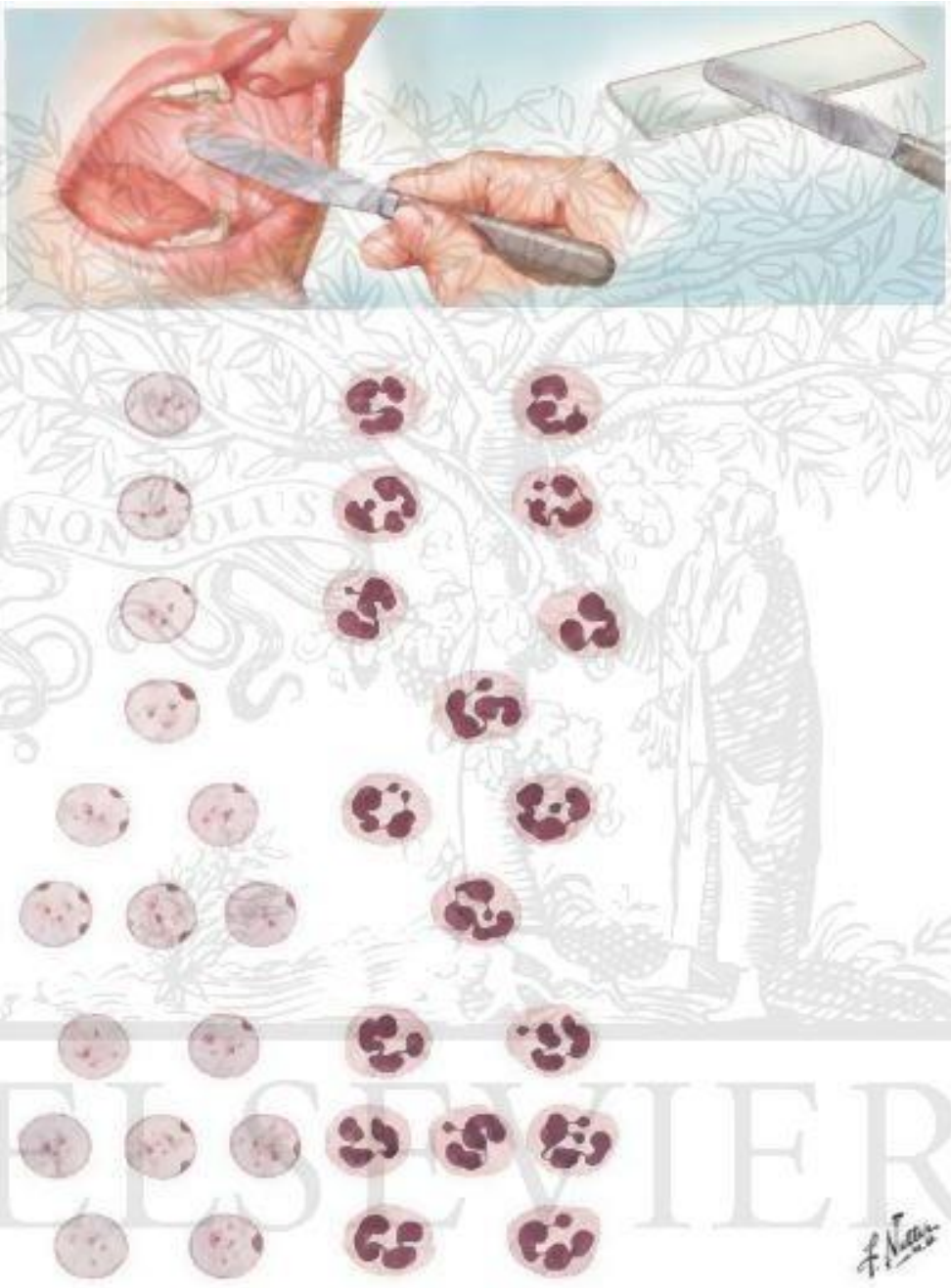
X
Y
Mary S. Gibbs (GNN)

GENETIC SEX

- Sex Linked Genes –X and Y chromosomes rarely cross over with autosomal chromosomes
- Temperature
  - Turtles and Tortoises
    - 16 to 28C = all females
    - 32C and greater = all males
  - Lizards
    - 16 to 28C = all males
    - 32C and greater = all females
  - Epigenetic Effect may involve aromatase gene

GENETIC SEX

- **X Chromosome carries 300+ genes – How does female carry a double dose of genes?**
  - **Inactivation of 1 X chromosome**
    - **Occurs at about 16 to 32 cell stage embryo**
    - **Detected by heterochromatic nuclear material (Mary Lyon 1961 and Murray Barr) – called Barr Body or sex chromatin**
      - **1 um diameter, feulgen stain +, lying against inner surface of nuclear membrane in most cells; against nucleolus in nerve cells**
      - **XO female – Turner Syndrome - No Barr Body**
      - **XYY Male – normal – No Barr Body**
      - **XXY, XXYY, XXXY, XXXXY – Kleinfelter Syndrome; Phenotypic male; Inactivation of all but 1 X, so 1, 1, 2, and 3 Barr Bodies, respectively**
      - **XXX or XXXX – Superfemale – 2 or 3 Barr Bodies, respectively**



GENETIC SEX

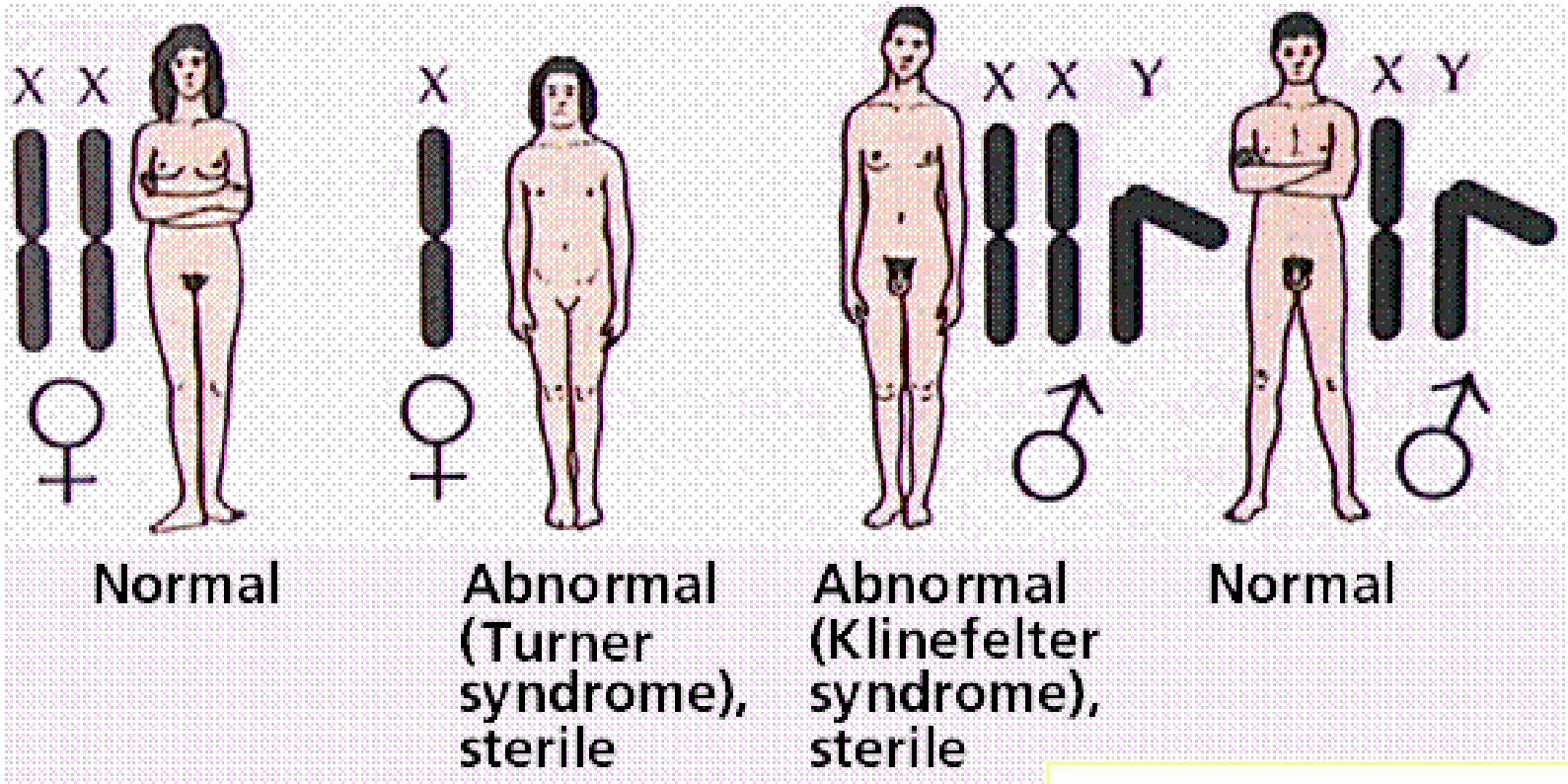
- Maternal Twins – Female twins less similar than males twins – differenential inactivation of X
- Tortoise Shell (Calico) Cats – all are female
  - B = Black; b = yellow on X chromosome so some clusters of cells are B (black), some are b (yellow): cat skin is then a mosaic of cells with differentially inactivated X chromosome

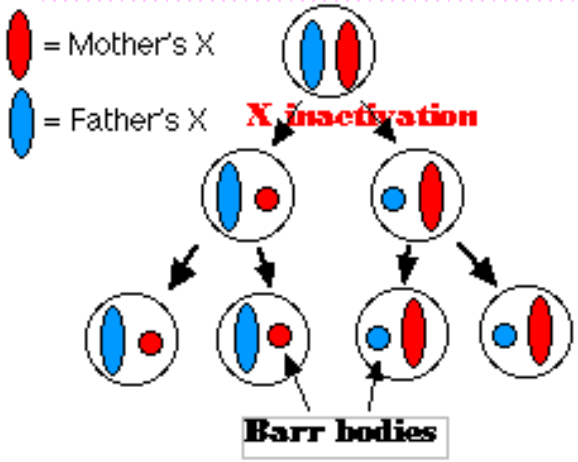
Primary sex determination

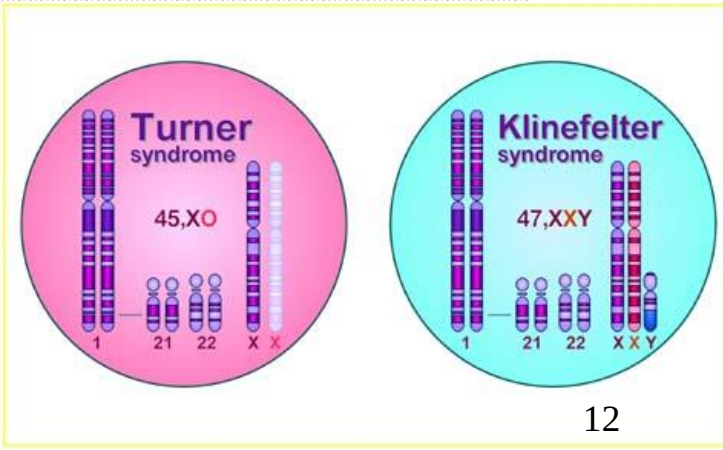
- Genetic (chromosomal) sex is determined at the moment the egg is **fertilized** by sperm
- Females XX
- Males XY
  - Y chromosome carries a testis determining factor (TDF) encoded by the *sex determining region Y* (*SRY)* gene.
  - Y chromosome determines maleness.
- Male and female gonads diverge from a common precursor termed the **bipotential gonad**.

Chromosomal Sex

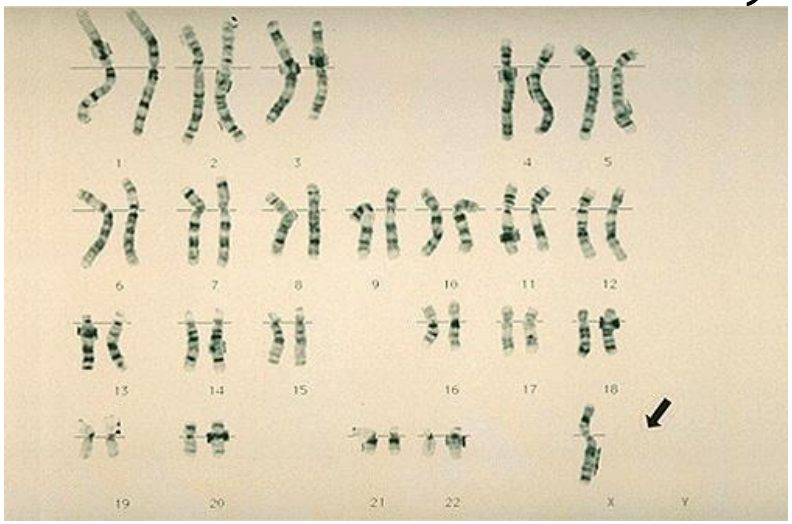
- **XY** (normal) or XXY or XXYY or XXXY or XXXXY = Male (testis)
- **XX** (normal) or XXX = Female (ovary)
- XO = Female with incomplete ovarian development
- XXY or XXYY or XXXY or XXXXY = testis but impaired sperm production
- Y must encode a factor that makes the bipotential gonad develop into a testis.

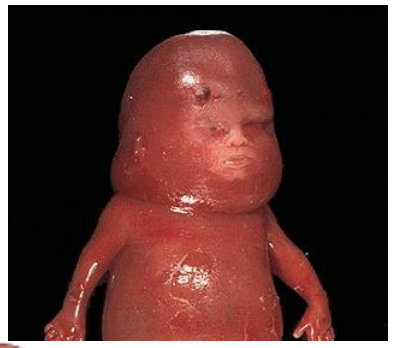

X X
X
X X Y
X Y
Normal
Abnormal (Turner syndrome), sterile
Abnormal (Klinefelter syndrome), sterile
Normal

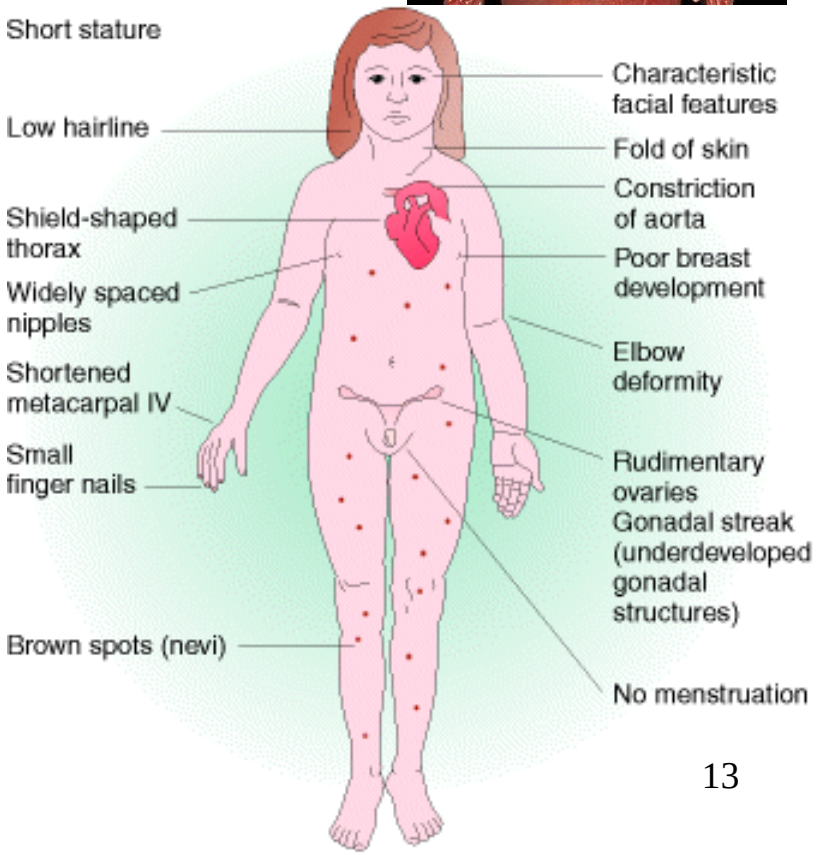

= Mother's X
= Father's X
X inactivation
Barr bodies


Turner syndrome
45,XO
1
21
22
X X
Klinefelter syndrome
47,XXY
1
21
22
X X Y

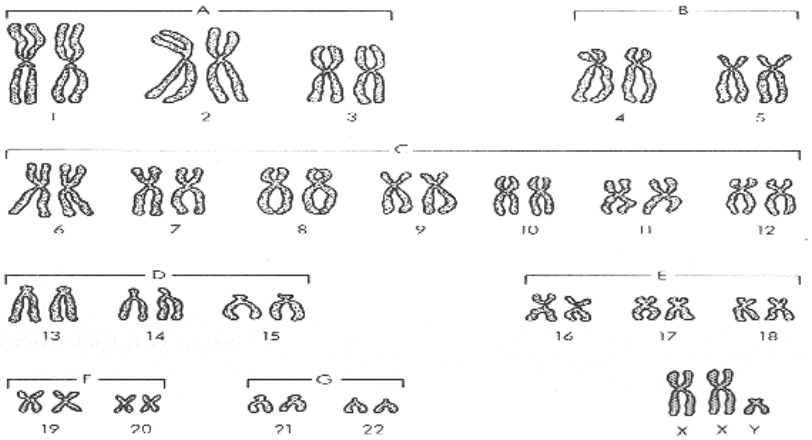
Turner's Syndrome (XO)
1 in 10,000 live births



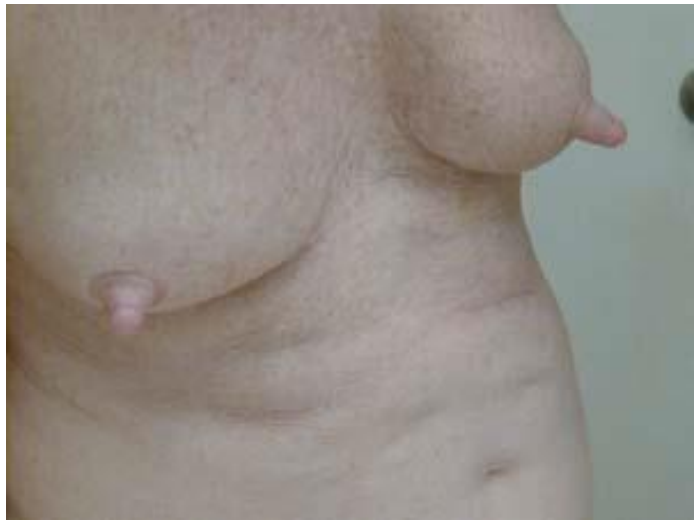


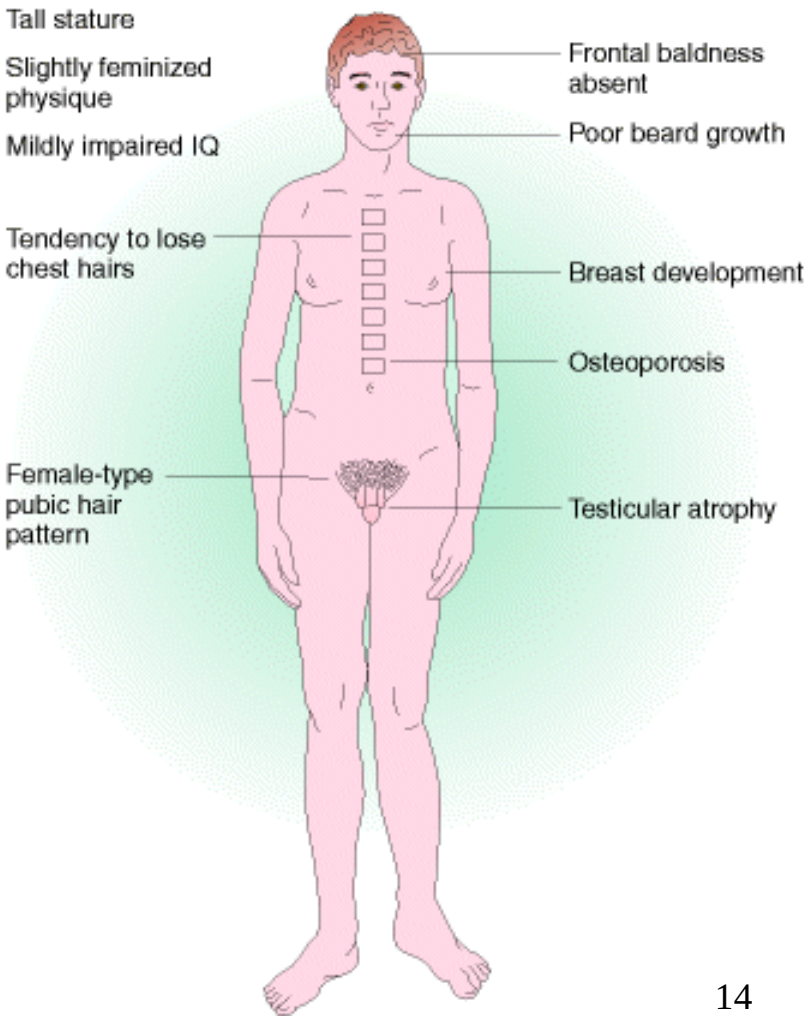


Klinefelter's Syndrome (XXY)
1 in 100,000 live births



Kleinfelter's Syndrome





Disorders of phenotypic sex

- Disorders in which **phenotypic sex is ambiguous** or completely in **disagreement with chromosomal and gonadal** sex

- Generally result from **failure of synthesis or action of hormones** that mediate male sexual differentiation or the **inappropriate synthesis** of androgens.

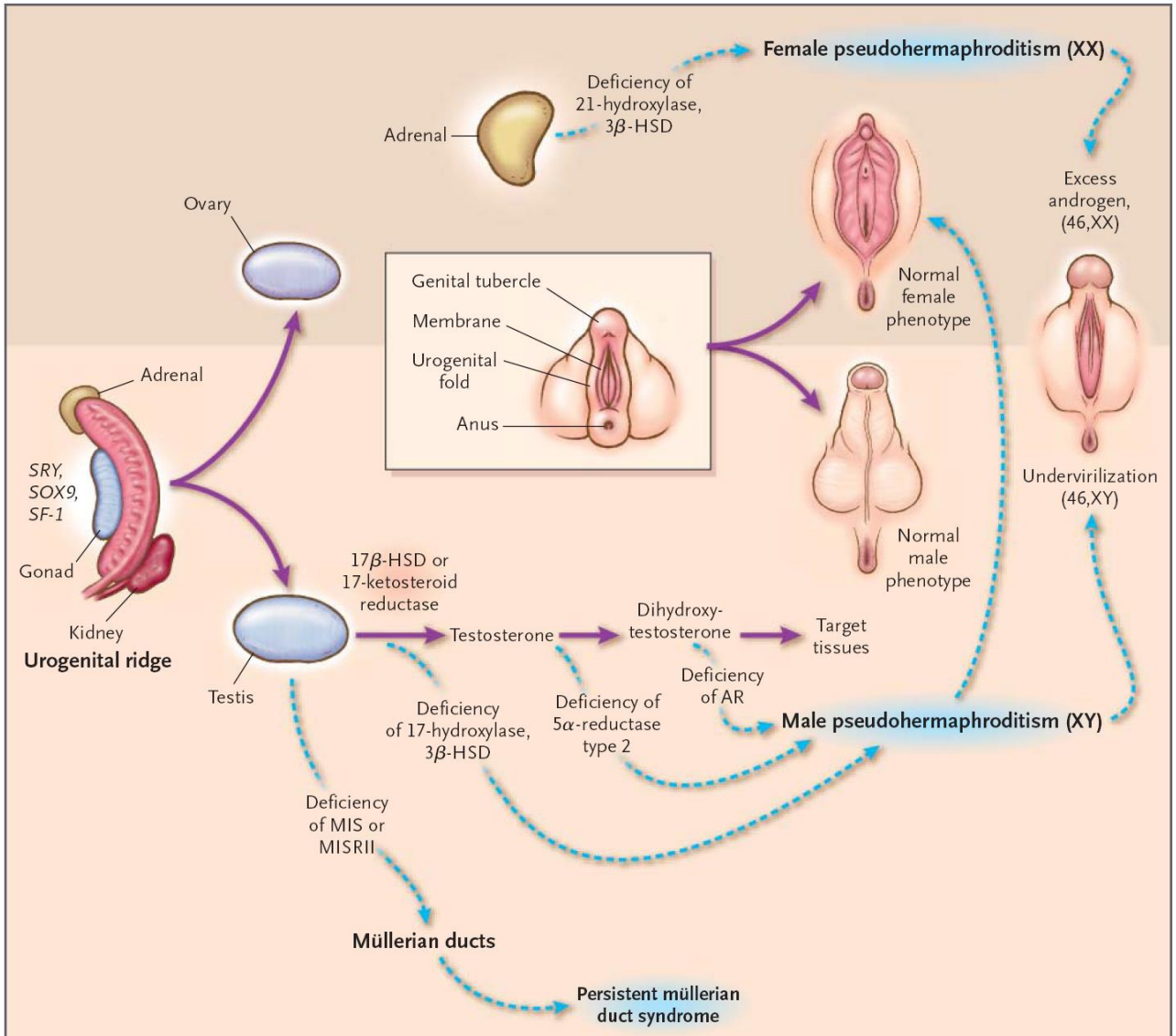


Figure 4. Functional Abnormalities of the Synthesis and Action of Hormones.

After the gonads have formed, reduced hormonal activity or signaling of specific receptors can lead to functional abnormalities of the reproductive tract, including persistent müllerian duct syndrome; male pseudohermaphroditism, causing undervirilization; and müllerian agenesis. After adrenal development, reduced enzymatic activity can result in female pseudohermaphroditism with excessive virilization. HSD denotes hydroxysteroid dehydrogenase, MIS müllerian inhibiting substance, MISRII müllerian inhibiting substance type II receptor, *SF-1* the gene for steroidogenic factor 1, *SRY* the gene for the sex-determining region of the Y chromosome, *SOX9* the gene for SRY homeobox 9, and AR androgen receptors.

N Engl J Med 2004;350:367-78.

Androgen Insensitivity Syndrome

- Can be partial.
- Can be complete.
- Can be caused by AR (androgen receptor) mutations or mutations upstream of AR.
- The classical phenotypical expression of AR mutation is the androgen insensitivity syndrome (AIS). In the most extreme form, the complete AIS (CAIS), the 46,XY individual presents at birth as a phenotypically normal girl. However, the patients have undescended testes and no Müllerian duct-derived structures.

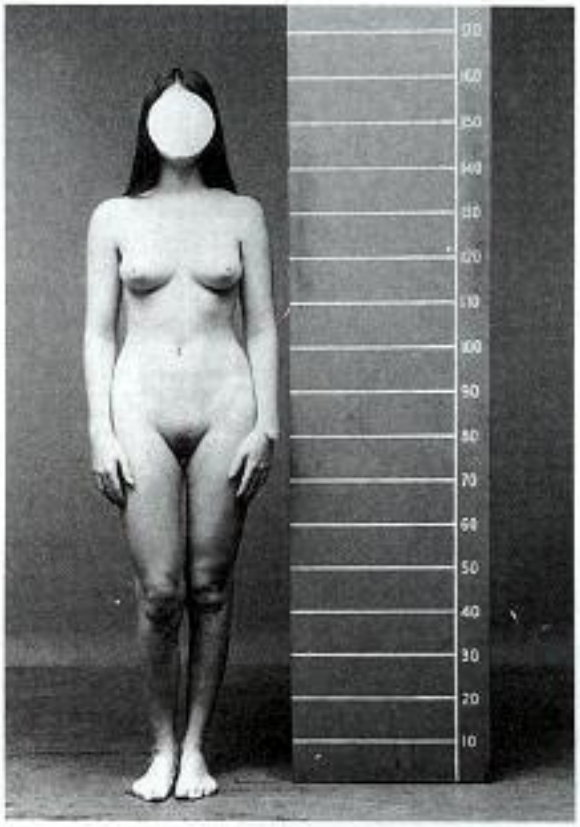


Figure 17.11. An XY individual with androgen insensitivity syndrome. Despite the XY karyotype and the presence of testes, such individuals develop female secondary sex characteristics. Internally, however, these women lack the Müllerian duct derivatives and have undescended testes. (Photograph courtesy of C. B. Hammond.)

Testicular Feminization Syndrome

A. Causes

1. Lack of androgen receptor (AR)
2. Mutation in AR
    a. Only respond to estrogen
3. 5a-reductase enzyme deficiency
    a. No Dihydrotestosterone
4. 5a-reductase enzyme affinity for NADPH is low or absent
    a. Low conversion of T to DHT

Partial androgen insensitivity

Imperato-McGinley Syndrome

- Described by Imperato-McGinley and Peterson (1976) in Dominican Republic
- XY children with syndrome have functioning testes with a blind pouch and enlarge clitoris and are raised as girls
- Androgens at puberty stimulate Androgen Receptor, 5a-reductase
  - Virilization of external genitalia occurs
- Urethra opens at base of penis (hypospadia)
  a. Precludes normal insemination
  b. Individuals are fertile and can sire offspring via artificial insemination

Congenital Adrenal Hyperplasia

A. Due to deficiency in 21-hydroxylase, 11b-hydroxylase, or 17a-hydroxylase

B. Genotype – XX

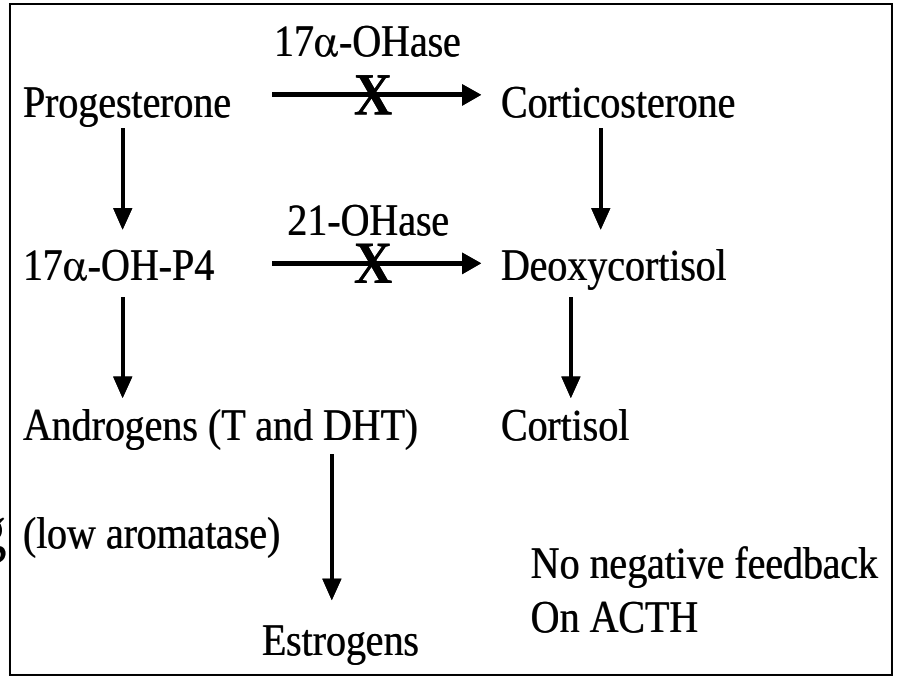
C. Phenotype – Male

Pseudohermaphrodite

1. Clitoris to Penis

2. Labioscrotal swelling

fuse to form scrotum

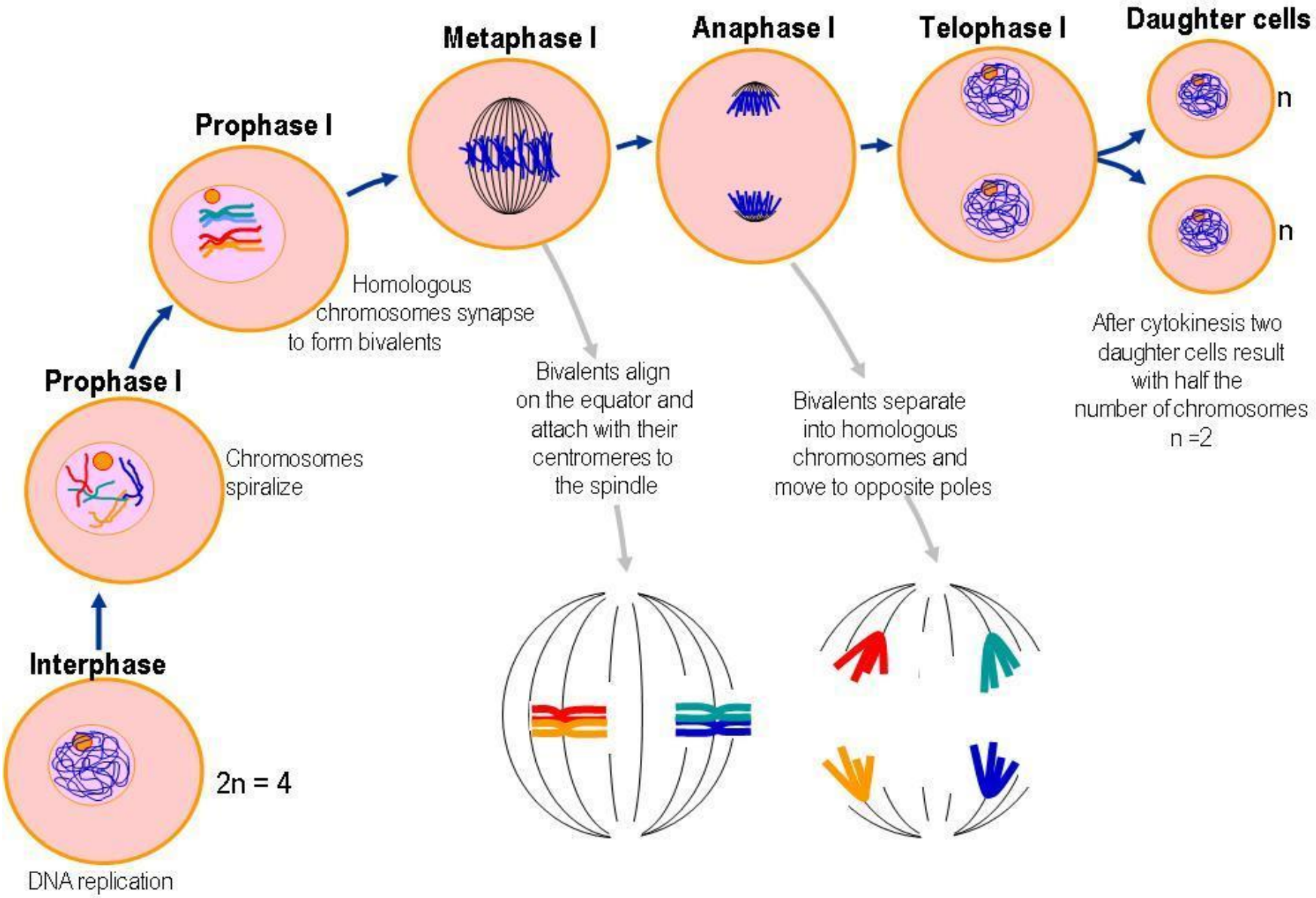


D. Treatment

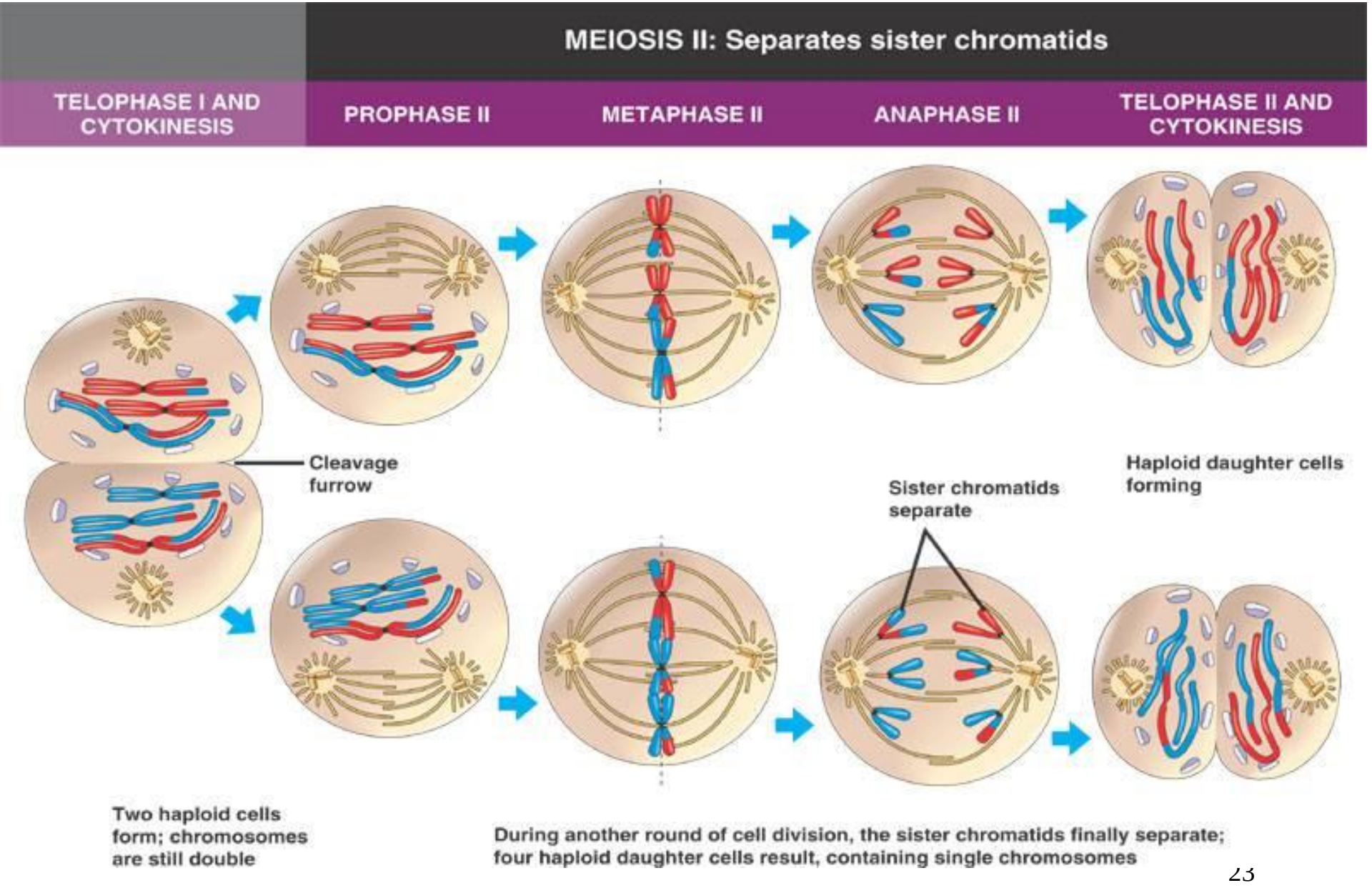
1. Corticosteroids

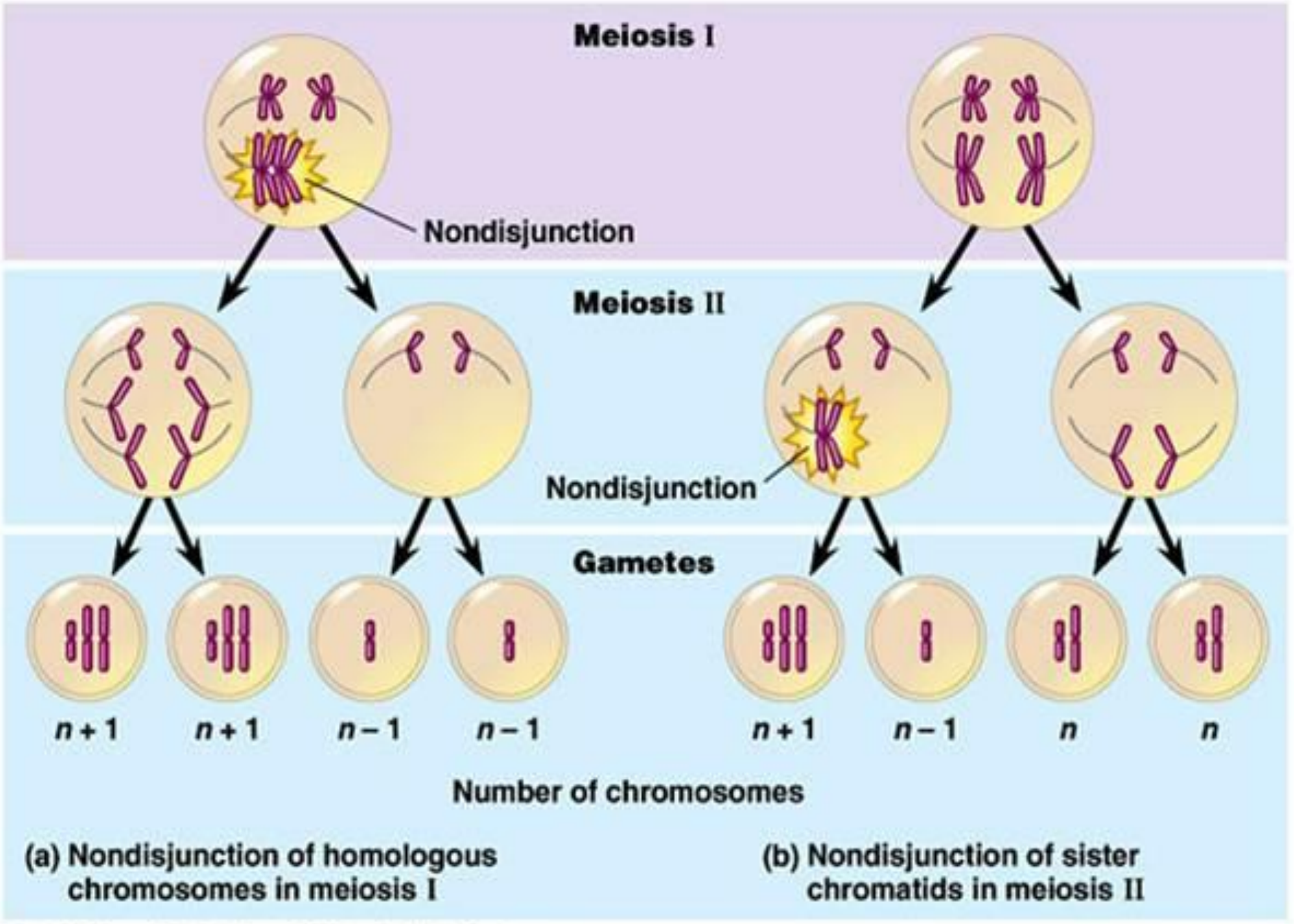
2. Plastic Surgery

Aneuploidy: Condition when nuclei contain chromosomes whose numbers are not true multiples of normal basic number,e.g triploidy, tetraploidy or 2N-1



NON-DYSJUNCTION OF SEX CHROMOSOMES





ANEUPLOIDY, GENETIC SEX AND PHENOTYPE

- POSSIBILITIES
  - X, XX, XXX AND XXXX OOCYTE
  - X, Y, XY, XXY, XXYY SPERM
  - GENOTYPE OF INDIVIDUAL CAN RANGE FROM
    - NORMAL XY TO ABNORMAL XXXXXXXYY

SEX RATIO: NUMBER OF MALES/100 FEMALES

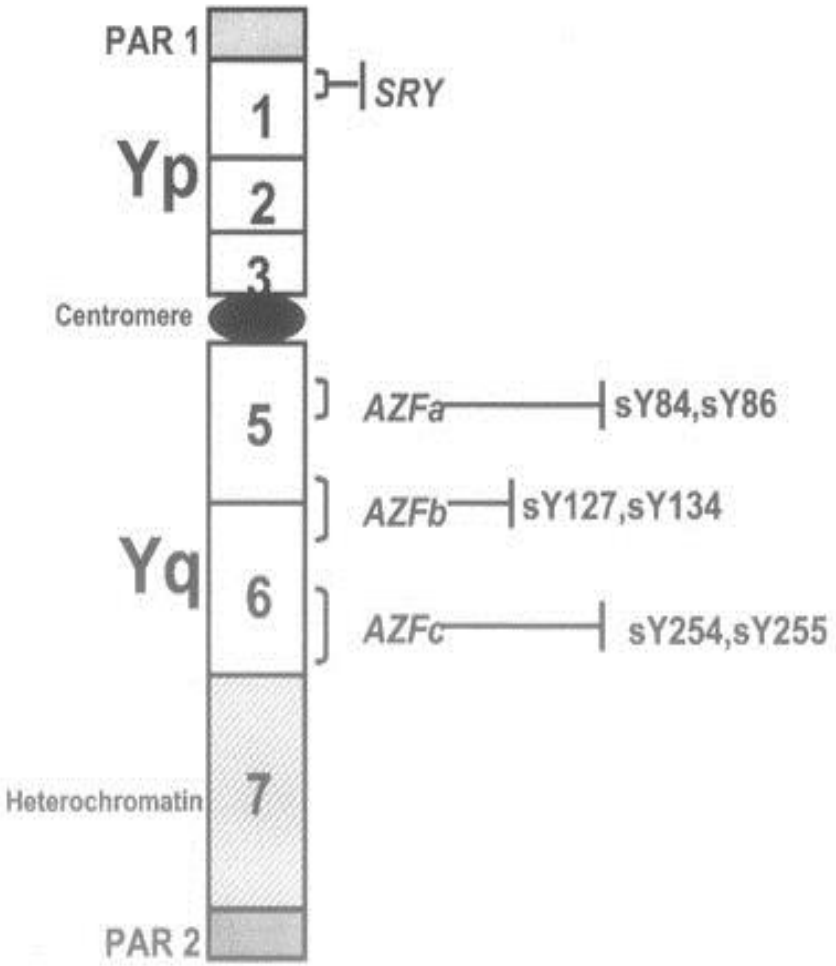
- PRIMARY – AT FERTILIZATION
- SECONDARY – AT BIRTH
- TERTIARY – AT PUBERTY

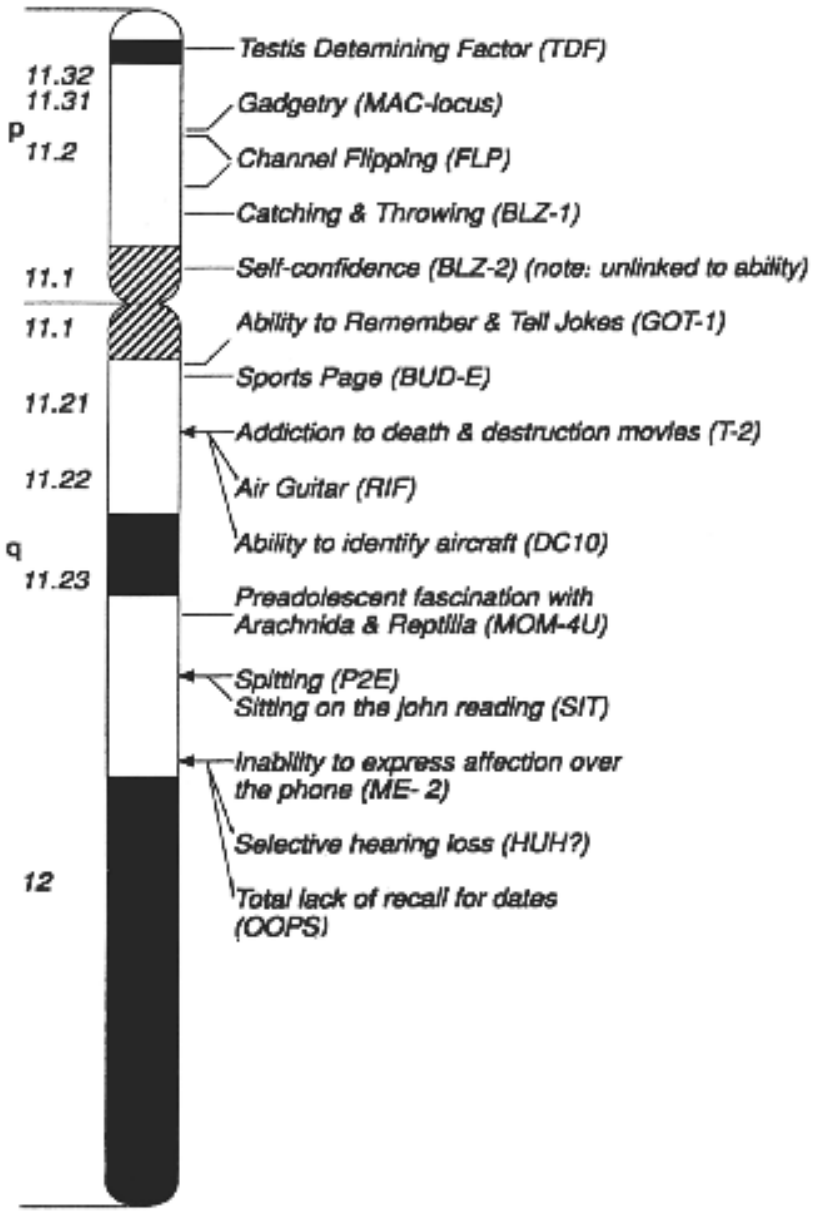
- FACTORS AFFECTING SEX RATIO
  - SELECTION – 110 TO 124 AND 110 TO 82 IN 25 GENERATIONS – HELEN KING WITH MICE
  - PARITY – 122 IN FIRST LITTERS TO 103 AT 4^{TH} LITTER IN RATS

SEX RATIO: NUMBER OF MALES/100 FEMALES

- Cattle:193 at Day 50-60 of Pregnancy and100 at Birth – Loss At Expense Of Male Embryos
- Three To Four Times As Many Male As Female Fetuses Aborted In Humans
- Control Of Sex Ratio: Laser Sorting Flourescence-Labeled (H33342 Flurochrome) Sperm Due To 3% Difference In Amount Of Dna In Favor Of X Chromosome

AZF – Azospermia Factors



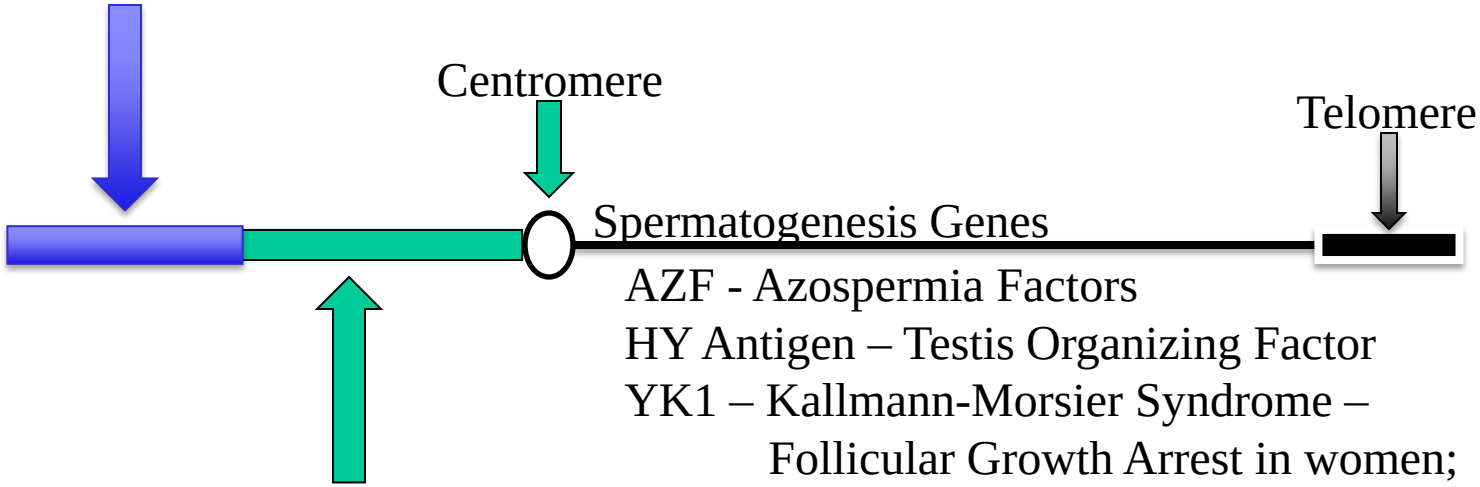


Y CHROMOSOME

*****Yp – Short Arm*****

**********Yq – Long Arm**********

Pseudoautosomal Genes
CSF2R
Blood Group Antigens



AZF - Azospermia Factors
HY Antigen – Testis Organizing Factor
YK1 – Kallmann-Morsier Syndrome –
Follicular Growth Arrest in women;
Nonfunctional in men

Sex Determining Genes
TDF/SRY – Testicular Determining Factor
RPS4Y – Ribosomal Protein
ZFY – Zinc Finger Y Transcription Factor
TSPY – Transcript Spermatid Specific Factor Y
CSF2R – Colony Stimulating Factor 2 – granulocyte and monocyte stimulating factor

The Y chromosome

- Presumably arose from an ancestral homolog of the X chromosome
- Retains regions of homology at its ends
- Permits the Y to pair with X during meiosis
- *SRY* gene is located near these ends
- Allows SRY domain to be transferred occasionally from Y to X chromosome
  - Leads to a 46,XX male (**one X has SRY**)
  - or 46,XY female (**Y lacks SRY**)

SRY: the Y chromosome sex determinant

In humans, major gene for the testis-determining factor (TDF) resides on the short arm of the Y chromosome

• Mapping of different Y fragments inherited by rare XX males, XY females and deleted in XY males permitted localization of TDF to short arm of human Y

>Individuals born with the short arm, but not long arm, of the Y are male
>Individuals born with long arm, but not short arm, of the Y are female

• These mapping analyses identified ***Sex Determining Region Y (SRY)*** **gene**

>Encodes a male-specific DNA sequence that encodes a protein of 223aa

> A transcription factor that contains a DNA-binding domain called the HMG (high mobility group) box

> HMG box found in several transcription factors and non-histone chromatin proteins that induces DNA transcription upon binding

Testis-Determining Factor

- **TDF (Testis-Determining Factor)** is a protein encoded by the *SRY* gene.
- If the ***SRY*** gene is **expressed**, then the **bipotential gonad** will become a **testis**, if **not expressed** then it will become an **ovary.**
- **Partial expression** of this gene results in **incomplete** gonadal **differentiation**.

Sry transgene in mice

The 14-kb region of DNA that encodes the *SRY* gene was microinjected into newly fertilized mouse oocytes

> XX embryos injected with *SRY* gene developed into phenotypic males

> However, these mice did not produce functional sperm

XXY mice and men do not produce sperm

Transgenic XX mice lack remainder of Y that contains genes for spermatogenesis

SRY is necessary for development of the mammalian testis

> Other testis forming genes are on the autosomes

> Autosome is any chromosome except sex chromosome

Box 1. A brief history of SRY

1959
The Y chromosome was identified as the dominant male determinant in humans and mice (for a review, see Ref. [1]). This finding initiated a search for *TDF* or *Tdy* on the Y chromosome. Several candidates were proposed (see below) and later refuted by genetic studies before the isolation of SRY.

1975
H–Y transplantation antigen. Because the Y chromosome was thought to carry few genes, it was proposed that H–Y must be TDF or Tdy.

Y chromosome deletions were eventually found, first in mice (1984) and then in humans (1987), where H–Y was lost, but male development was not.

1982
Bkm (banded krait minor-satellite). A repetitive DNA sequence found on the mouse Y chromosome with similarity to repeats found on a snake heteromorphic (W) chromosome.

Similar repeats could not be readily identified on other mammalian Y chromosomes, including the human Y.

1987
ZFY, a zinc finger nucleic-acid-binding protein, was proposed to be *TDF* owing to its presence within a 280-kb Y-unique sequence in an XX male. It also was deleted from an XY female carrying X,t(Y:22).

All eutherians have a close homologue on the X chromosome (ZFX), which is ubiquitously expressed. ZFY is also ubiquitously expressed in humans. Marsupial homologues are autosomal (1988). Mouse Zfy1 or Zfy2 are normal in XY females (1990).

1990
SRY was identified in a search for conserved sequences within a 35-kb Y unique region found in four XX humans showing some male characteristics. The mouse *Sry* was found to be present in the smallest region of the mouse Y chromosome associated with male development, but was missing in Tdy^{m1} allele (an 11-kb deletion from mouse Y). In line with predictions, *Sry* expression was observed within somatic cells of XY indifferent gonads. Point mutations were discovered within *SRY* in several XY females, indicating that *SRY* is required for male development in humans. Moreover, *SRY* lies within a second deletion in the X,t(Y:22) female.

1991
A 14-kb mouse genomic fragment carrying *Sry* gave XX male development when introduced as a transgene into mice, demonstrating that *Sry* was not only necessary but, rather, sufficient for testis and subsequent male development. The XX male mice were predictably sterile because postnatal spermatogonia cannot tolerate the presence of two copies of the X chromosomes and because other Y-linked genes are required for spermatogenesis.

Sekido & Lovell-Badge, Trends in Genetics 2008; 25:19

A.

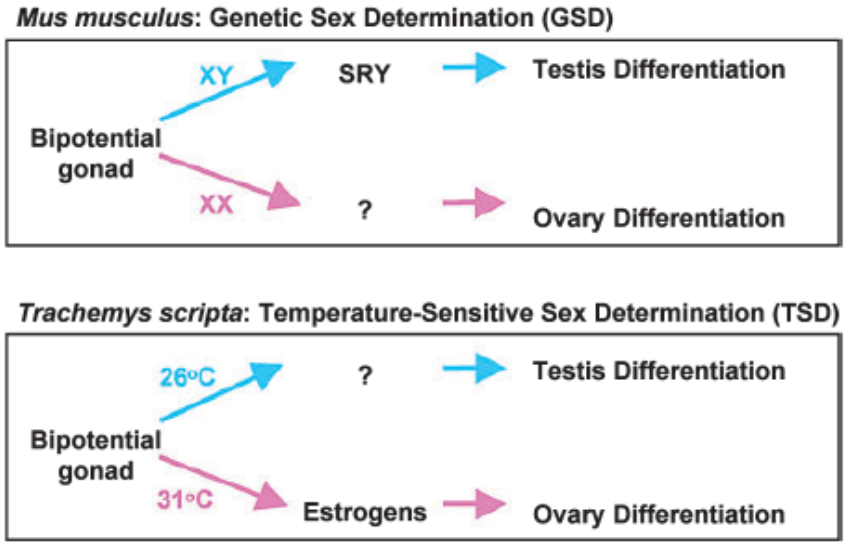
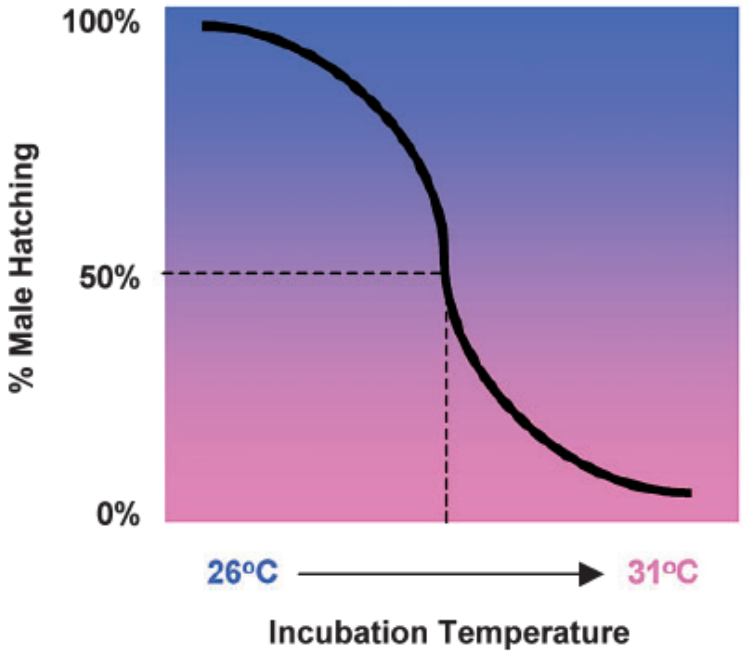
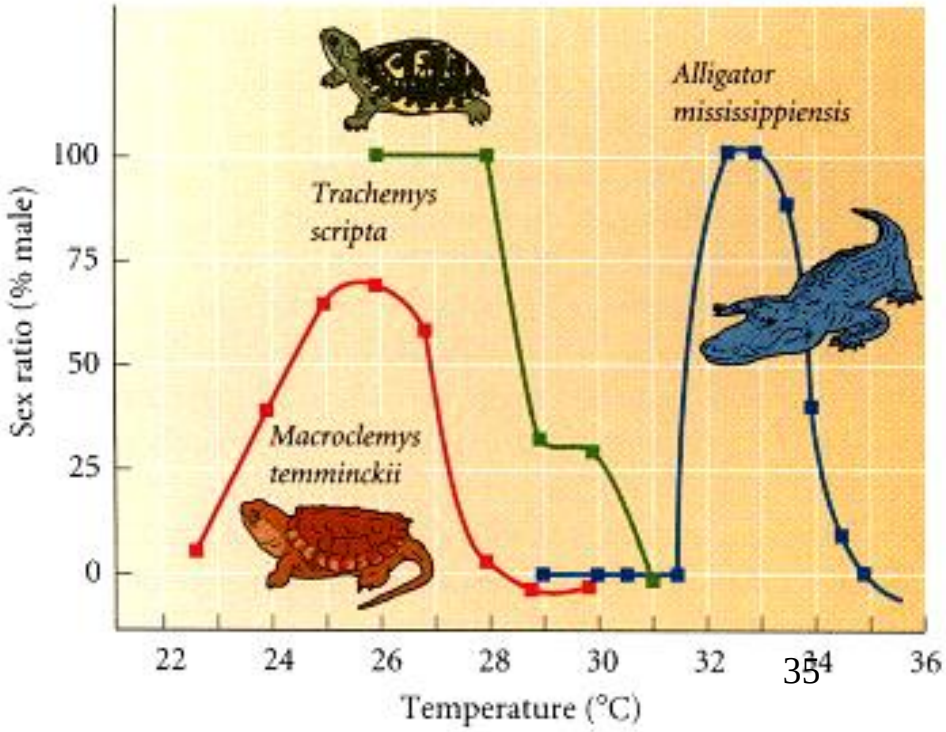


Fig. 1. **A: Current sex determination paradigms for mouse (*Mus musculus* with a genetic sex determination mechanism) and red-eared slider turtle (*Trachemys scripta* with a temperature-sensitive sex determination mechanism). B: Diagram of TSD in *T. scripta*: Nearly 100% of *T. scripta* eggs incubated at 26°C develop as males; whereas incubation at 31°C produces nearly 100% females.** At median temperatures ~50% develop as male, and 50% as female.

J. Biochem. **138**, 5–12 (2005)
DOI: 10.1093/jb/mvi097

B.





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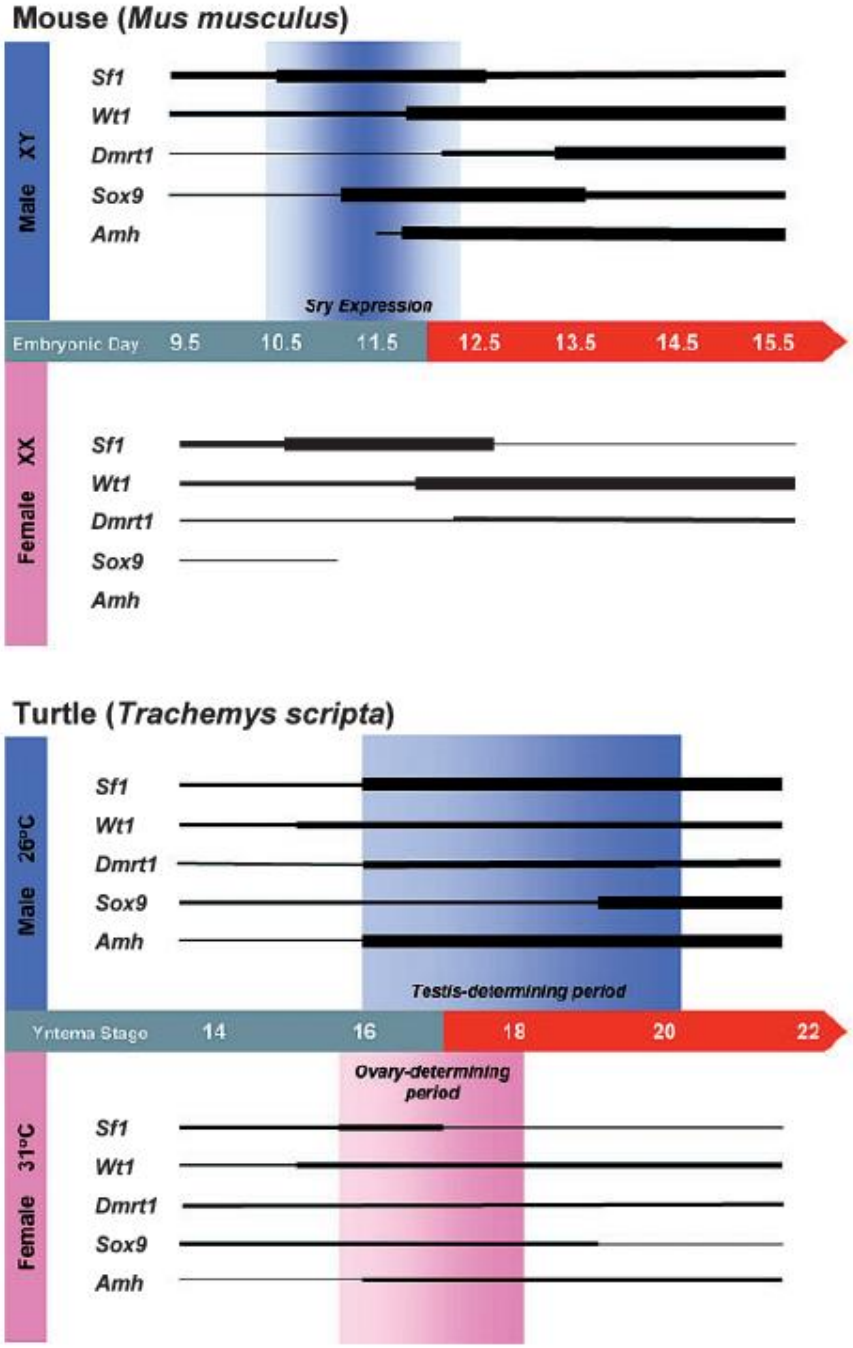

Fig. 2. **Diagrammatic expression profiles of genes critical for sex determination during embryogenesis in mouse (*Mus musculus*) and red-eared slider turtle (*Trachemys scripta*).** Relative expression is indicated by the thickness of lines (thicker lines indicate stronger expression and *vise versa*). It is valid only to compare expression levels of a given gene between sexes, not between genes or species. Red bars that highlight the developmental stage indicate the appearance of morphological differences in gonads between sexes. Blue and pink shaded areas represent gene or temperature sensitive periods. anti-Müllerian hormone, *AMH*; *Drosophila Doublesex* and *C. elegans Mab-3* Related Transcription factor 1, *DMRT1*; genetic sex determination, GSD; temperature-sensitive sex determination, TSD; Sex-determining region of the Y chromosome, *Sry*; *Sry*-like HMG-box protein 8, *SOX8*; Sry-like HMG-box protein 9, *SOX9*, Steroidogenic factor 1, *SF1*; Wilm's tumor gene 1, *WT1*.

WT1 (Wilms Tumor 1)

- *WT1* encodes a zinc finger transcription factor
  - 24 protein isoforms due to alternative splicing
- ***Wt1*** -/- does not form kidney, adrenal gland, or gonad
- Specifies coelomic epithelial cells in the urogenital ridge
- Functions upstream of two orphan nuclear receptors ***SF1*** (steroidogenic factor 1) and ***DAX1*** (dosage-sensitive sex reversal, adrenal hypoplasia congenita, X chromosome)

*SF1 (*Steriodogenic Factor 1)

- SF1 is an orphan nuclear hormone receptor
- SF1 regulates expression of multiple genes in male differentiation, steroidogenesis, and reproduction
  - Stimulates **Mullerian Inhibitory Factor** (Anti-Mullerian Hormone), P450 steroid hydroxylases, and 3βHSD
- *Sf1*-/- mice
  - Neither XX nor XY form adrenal glands or gonads
  - Rudimentary adrenal gland and gonads undergo apoptosis
  - Die at birth
- WT1 and SF1 interact in mice and this enhances SF1 target gene expression (*MIS, DAX1*)

DAX1

- *DAX1* encodes a nuclear hormone receptor
  - Lacks DNA binding domain (protein-protein interactions)
  - Transcriptional repressor that interacts with SF1
- *DAX1* mutations associated with adrenal hypoplasia and **hypogonadotropic hypogonadism**
  - Impaired development of pituitary and gonads
- Antagonizes synergy between WT1 and SF1 in mice
  - Inhibits transcription of SF1 target genes
- *Dax1-/-*
  - Gonadal defects in testis cord morphogenesis, peritubular myoid cell differentiation, and spermatogenesis
- Both SF1 and DAX1 are independently important for normal male gonadal differentiation

SOX9 **(**Sry related, HMG box)

- One of the autosomal genes involved in sex determination is *SOX9*
  - Encodes a putative transcription factor that contains a SRY-like HMG box
  - Also other family members (*SOX8*)
- **XX humans who have an extra copy of *SOX9* develop as males, even though they have no *SRY* gene**
- **Individuals having only one copy of SOX9 have campolemic dysplasia**
  - Disease involves numerous skeletal and organ systems
  - 75% of XY patients with this syndrome develop as phenotypic females or hermaphrodites

Sox 9

- SOX9 is essential for testis formation
  - Sox9 is expressed at low level in bipotential gonad
  - Sox9 expression increases in the male genital ridge and becomes absent in the female genital ridge
  - After sex differentiation, Sox9 is expressed in Sertoli cell
  - Sox9 expression is expressed slightly after SRY expression
  - Sox9 protein binds to a promoter site on the MIS gene
- Sox9 is found in all studied vertebrates
  - May be more of a central sex determination gene, because *Sry* is limited to mammals

Supporting Cells

- Sertoli cells and granulosa cells may originate from the same precursor
- XX-XY Chimera experiments
  - Sertoli cells were the only cell type requiring *Sry* in a cell autonomous manner
  - XX cells are preferentially excluded from the Sertoli population
- Sertoli cells direct differentiation of Leydig cells
  - *Dhh* (desert hedgehog) secreted protein from Sertoli act on Leydig Cells to stimulate differentiation

Supporting Cells

- Endothelial cells orginating from the mesonephros are induced by a chemoattractant released by male gonad

- Mesonephric cell migration can be induced by FGF9 (fibroblast growth factor 9) and MIS

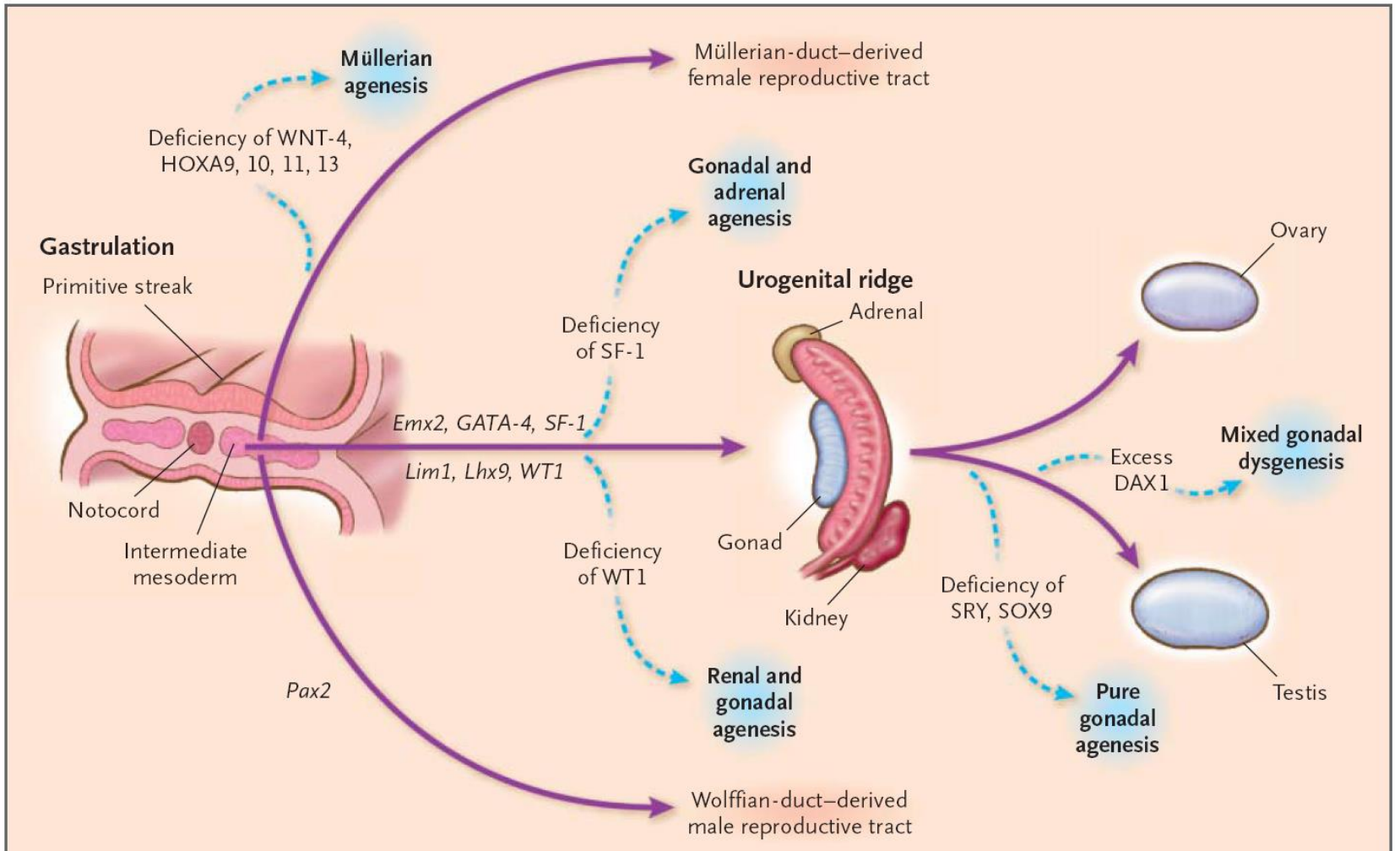

Figure 3. Syndromes of Dysgenesis during the Development of the Urogenital Ridge.

Mutations in various genes can lead to a variety of syndromes of dysgenesis involving the müllerian or wolffian ducts, gonads, kidneys, and adrenal glands as a result of a deficiency or excess of the proteins shown. *DAX1* denotes the gene for duplicated in adrenal hypoplasia congenita on the X chromosome 1; *Emx2* the empty spericles homeobox gene 2; *GATA-4* the gene encoding a protein that binds to a GATA DNA sequence; HOXA homeobox protein; *Lim1* a homeobox gene important for limb development; *Lhx9* a lim homeobox family member; *PAX2* paired box homeotic gene; *SF-1* the gene for steroidogenic factor 1; SRY sex-determining region of the Y chromosome; SOX9 SRY homeobox 9; Wnt-4 a protein that induces the development of the müllerian mesenchyme; and *WT1* Wilms' tumor-suppressor gene 1.

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N Engl J Med 2004;350:367-78.

Complete gonadal dysgenesis

- Wilms Tumor Related 1 (WT1)
  - First autosomal gene linked to 46,XY
- Wilms tumor=an embryonic kidney tumor
- Two distinct syndromes
  - Denys-Drash=gonadal and urogenital abnormalities along with diffuse mesangial sclerosis
  - Frasier=gonadal dysgenesis, impaired virilization, and focal glomerular sclerosis, but do not develop Wilms tumor

Complete gonadal dysgenesis

- 46,XY with abnormally formed gonads, were on the path to testis differentiation=gonadal streaks
- No androgen produced
- Wolffian ducts regress
- Müllerian ducts develop due to lack of MIS
- Female external genitalia
- Feminizing puberty with estrogen therapy

Complete gonadal dysgenesis

- Steroidogenic Factor 1 (SF1)
- SF-1 is required for the development of the indifferent gonads
- Mice lacking SF-1 exhibit adrenal and gonadal agenesis-have female internal and external urogenital tracts
- Studies suggest that SF-1 in humans plays roles in embryonic development of the adrenal glands and gonads

Complete gonadal dysgenesis

- DAX-1 & Dosage-Sensitive Sex Reversal
- Duplication of the short arm of the X
- DAX-1 is mutated in boys with adrenal hypoplasia congenita (AHC)
- Exhibit adrenal insufficiency
- Have a compound hypothalamic/pituitary defect
- Will lead to sex reversal

Pure gonadal dysgenesis

- Have a normal 46,XX or 46,XY
- Normal height, no associated somatic defects
- Gonadal development is arrested before MIS and androgens are produced
- Bilateral streak gonads are associated with an immature female phenotype

Table 1. Mutations in Genes Involved in Sex Determination and Development and Associated with Intersex Anomalies.

Gene (Locus)	Protein and Proposed Function	Mutant Phenotype
WT1 (11p13)	Transcription factor	Frasier syndrome, Denys–Drash syndrome with Wilms' tumor
SF-1 (9q33)	Transcription factor, nuclear receptor	Gonadal and adrenal dysgenesis
SOX9 (17q24)	High-mobility-group transcription factor	Campomelic dysplasia, male gonadal dysgenesis or XY sex reversal
DAX1 (Xp21.3)	Transcriptional regulator, nuclear-receptor protein	Gonadal dysgenesis, congenital adrenal hypoplasia
SRY (Yp11)	High-mobility-group transcription factor	Gonadal dysgenesis
MIS, or *AMH*, type II receptor (12q12–13)	Serine threonine kinase receptor	Persistent müllerian duct syndrome
MIS, or *AMH* (19p13)	Secreted protein, causes regression of fetal müllerian duct; Leydig-cell inhibitor	Persistent müllerian duct syndrome
AR (Xq11–12)	Androgen receptor, a ligand transcription factor	Male pseudohermaphroditism, complete or partial androgen insensitivity syndrome
HSD17B3 (9q22)	17β-Hydroxysteroid dehydrogenase, 17-ketosteroid reductase 3	Male pseudohermaphroditism
SRD5A2 (5p15)	5α-Reductase type 2	Male pseudohermaphroditism*
CYP17 (10q24–25)	17-Hydroxylase: 20–22 lyase	Male pseudohermaphroditism
CYP21 (6q21.3)	21-Hydroxylase	Congenital adrenal hyperplasia, female pseudohermaphroditism
HSD3B2 (1p13.1)	3β-Hydroxysteroid dehydrogenase type II	Congenital adrenal hyperplasia
CYP11B1 (8q24)	11β-Hydroxylase	Congenital adrenal hyperplasia
StAR (8p11.2)	Steroidogenic acute regulatory protein	Congenital lipoid adrenal hyperplasia

* Virilization may occur at puberty.

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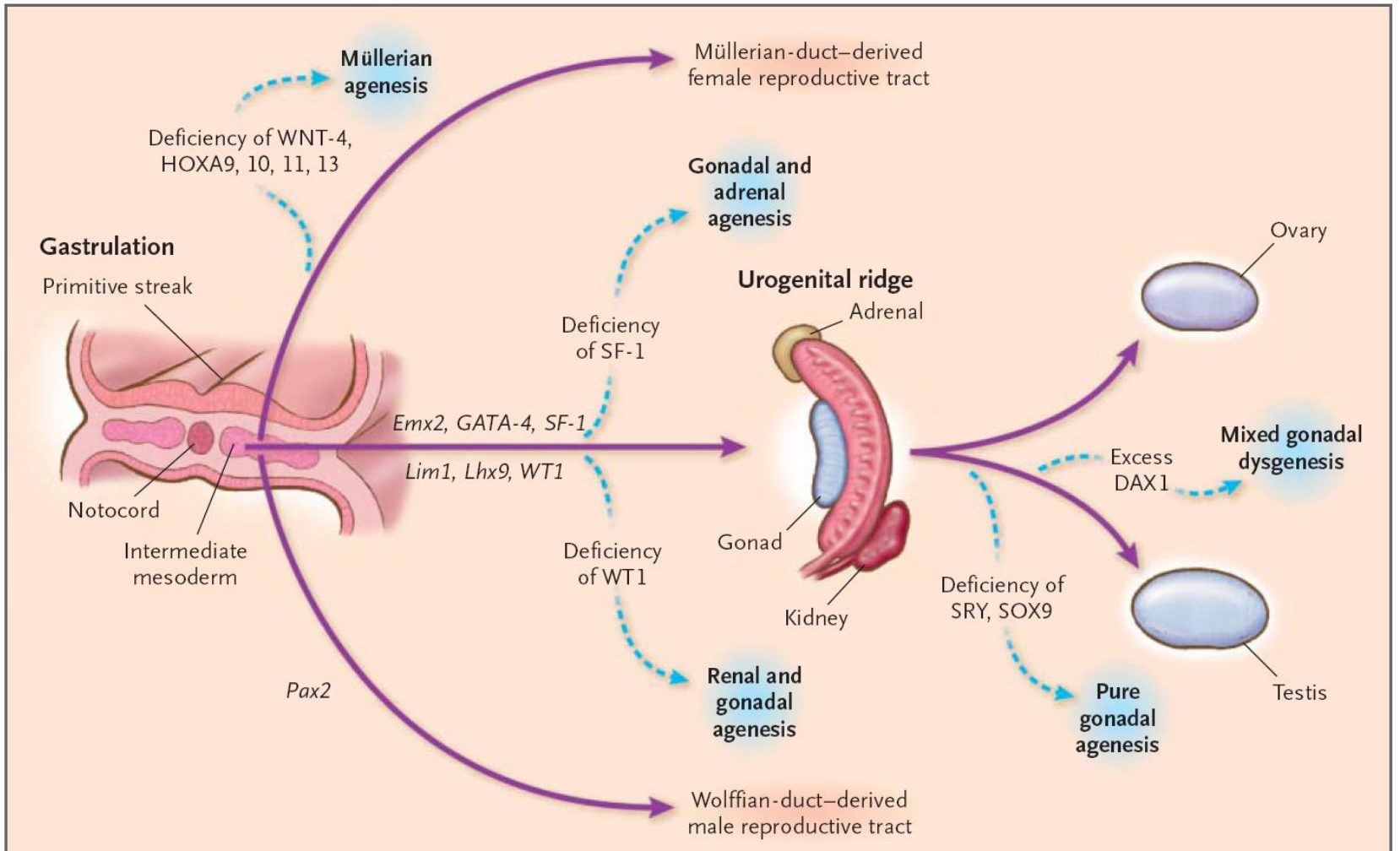

Figure 3. Syndromes of Dysgenesis during the Development of the Urogenital Ridge.

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Embryonic Development of Ovary
FIGα – female germ cell transcription factor

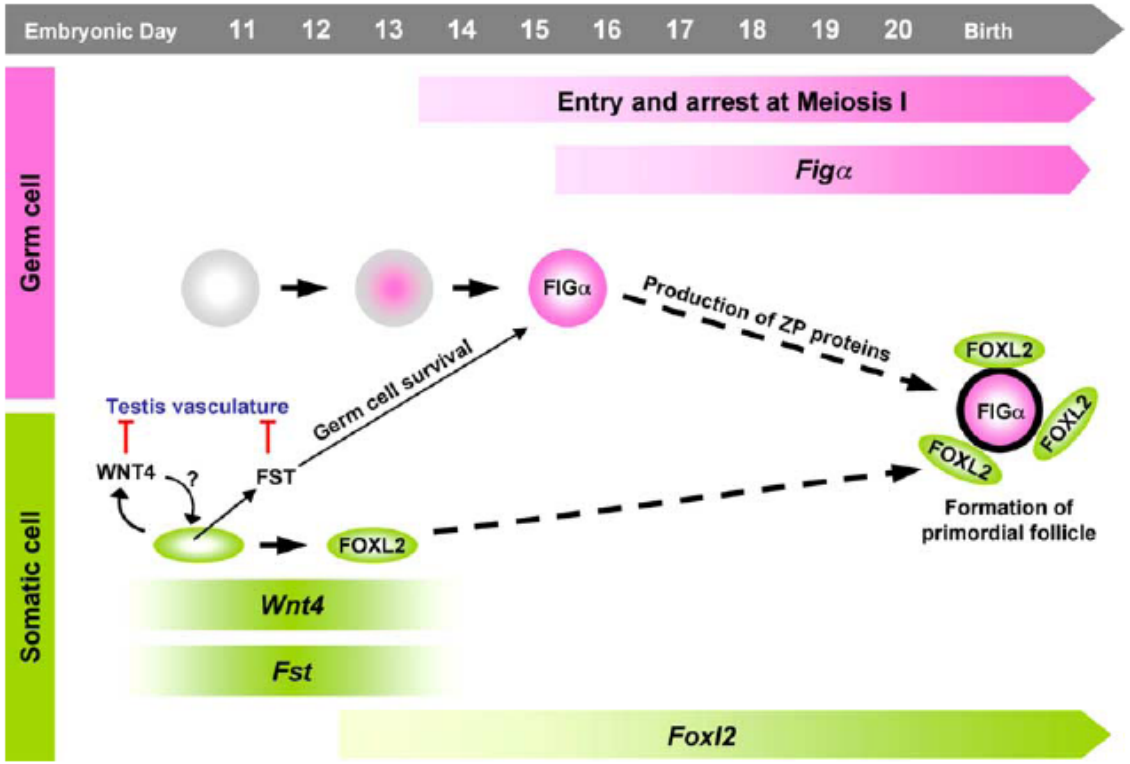

Fig. 1. A time course of events during embryonic development of the mouse ovary from embryonic day 11 (E11) to birth. Female germ cells (in pink) enter meiosis I around E13.5 and arrest at the dictyate at birth. At ~E15, *Figα,* a transcription factor specific for female germ cells, begins to be expressed and is essential for production of the zona pellucida (ZP) proteins and formation of primordial follicles at birth. In the somatic cell lineage (in green), WNT4 is produced which is postulated as an autocrine factor to induce the subsequent production of follistatin (FST) starting at E11.5. WNT4 and FST antagonize the formation of testis vasculature and at the same time, maintain the survival of female germ cells at ~E16. FOXL2, a transcription factor specific for female somatic cells, start to appear at ~E12. FOXL2 is critical for further differentiation of pregranulosa cells. Horizontal bars represent the expression of specific genes and occurrence of molecular events.

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WNT4: Ovary determining gene?

- WNT4 is a member of WNT family
- *Wnt4* expressed in mouse genital ridge while bipotential
- Wnt4 expression becomes undetectable in XY gonads, but is maintained in XX gonads
- *Wnt4*-/-
  - the ovary fails to form properly
  - Cells express testis-specific markers, including MIS and testosterone-producing enzymes
  - Masculinized with active Wolffian duct development and no Müllerian duct
- SRY may repress WNT4 expression in the genital ridge

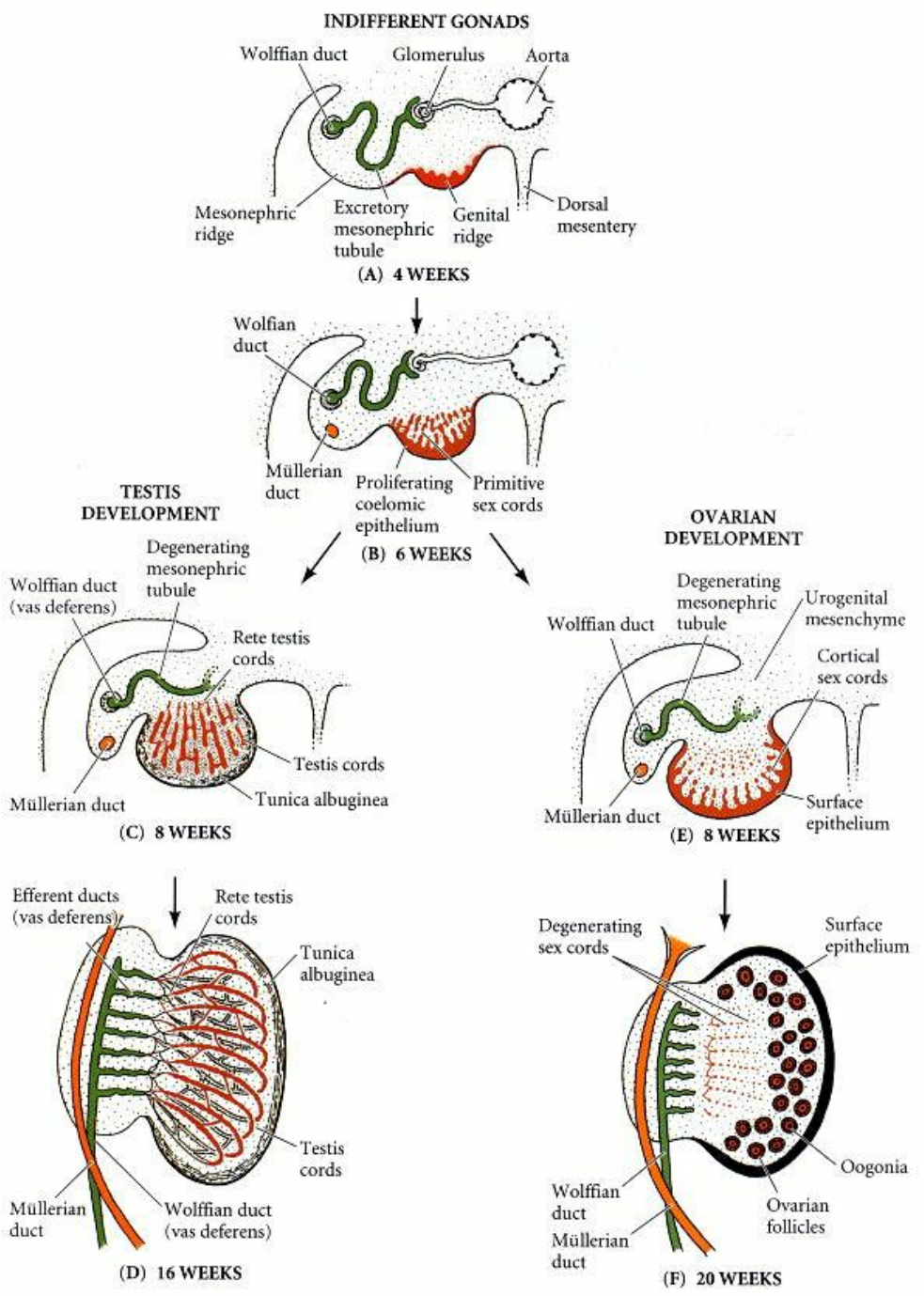
GONADAL SEX

- Initially, gonads are identical in both sexes, termed indifferent gonads
- Both gonads develop from the urogenital ridges
- There is **no difference in male and female development during the first six weeks after conception**, both sexes possess a **mesenephros** (protokidney) on which a ridge of **bipotent** tissue called the **germinal ridge** forms
- Bipotential gonad can develop into a testis or an ovary

Gonadal and Tract Development

- Intermediate mesoderm in developing embryo differentiates into urogenital ridge
- Urogenital ridge develops into
  - Gonads: ovary (*female*) or testis (*male*)
  - Wolffian duct = mesonephric duct (*male*)
  - Müllerian duct = paramesonephric duct (*female*)
- Both duct systems develop in early embryo during the "sexually indifferent" stage under the control of sex-independent genes
- Sex-dependent signals evoke gonadal identity and determine which duct will develop and which will regress

Differentiation of human gonads



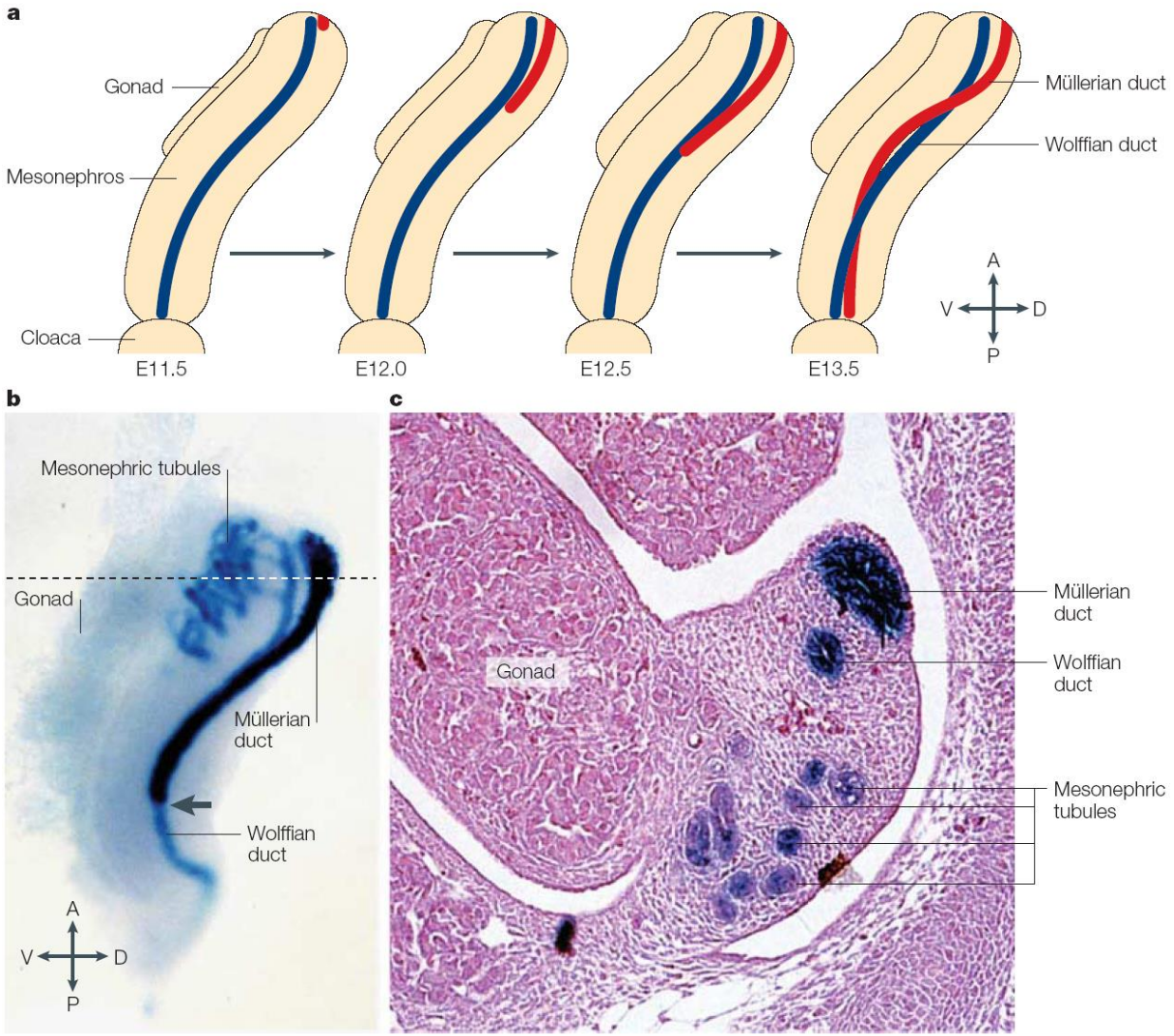


Figure 2 | **Formation of the Müllerian ducts. a** | Schematic diagram of Müllerian duct formation in mammals. The Müllerian duct forms as an invagination of the surface epithelium of the MESONEPHROS at around embryonic day (E) 11.5 in mice and this epithelial invagination extends posteriorly until it reaches the CLOACA at ~E13.5. **b** | The extending epithelium of the Müllerian duct is visualized at E12.5 by *Lim1* (*Lhx1*)–*lacZ* expression[11]. Note that the Wolffian duct (blue) has reached the cloaca posteriorly, but the Müllerian duct is still in the process of extending posteriorly. The grey arrow points to the posterior tip of the extending Müllerian duct. **c** | Cross section of the gonadal/mesonephric region (dashed line in **b**). Blue staining by *Lim1–lacZ* expression is observed in the epithelium of the Wolffian and Müllerian ducts and the mesonephric tubules. A, anterior (cranial); D, dorsal; *Lim*, *lin-11*, *Isl1* and *mec-3* transcription-factor homologue; P, posterior (caudal); V, ventral. Panel **c** adapted from REF. 11 © (2003) The Company of Biologists Ltd.

Nature Reviews Genetics 4, 969-980 (2003)

Gonads and Germ Cells

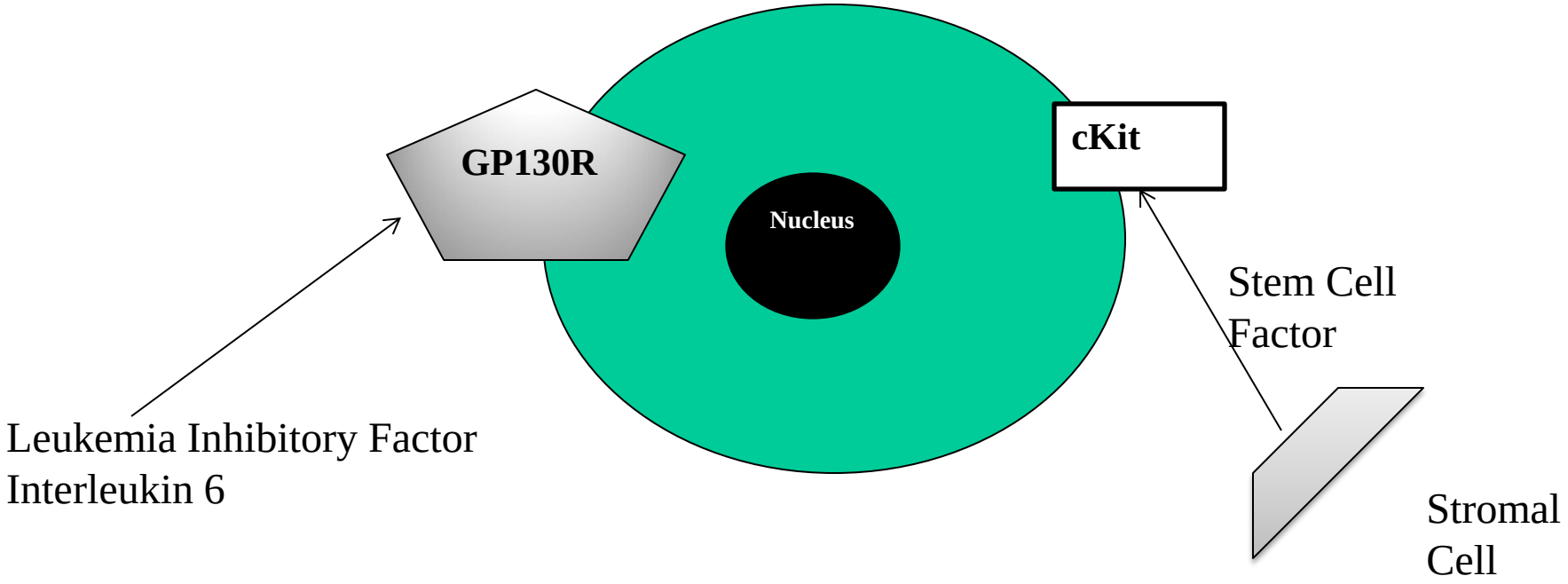
- Each gonad arises from a *gonadal ridge*, a thickening of intermediate mesoderm and overlaying coelomic mesothelium that develops ventromedial to the mesonephric kidney. The parenchyma of each gonad consists of **supporting cells** and **germ cells**:
  - *supporting cells* are derived from invading coelomic mesothelial cells, augmented by cells from disintegrating mesonephric tubules; the invading cells form cellular cords (gonadal cords) that radiate into gonadal ridge mesoderm
  - **primordial *germ cells* arise from yolk sac endoderm**; they **migrate to the gut and then through dorsal mesentery** to reach the *gonadal* ridge
- Germ cells **proliferate** and **migrate into cellular cords** to become surrounded by supporting cells (germ cells that fail to enter a cellular cord undergo degeneration).

Primordial Germ Cells

- Larger than somatic cells
- Large round nucleus
- Few organelles – clear cytoplasm
- Prominent nucleoli
- Enriched in:
  - Alkaline phosphatase
  - Glycogen
  - Esterases
- Mirgration – Ameboid and Blood
  - Telopherone – chemotaxis for PGC to Urogenital Ridge

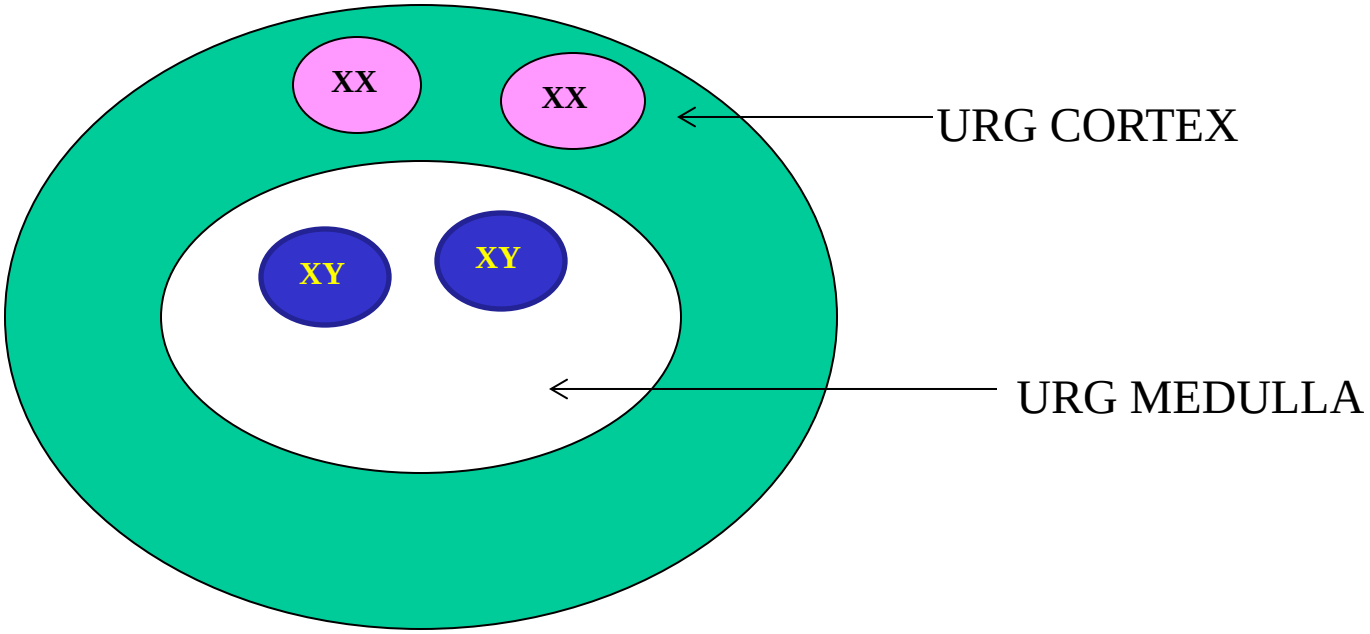
Primordial Germ Cells

- Mouse
  - Day 8.5 – 50 to 100 PGC in UGR
  - Day 13.5 – 25,000 to 30,000 PGC in fully colonized PGC



Absence of GP130R and cKIT = Cell Death

Urogenital Ridge and PGC Migration



XX MIGRATION INTO MEDULLARY PORTION = DEATH
XX AND XY GIVES RISE TO OVOTESTIS, E.G. INTERSEX PIG

Freemartin = female calves born co-twin to males are sterile due to colonization of URG with XX and XY PGC.

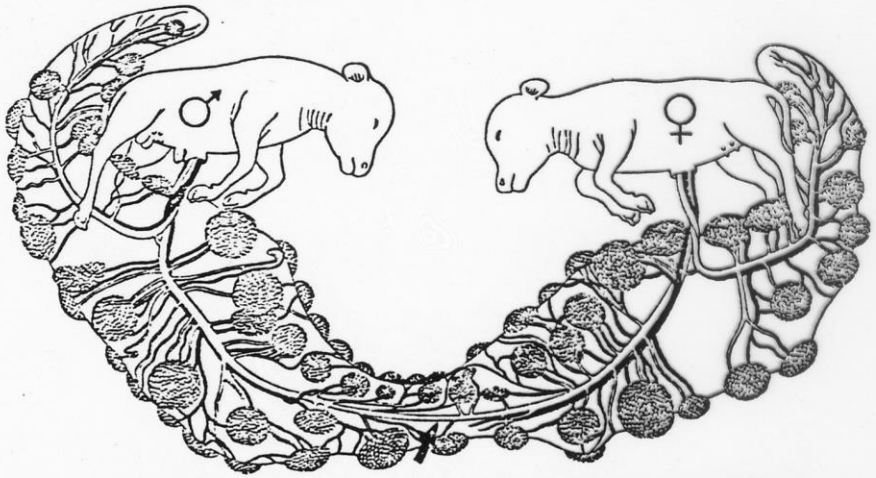


Fig. 2-4. The cause of the freemartin condition. When twin fetuses of opposite sex share a common circulation as a result of fusion of placental blood vessels (arrowed), some substance passes from the male to the female, causing partial sex-reversal of her ovaries. This interferes with the normal development of the female reproductive tract, so that the animal is sterile. (From F. R. Lillie. *J. Exp. Zool.* 23, 271 (1917).)

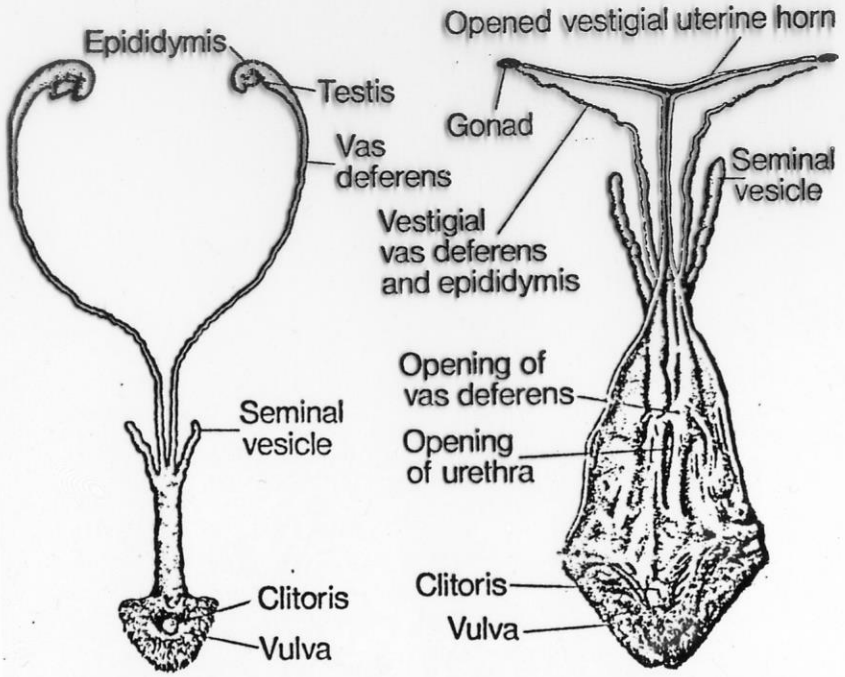
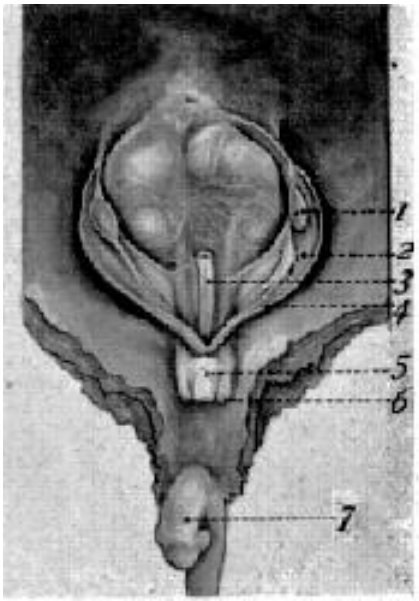


Fig. 2-5. The reproductive tracts of two bovine freemartins, showing the great variability that exists. On the left is the tract of a newborn animal described by Lillie with complete masculinization of the internal genitalia. The gonads, although very small, have been completely transformed into testes and have descended into the inguinal canals. The epididymi, vasa deferentia and seminal vesicles are well developed, and all remnants of the Mullerian duct have been completely suppressed. However, the external genitalia are still female in appearance, apart from slight hypertrophy of the clitoris. On the right is the reproductive tract of an adult freemartin, dissected and drawn for John Hunter 200 years ago. The gonads are extremely small, and both male and female duct systems are present, but incompletely developed. The external genitalia are those of a normal cow, although the clitoris is slightly hypertrophied.

Freemartinism



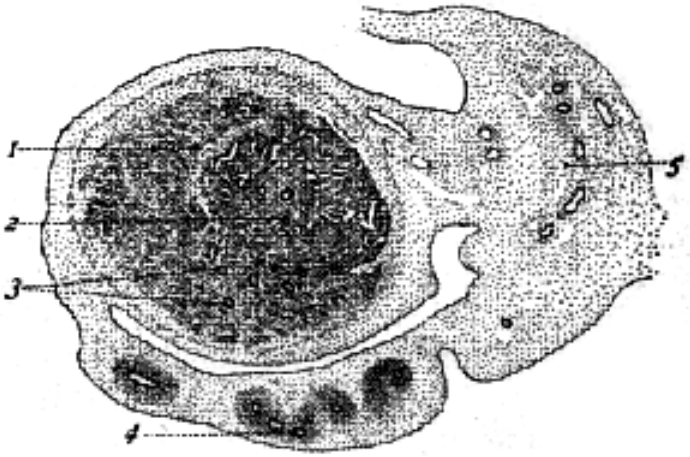


Figure 1. Masculinization of the XX bovine gonads and genital tract in freemartins. (A) Urogenital system of a sterile freemartin (7.5 cm long). 1. gonad; 2. Wolffian body; 3. rectum; 4. genital duct; 5. allantois; 6. umbilical artery; 7. enlarged clitoris. (B) Cross-section through gonadal region of a freemartin (21.5 cm long). 1, sex cord resembling medullary (male) cord; 2, rete; 3, sex cords resembling seminiferous subules; 4, Wolffian duct in fold of peritoneum overhanging the gonad; 5, rudiment of the Wolffian body. (A after Lillie, 1917; B after Chapin, 1917.)

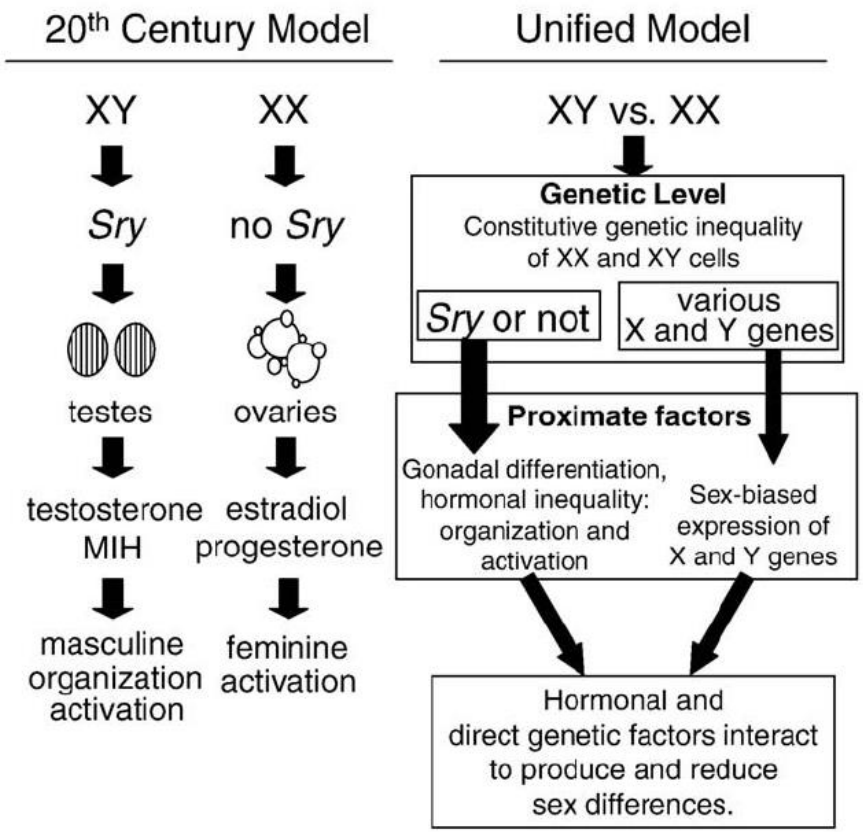


Fig. 1. Contrast between the predominant 20th century model to explain sex differences in the phenotype of tissues, with a revised model. In the 20th century model, the sexual differentiation of the gonads is ascribed to the male-specific effect of the Y-linked gene *Sry*. Once the gonads have differentiated, they secrete different sex steroid hormones. Based on the organizational–activational framework of Phoenix et al. (1959), the testicular hormones testosterone and Müllerian inhibiting hormone (MIH) act on diverse tissues (e.g., genital tracts and brain) in the fetal/neonatal male to cause masculine patterns of development resulting in permanently sexually differentiated substrates. Later in life, ovarian and testicular hormones act differentially on those substrates to create further sex differences in phenotype. In contrast, the unified model recognizes that *Sry* and other (to be identified) X and Y genes occupy the same primary logical level because they are all unequally encoded by the sex chromosomes in males and females. Some X and X genes act in a sex-specific manner, on the gonads and other tissues, to cause sex differences in XX and XY cells. *Sry* plays a dominant role by setting up the life-long sex difference in secretion of gonadal hormones, which have organizational and activational effects on the brain and other tissues. Because of the independent sex differences in sex chromosome genes, and in hormonal secretions, the various sex-specific factors interact in one of several ways. Their effects are synergistic (as for example when Y factors and testicular testosterone both push the male's tissues to function differently than in females), or they counteract each other to reduce sex differences (for example when the female-specific process of X-inactivation shuts down one X chromosome in each female cell to counteract the female bias in X gene expression that would otherwise occur). With minor modification the schema shown here can apply equally to birds or other groups that have a constitutive sexual imbalance of sex chromosome genes, by substituting species-appropriate sex determining gene(s) for *Sry*.

Domestic Animals

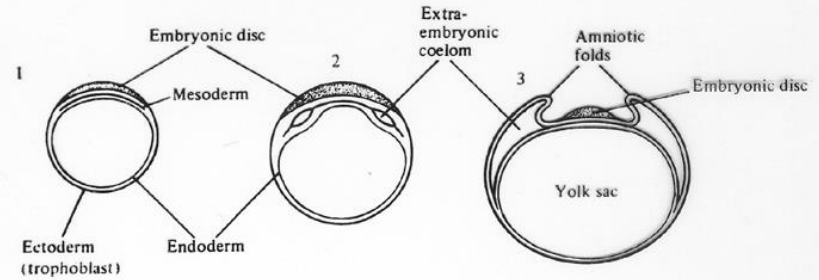
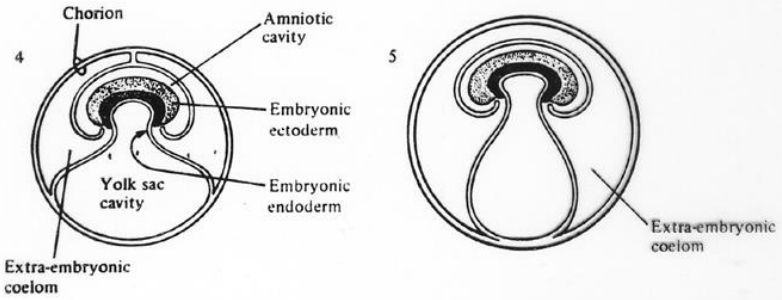
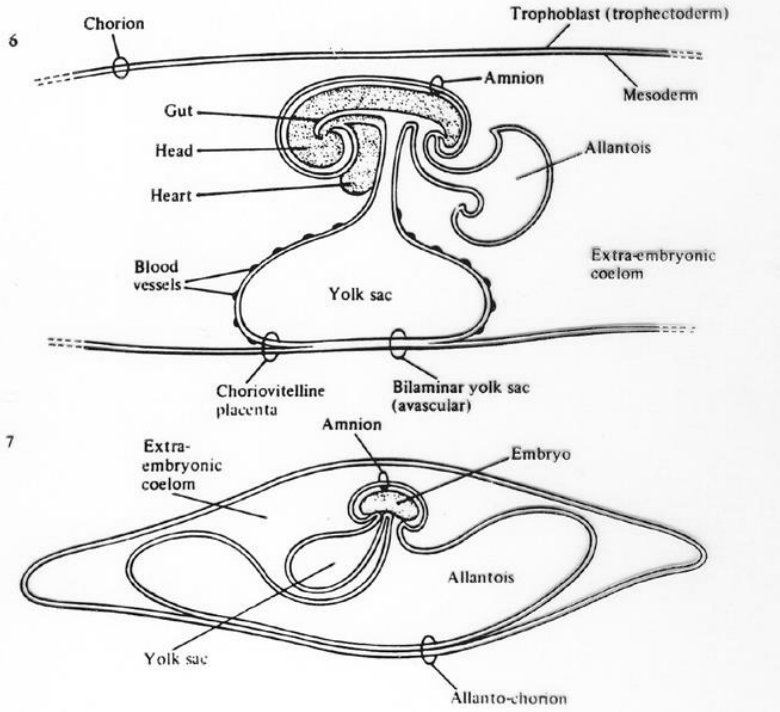


Fig. 2.14. Pattern of development of the fetal membranes in a generalized mammal, based largely on the arrangement seen in the pig. In 5, 6 and 7, the stages shown are just before and during the elongation of the embryonic vesicle, which occurs in the long dimension of the uterus. (From J. S. Perry. *J. Reprod. Fert.* **62**, 321–35 (1981).)





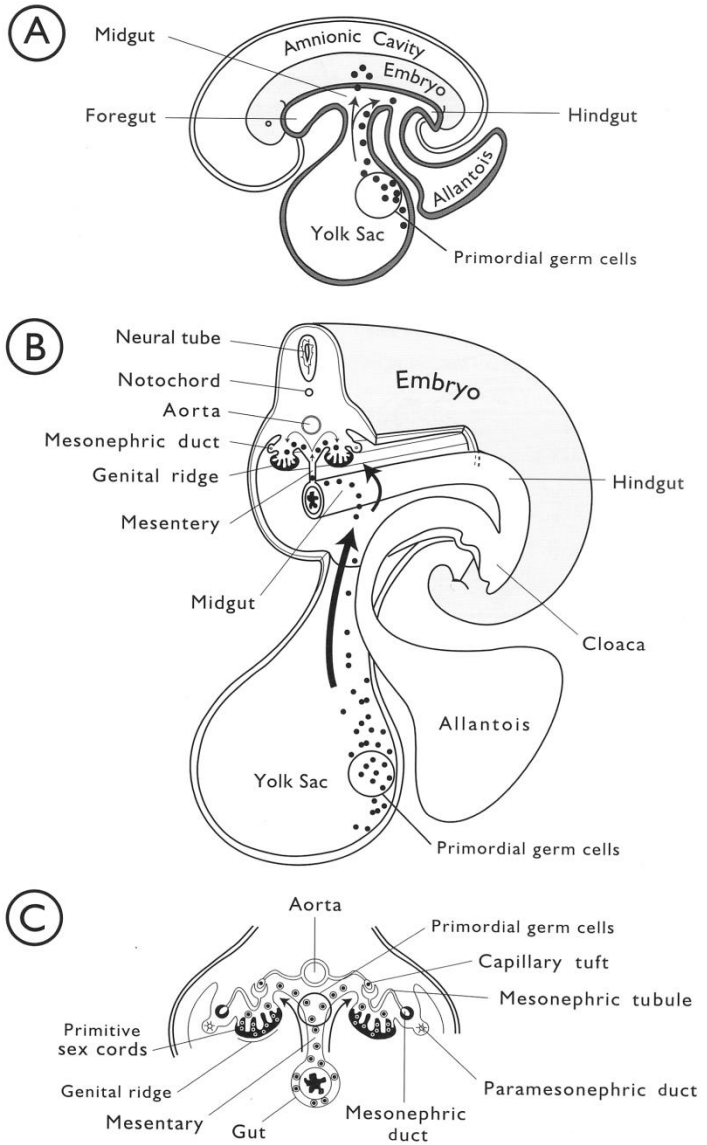


Figure 4-3. Migration of primordial germ cells from the yolk sac into the genital ridge (the indifferent gonad). **A**. Migration of primordial germ cells from the yolk sack into the midgut region of the embryo as seen from a lateral view. **B**. Primordial germ cells migrate by ameboid motion around the midgut, enter the mesentery and take up residence in the genital ridge. **C**. A transverse section showing migration of primordial germ cells being incorporated into the sex cords of the indifferent gonad. Notice the close relationship between the paramesonephric duct, the mesonephric duct and tubule and the genital ridge. Modified from Dyce, Sack and Wensing, *Textbook of Veterinary Anatomy*, 2nd Edition, with permission from W.B. Saunders Co.

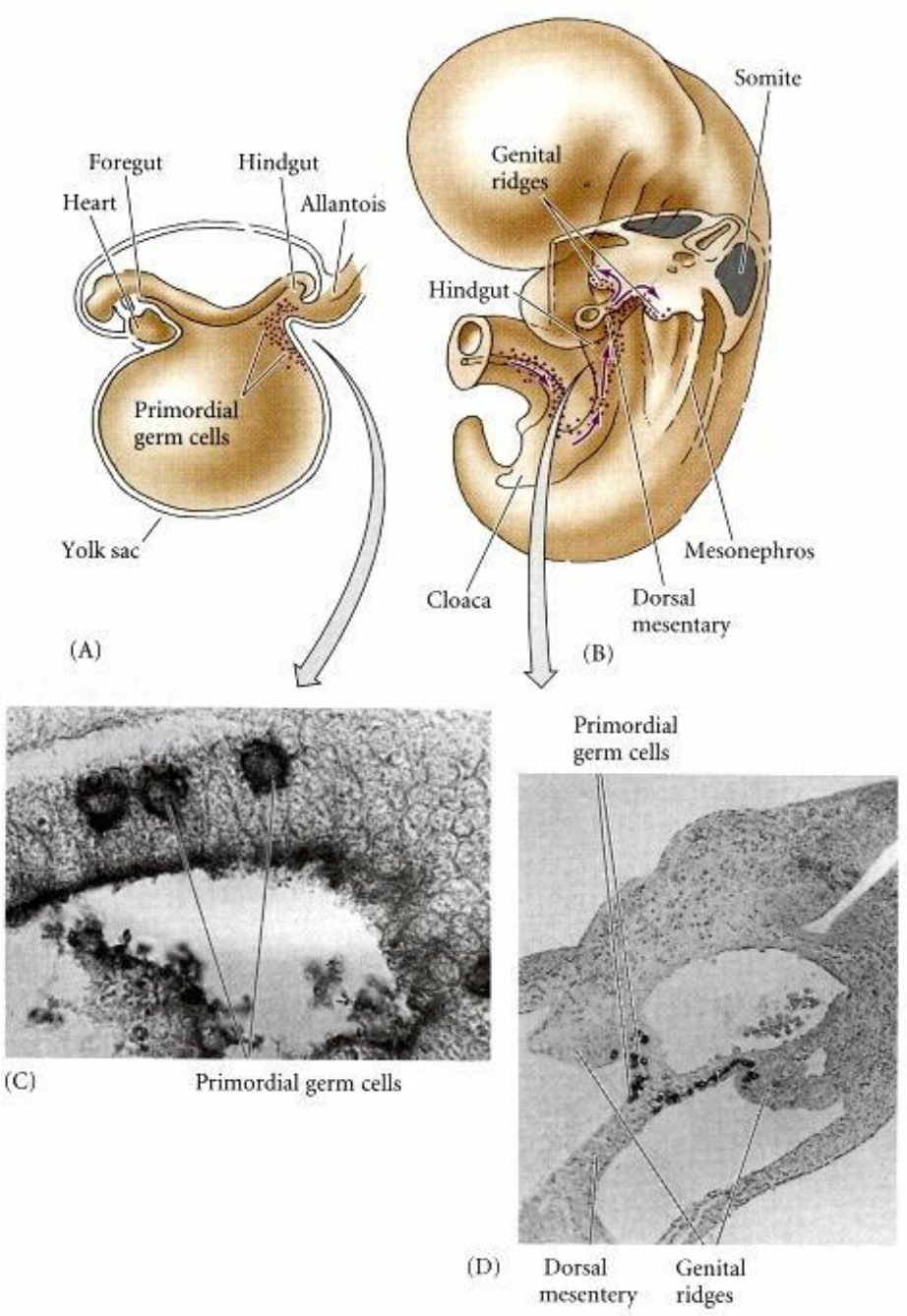


Figure 19.6. Pathway for the migration of mammalian primordial germ cells. (A) PGCs seen in the yolk sac near the junction of the hindgut and allantois. (B) The PGCs migrate through the gut and, dorsally, up the dorsal mesentery and into the genital ridges. (C) Four large PGCs in the hindgut of a mouse embryo (near the allantois and yolk sac) stain positively for high levels of alkaline phosphatase. (D) Such alkaline phosphatase-staining cells can be seen migrating up the dorsal mesentery and entering the genital ridges. (A and B from Langman 1981; C from Heath 1978; D from Mintz 1957; photographs courtesy of the authors.)

Adhesion molecules for mouse primordial germ cells.

• Recent findings indicate that the adhesion-dependent allocation of the PGC precursors to a niche within the epiblast and the forming extraembryonic mesoderm during the pre-gastrulation period is crucial for their commitment.

• PGC migration and homing within the gonadal ridges require integrated signals involving contact of PGCs with extracellular matrix molecules and cellular substrates or repulsion from them, adhesion among PGCs themselves and attraction by the developing gonads.

• A number of adhesion, or putative adhesion molecules, have been identified in mammalian PGCs, mainly in the mouse.

• These include:

- **cadherins** (E-P- and N-cadherins)
- **integrins**
- **IgG superfamily** (PECAM-1)
- Also **oligosaccharides** (LewisX) and **growth factor receptors** (c-Kit)

• Adhesion molecules control not only the germ cell lineage and PGC migration but also the PGC differentiation fate itself.

De Felici M, Scaldaferri ML, Farini D. Front Biosci. 2005; 10:542-51

Colonization of Urogenital Ridge by PGC

- Human – 150 days
- Pigs – 35-40 days
- Cattle – 40-60 days
- Human –
  - 24-30 days – 1,700
  - 60 days – 600,000
  - 150 days – 7 million
  - Birth – 1,000,000
  - Puberty – 300,000 to 400,000

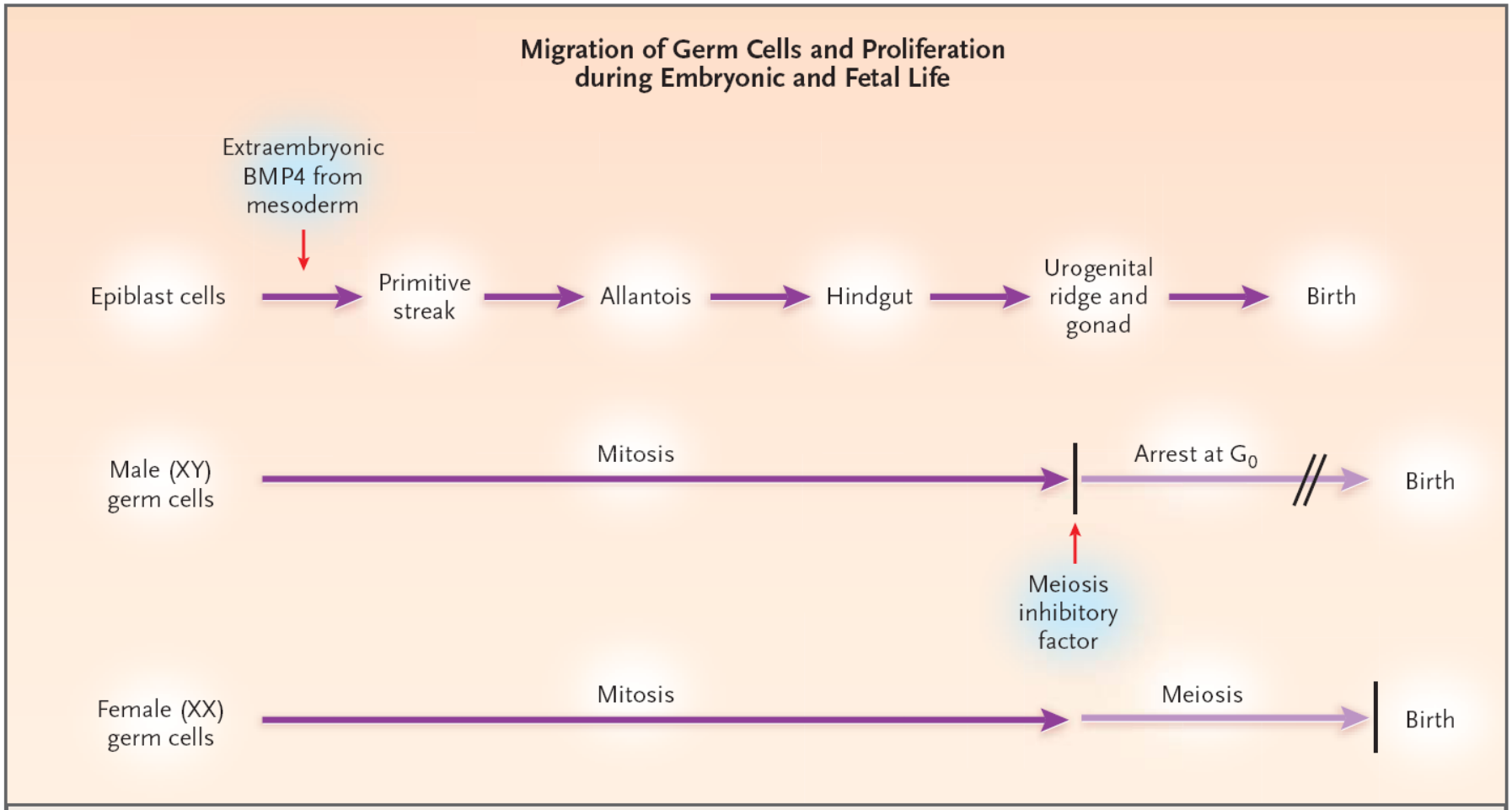


Figure 2. Migration and Proliferation of Germ Cells during Embryonic and Fetal Life.

Germ cells are first detected in the epiblast, where they are activated by bone morphogenetic protein 4 (BMP4) from the extraembryonic ectoderm. Migration occurs through the primitive streak to the base of the allantois, where alkaline phosphatase–positive cells can be detected. Subsequently, the cells migrate to the urogenital ridge, where the gonads form. The 46,XY and 46,XX germ cells undergo different patterns of mitosis and meiosis.

MacLaughlin and Donahoe N Engl J Med 2004;350:367-78.

Brennan & Capel, Nat Rev Genet 2004; 509

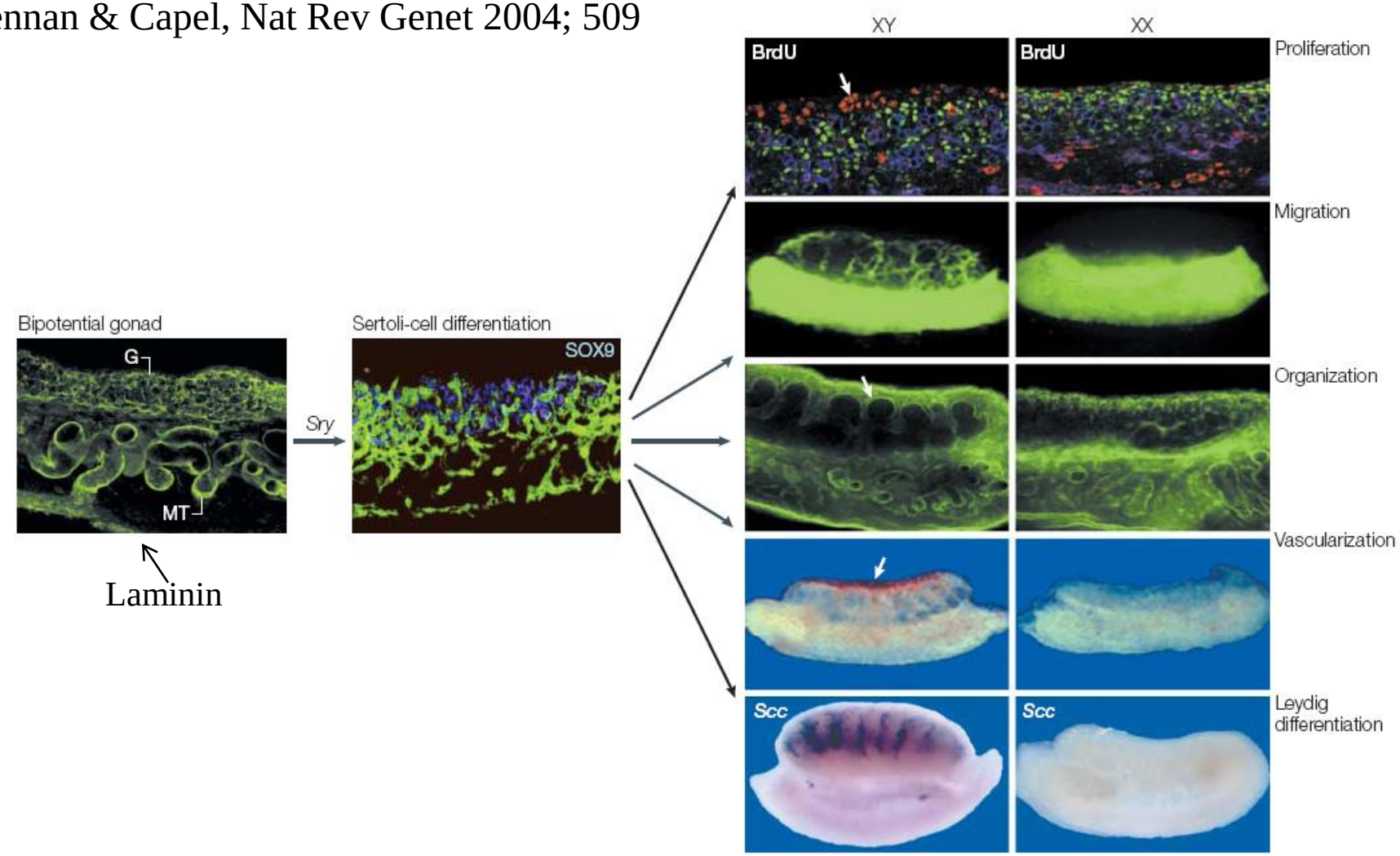


Figure 4 | **Cellular events downstream of *Sry* rapidly organize testis structure.** At the bipotential stage (10.5–11.5 days post coitum; dpc), no obvious morphological features distinguish XX and XY gonads. Antibodies against laminin (green) outline all cells in the gonad (G) and also label the basal lamina of mesonephric tubules (MT) in XX and XY samples. In XY gonads, *Sry* upregulates nuclear SOX9 (blue) in pre-Sertoli cells, and initiates Sertoli-cell differentiation by 11.5 dpc (germ cells and vasculature are labelled with platelet endothelial cell adhesion molecule (PECAM); green). Between 11.5–12.5 dpc, male-specific pathways activate marked morphological and cellular changes in the XY gonad (left column) that do not occur in the XX gonad (right column). These include an upregulation of proliferation in coelomic epithelial cells (measured by BrdU incorporation; red, arrow); migration of cells from the mesonephros (detected in recombinant cultures between a wild-type gonad and a mesonephros in which all cells express GFP; green); structural organization of testis cords (detected by laminin deposition; green); male-specific vascularization (red; blood cells are visible in the light microscope; arrow); and Leydig-cell differentiation (detected by RNA *in situ* hybridization for the steroid enzyme, *Scc*). BrdU image pair repoduced with permission from REF. 29 © (2000) The Company of Biologists Ltd. XY migration image and vascular image pairs reproduced with permission from REF. 77 © (2002) Elsevier Science.

Brennan & Capel, Nat Rev Genet 2004; 509

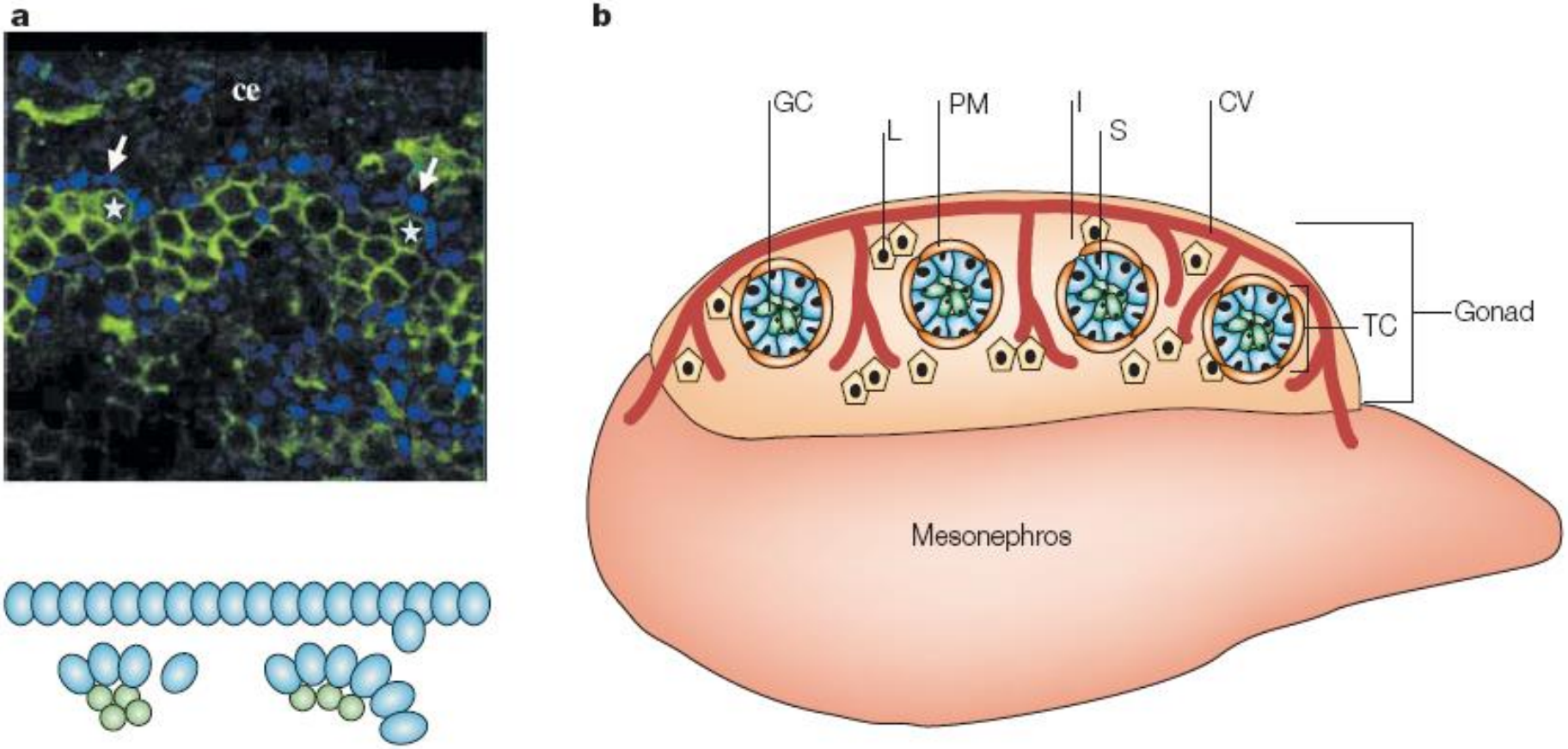


Figure 2 | **Compartmentalization of the testis. a** | At the earliest stages of testis organogenesis (11.75–12.0 days post coitum; dpc), Sertoli cells (stained with SF1 antibody; blue) polarize and begin to aggregate around clusters of primordial germ cells (stained with PECAM antibody; asterisk) to initiate development of testis cords. ce, coelomic epithelium. **b** | Between 11.5–12.5 dpc, the cells of the testis are organized into two functional compartments: testis cords (TC) and the interstitial space (I) outside the cords. Within testis cords, Sertoli cells (S; blue) surround germ cells (GC; green). A basal lamina is deposited between Sertoli cells and peritubular myoid cells (PM). The interstitial compartment contains Leydig cells (L; yellow) and the coelomic vessel (CV; red), with branches that extend between cords.

Brennan & Capel, Nat Rev Genet 2004; 509

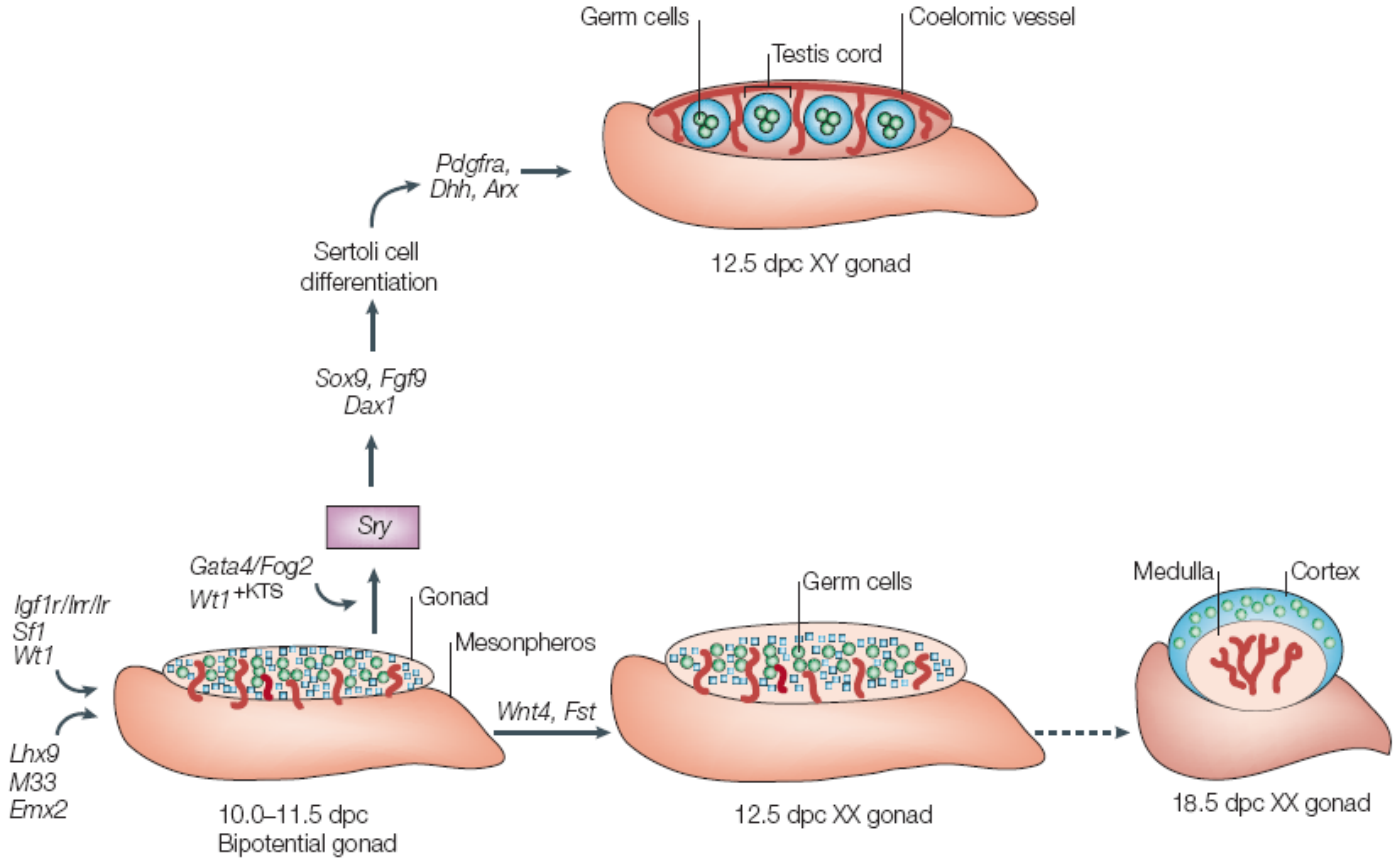


Figure 1 | **Genetic pathways with a characterized functional role in the divergent development of XX and XY gonads.** Several factors are required between 10.5–11.5 days post coitum (dpc) for the outgrowth of the early bipotential gonad by preventing apoptosis or promoting cell proliferation (*Sf1*, *Wt1*, *Lhx9*, *M33*, *Emx2*, *Igf1r/Ir/Irr*). Between 10.5–12.0 dpc, GATA4/FOG2 and WT1+KTS are implicated in the activation of *Sry* expression in the XY gonad. *Sry* expression diverts the XY gonad towards the testis fate. *Sox9*, *Fgf9* and *Dax1* are implicated in the early steps of the male pathway after the initiation of *Sry* expression. Downstream signalling pathways promote the rapid structural changes that characterize early testis development (*Pdgf*, *Dhh*, *Arx*). By contrast, few morphological changes are apparent in the XX gonad until near birth (18.5 dpc), when ovarian follicles begin to form in the ovarian cortex. *Wnt4* and *Fst* are the only two genes with characterized functions in early ovarian development. *Arx*, aristaless related homeobox; *Dax1*, nuclear receptor subfamily 0, B1 (Nr0b1); *Dhh*, desert hedgehog; *Emx2*, empty spiracles homologue 2; *Fgf9*, fibroblast growth factor 9; *Fog2*, zinc finger protein, multitype 2 (*Zfpm2*); *Fst*, follistatin; *Gata4*, GATA binding protein 4; *Igf1r*, insulin-like growth factor 1 receptor; *Ir*, insulin receptor; *Irr*, insulin receptor-related receptor; *Lhx9*, LIM homeobox protein 9; *M33*, chromobox homologue 2 (*Cbx2*); *Pdgf*, platelet-derived growth factor; *Sf1*, nuclear receptor subfamily 5, group A member 1 (*Nr5a1*); *Sox9*, *Sry*-like HMG-box protein 9; *Wnt4*, wingless-related MMTV integration site 4; *Wt1*, Wilms tumour homologue.

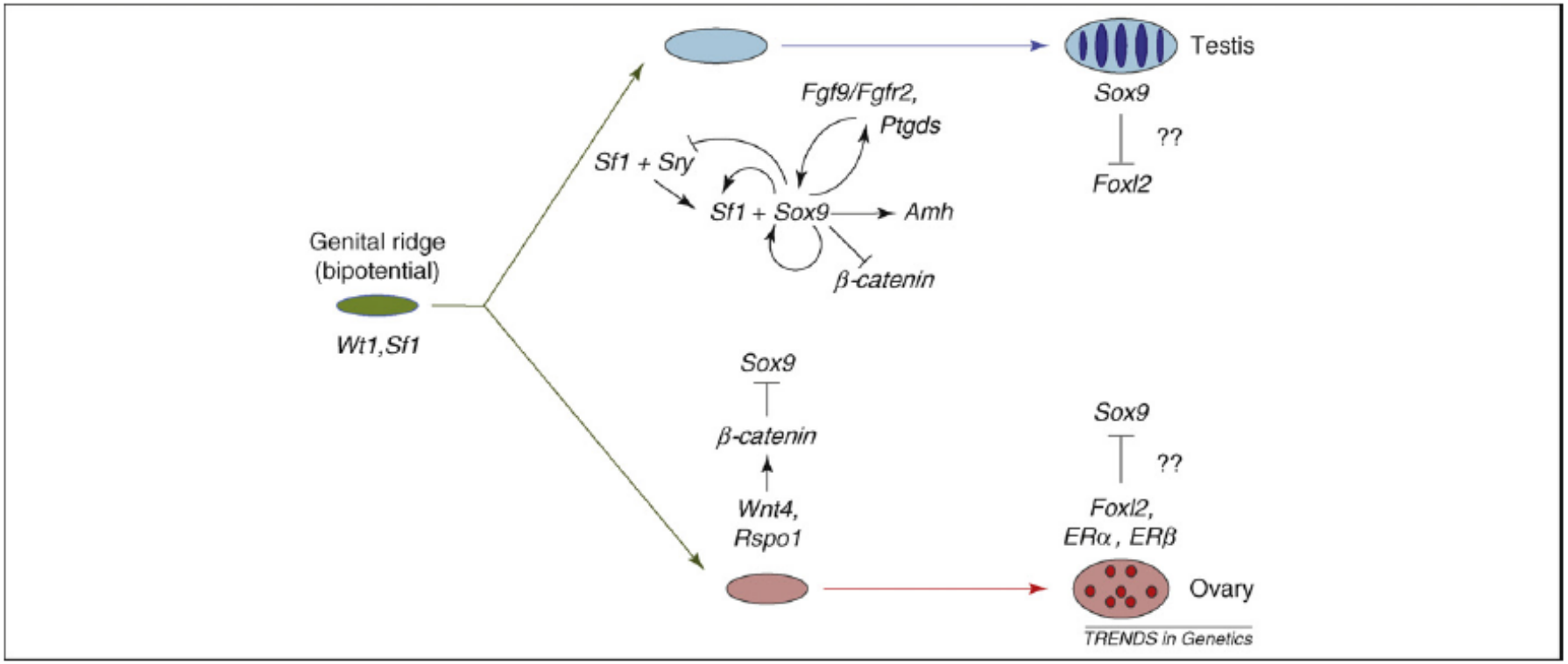


Figure 3. The molecular and genetic events in mammalian sex determination. The bipotential genital ridge is established by genes including *Sf1* and *Wt1*, the early expression of which might also initiate that of *Sox9* in both sexes. *β-catenin* can begin to accumulate as a response to Rspo1–Wnt4 signaling at this stage. In XX supporting cell precursors, *β-catenin* levels could accumulate sufficiently to repress SOX9 activity, either through direct protein interactions leading to mutual destruction, as seen during cartilage development [91], or by a direct effect on *Sox9* transcription. However, in XY supporting cell precursors, increasing levels of SF1 activate *Sry* expression and then SRY, together with SF1, boosts *Sox9* expression. Once SOX9 levels reach a critical threshold, several positive regulatory loops are initiated, including autoregulation of its own expression and formation of feed-forward loops via FGF9 or PGD_2 signaling. If SRY activity is weak, low or late, it fails to boost *Sox9* expression before β-catenin levels accumulate sufficiently to shut it down. At later stages, FOXL2 increases, which might help, perhaps in concert with ERs, to maintain granulosa (follicle) cell differentiation by repressing *Sox9* expression. In the testis, SOX9 promotes the testis pathway, including *Amh* activation, and it also probably represses ovarian genes, including *Wnt4* and *Foxl2*. However, any mechanism that increases *Sox9* expression sufficiently will trigger Sertoli cell development, even in the absence of SRY.

Rspo1 – transcription factor activating Wnt genes

Sekio & Lovell-Badge, Trends Genet 2009; 25:19

Human, Wk Px	Mouse, Day Px	Event in Gonadogenesis
4	7.5	PGC recognizable
4-7	8-12.5	PGC proliferate and migrate
4	9	Mesonephric Duct and Tubles Form
4-5	9	PGC to Hindgut
4-5	9	Wolffian Duct and Tubules Form
5	9	Urogenital Ridge Forms
6	10-11	PGC to Dorsal Messentary
6	11	Undifferentiated gonad recognizable
6	11	Wolffian Duct Forms
7	11-12	PGC to Gonads
7	12.5	PGC Migration Finished
7-8	12	Ovary and Testes Recognizable
8-49	12-13	Germ Cells Proliferate
8.5-49	13-15	Oocytes arrest Prophase I; Spermatogonia Mitosis Ceases

BRAIN SEX

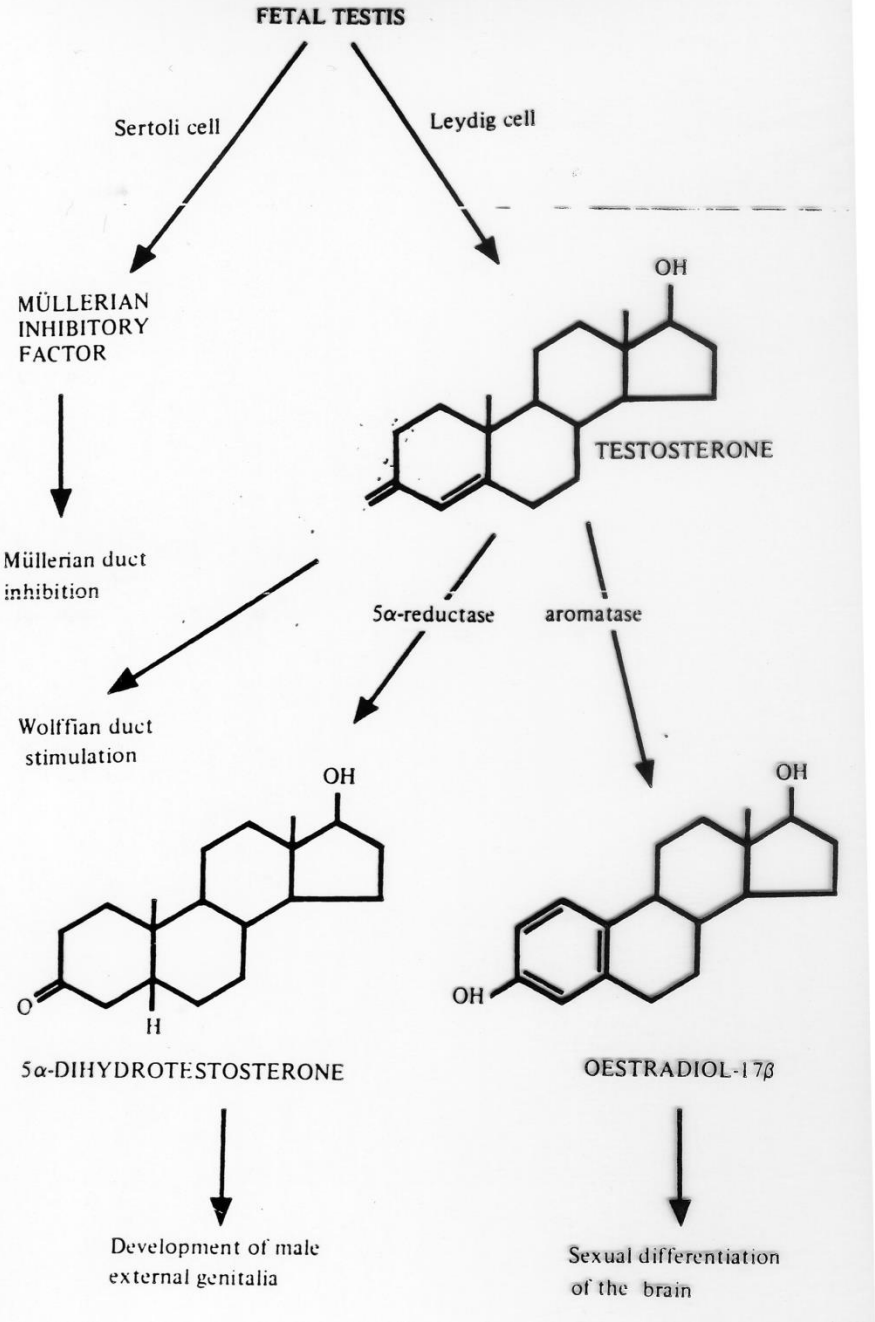
Brain Differentiation

- ***Organization/Activation Hypothesis***
  - Sex hormones act in fetus or neonate to organize the nervous system in a sex-specific manner, but the same hormones have transient activational effects in the adult.

- Prenatal or neonatal exposure to particular steroid hormones elicits permanent sex-specific changes in the central nervous system/hypothalamus

- Charles Barraclough and Roger Gorski
  - Found that the cyclic secretion of LH by the adult female rat pituitary is dependent on the lack of testosterone during the first week of life
  - LH secretion pattern is not cyclical if testosterone is administered on Days 1 through 4 of postnatal life of rats
  - Conversely, LH secretion in males castrated at birth is cyclical

Fig. 3.10. Hormonal activities of the mammalian fetal testis.



Brain Differentiation

- Estradiol: chiefly responsible for determining male brain pattern
  - Testosterone is converted to estradiol by P450 aromatase
  - Aromatase is active in the hypothalamus and limbic system
  - Fetal environment is replete with estrogens from the gonads and placenta
- How is the female fetus protected from these estrogens?
  - α-fetoprotein is made by fetal liver is present in blood and cerebrospinal fluid
  - α-fetoprotein binds and inactivates estrogen, but not testosterone
- Problems with hypothesis
  - Male mice lacking ESR1 retain male-specific morphology in brain (ESR2)
  - Male mice lacking aromatase are capable of breeding
- Extrapolating from rats and mice to humans and livestock species is often flawed

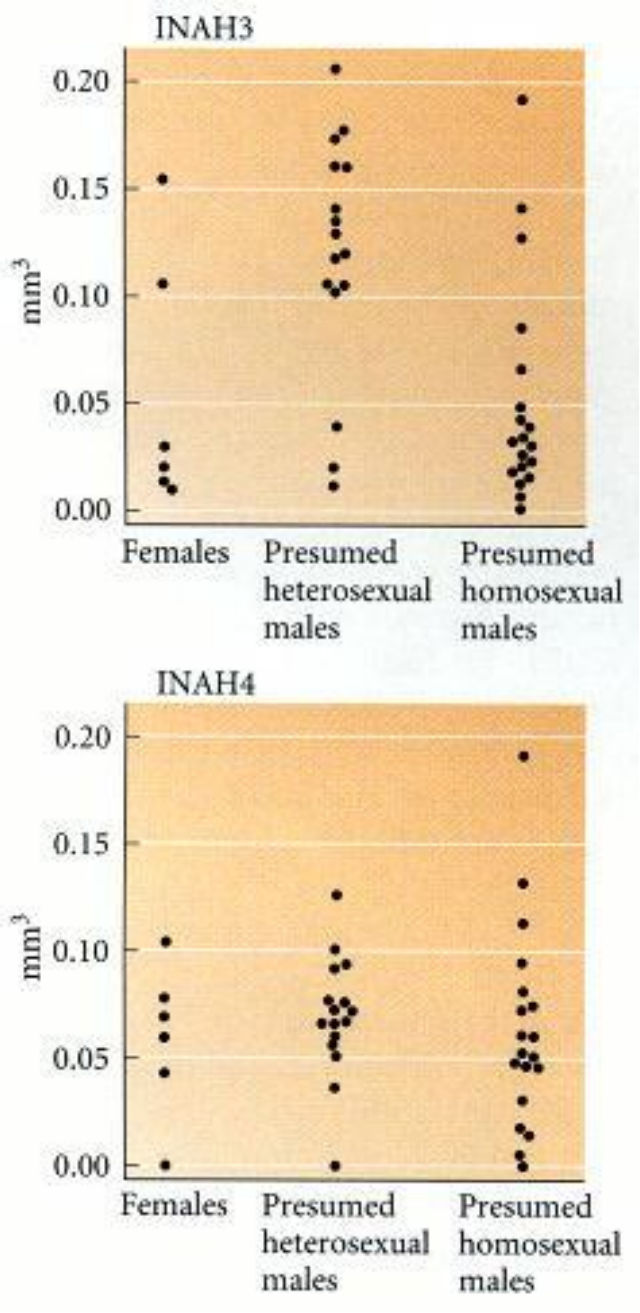


Figure 17.14. A portion of the data claiming a biological basis for homosexuality. INAH4 and INAH3 are groups of hypothalamic neurons. INAH4 shows no sexual dimorphism in volume, while INAH3 shows a statistically significant clustering, although the range is similar. INAH3 from autopsies of "homosexual" male brains cluster toward the female distribution. LeVay 1991.)

Sexually dimorphic nucleus (SDN): cluster of cells in preoptic area of hypothalamus of the brain believed to be related to sexual behavior in animals. The volume of SDN is about twice as large in males due to greater number and size of cells in male SDN. SDN and its homologues exist in Human and other animal brains, including the third interstitial nucleus of anterior hypothalamus (INAH3) in humans, ovine sexually dimorphic nucleus (oSDN) in the medial preoptic area/anterior hypothalamus (MPOA/AH) in sheep, sexually dimorphic nucleus in the preoptic area (SDN-POA) in rats, anterior hypothalamic nucleus (AHdc) in macaques, specific areas in medial preoptic nucleus (POM).

Behavioural Sex

- Polygynous males – 1 male/multiple females
  - Aggressive
  - High degree of sexual dimorphism of physique to accommodate combat- antlers, canine teeth etc
- Monogamous males – little sexual dimorphism
- Castration – abolishes normal sexual behavior in males and females – restored with E2 and T4
- Social Aggression due to T4 and dose-dependent

Legal Sex

- TO BE DETERMINED
  - GENETIC SEX
  - PHENOTYPIC SEX
  - LEAGAL, ETHICAL AND MORAL ISSUES FOR COURTS TO DECIDE AND PUBLIC TO ACCEPT