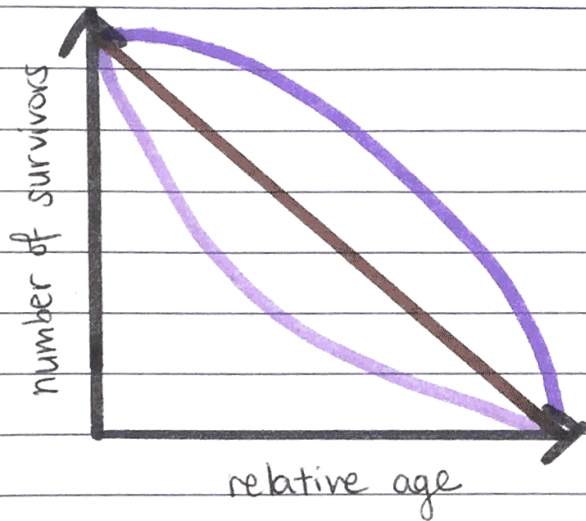


# CONTENTS

1	Survivorship	19	Cell Cycle
2	Reproduction	20	Mitosis & Meiosis
3	Reproduction	21	DNA VS RNA & W-C Model
4	Reproduction: Humans	22	DNA Replication Process
5	Reproductions: Humans	23	Prokaryotes VS Eukaryotes
6	Reproductive System: Male	24	Mitosis VS Meiosis
7	Reproductive System: Female	25	PCR
8	Sperm VS Ovum	26	Mendelian Genetics
9	Menstrual Cycle	27	Crosses & Sex-Linked Diseases
10	Menstrual Cycle	28	Errors & Mutations
11	Fertilisation & Gastrulation	29	Cancer & Checkpoints
12	Pregnancy	30	Stem Cells
13	Differentiation & Structure	31	Mutation & Cloning
14	Amniotic Fluid & Umbilical Cord & Placenta	32	Cloning Process & MCS
15	AF & VCLP	33	GMOs
16	DNA & RNA	34	Results
17	Condensation & Replication	35	Reprogramming
18	Replication Theories	36	Bio SPA

## SURVIVORSHIP



### 3 TYPES

- 1:** late loss. mortality =  $\downarrow$  infant & juvenile,  $\uparrow$  elders
- 2:** constant loss. mortality rate constant thruout life.
- 3:** early loss. mortality =  $\uparrow$  infant & juvenile,  $\downarrow$  elders (lack)

**1**

**2**

CLIMATE: variable, unpredictable  
 MORTALITY: density independent  
 SURVIVORSHIP: see above diagram  
 POP<sup>n</sup> SIZE: fluctuates wildly  
 COMPETITION: variable, lax, generalist  
 LIFE LENGTH: short  
 SELECTION FAVOURS: rapid dup<sup>+</sup>, early reproduction, small body size,  $\uparrow$  offspring  
 LEADS TO: productivity

**3**

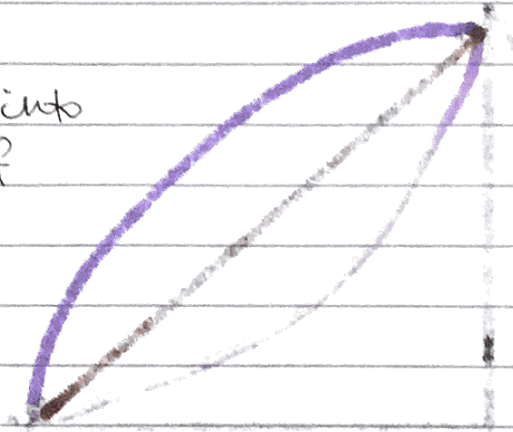
### 3 TYPES

CLIMATE: fairly constant/predictable  
 MORTALITY: density dependent  
 SURVIVORSHIP: see above diagram  
 POP<sup>n</sup> SIZE: constant, equil. w/ enviro.  
 COMPETITION: keen, specialist  
 LIFE LENGTH: long (generally)  
 SELECTION FAVOURS: slower dup<sup>+</sup>, later reproduction, large body size,  $\downarrow$  offspring  
 LEADS TO: efficiency

# REPRODUCTION

## BINARY FISSION

- ↳ separation of a parent into 2 or more individuals of about equal size
- eg. sea anemones



## BUDDING

- ↳ 1 or more individuals formed out of an original
- ↳ offspring grows out of body of parents
- eg. hydra

## FRAGMENTATION

- ↳ parents break into different distinct pieces
- ↳ each piece forms new individuals by body part regen.
- eg. planarians

## PARTHOGENESIS

- ↳ growth/development of embryos occurs without fertilisation
- eg. new mexico whiptail

## HERMAPHRODISM

- ↳ existence of reproductive organs in both sexes in same individuals at same/different times
- eg. salamanders

# SEXUAL REPRODUCTION

definition: requires 2 parents who donate genes to the young  
results in genetically unique offspring

features: fusion of gametes  
↑ common in higher organisms  
↑ genetic variability among offspring  
↳ variations = ↑ ability to adapt to changing conditions  
↑ energy expenditure in producing egg/sperm + mating

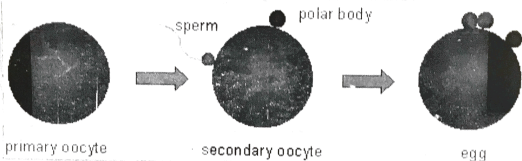
# ASEXUAL REPRODUCTION

definition: creation of genetically identical offspring  
by lone parent w/o fertilisation or gametes

features: offspring produced by mitosis  
organisms living in isolation can reproduce w/o mating  
rapid reproduction, ↓ energy expenditure  
↑ survival rate in stable enviro.  
↳ effective way to reproduce if well-suited to enviro,  
↑ expand population, exploit available resources  
↳ if enviro. becomes unfavourable,  
all individuals affected, ↑ risk of extinction



# SEXUAL REPRODUCTION: HUMANS



1 **capacitation**  
essential change to the sperm surface

2 at the secondary oocyte, the **acrosome reaction** (release of enzymes of the acrosome) digests a narrow path through the follicle path (zona radiata) and through the zona pellucida

3 **fusion** between posterior region of sperm head and the secondary oocyte plasma membrane, leading to **entry into cytoplasm** (sperm head engulfed)

4 **cortical reaction**: exocytosis of cortical granules alters zona pellucida to prevent further entry of sperm

5 the secondary oocyte is stimulated to complete meiosis II, the two haploid nuclei form, and a **zygote is formed**

zona pellucida (glycoprotein barrier)

plasma membrane of secondary oocyte  
cortical granules

fertilisation

stages of fertilisation

fertilized egg

2-cell stage

morula stage

egg  
dypmt

cavity

inner cell mass

outer cell layer (future chorion)

blastocyst (blastula stage)

embryonic disk (future embryo)

chorion

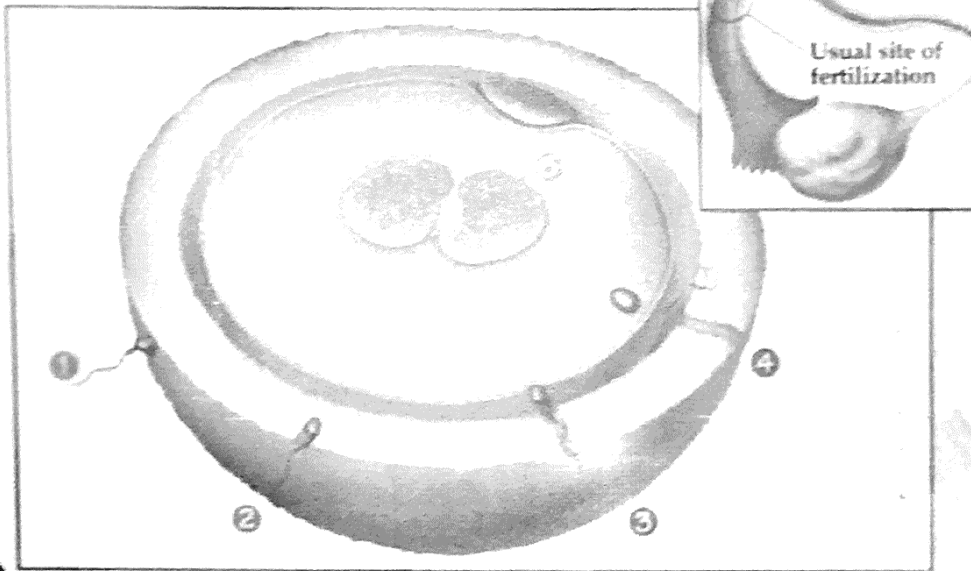
amnion

yolk sac

uterine lining (endometrium)

# If fertilisation occurs ...

## Fertilization Process

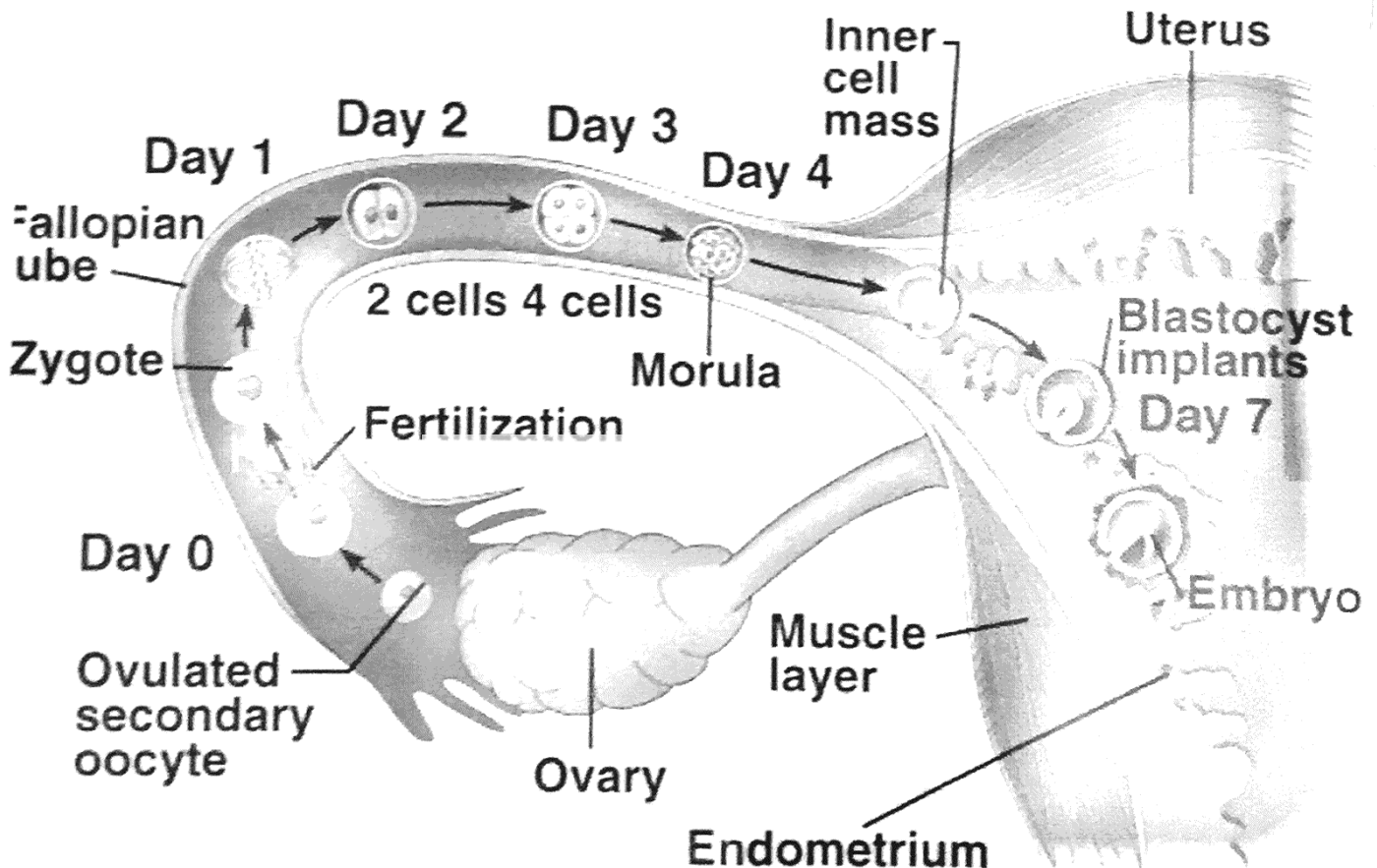


After the sperm enters the uterus, they swim into the fallopian tube. When they encounter an egg that has been released from the ovary, one sperm may enter the egg. The union of a sperm and egg forms a zygote.

- |                                 |  |
|---------------------------------|--|
| ① Sperm binds to egg            | ④ Sperm enters egg                                       |
| ② Sperm begins to penetrate egg | ⑤ Chromosomes from sperm and egg unite to form pronuclei |
| ③ Sperm penetration continues   |  |

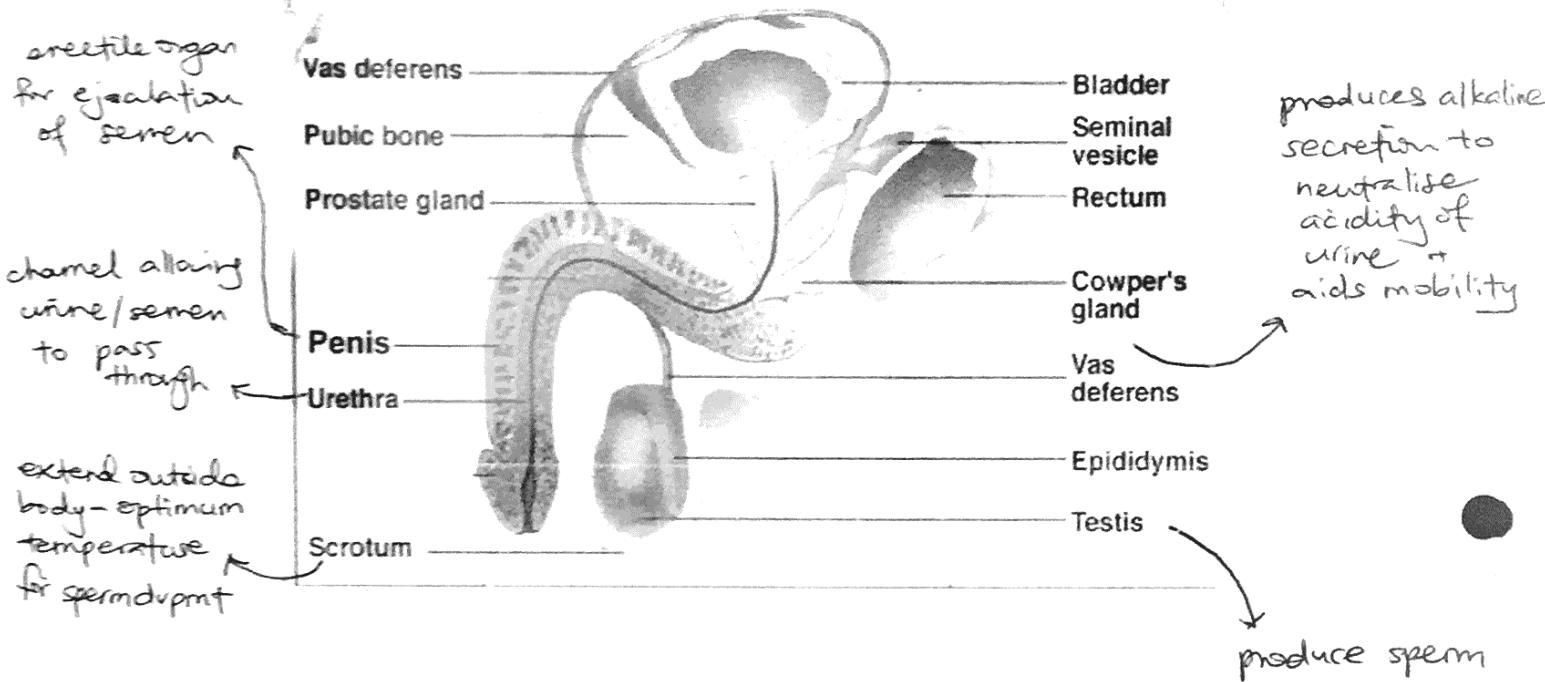
more info  
re:  
fertilization  
process

## From ovulation to implantation



# REPRO. SYSTEM: MALE

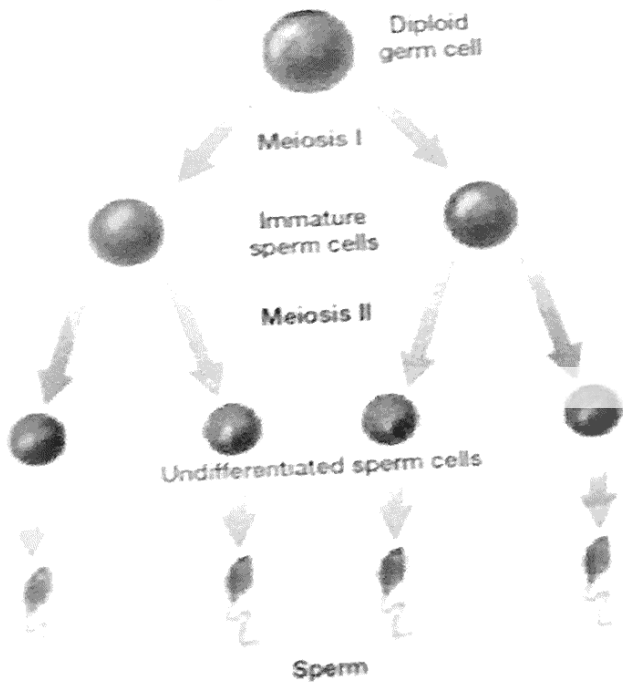
## Male Sexual & Reproductive Organs



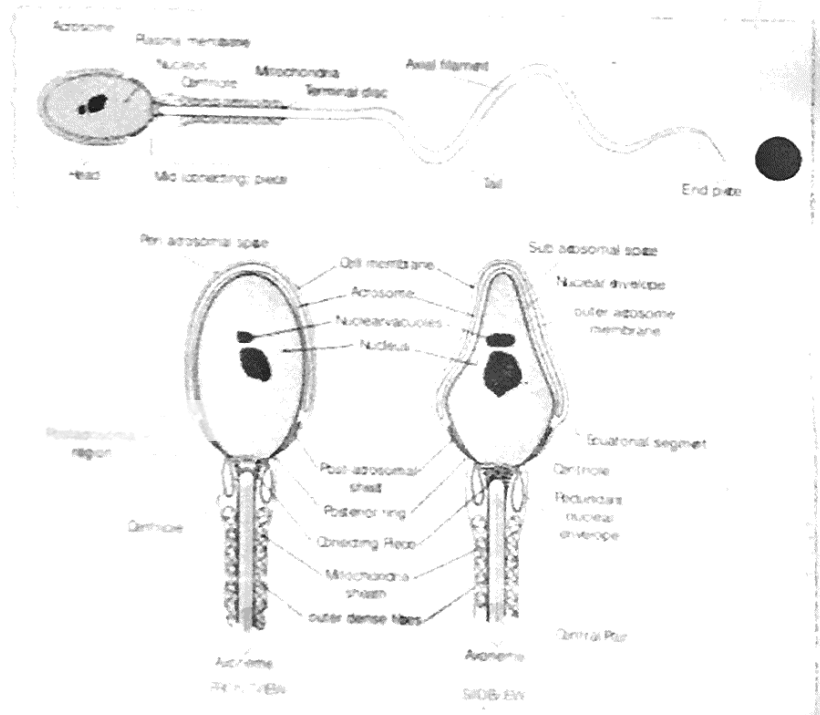
Functions:

- produce/maintain/transport sperm & semen
- discharge sperm within female system
- produce/secrete male sex hormones

### Spermatogenesis



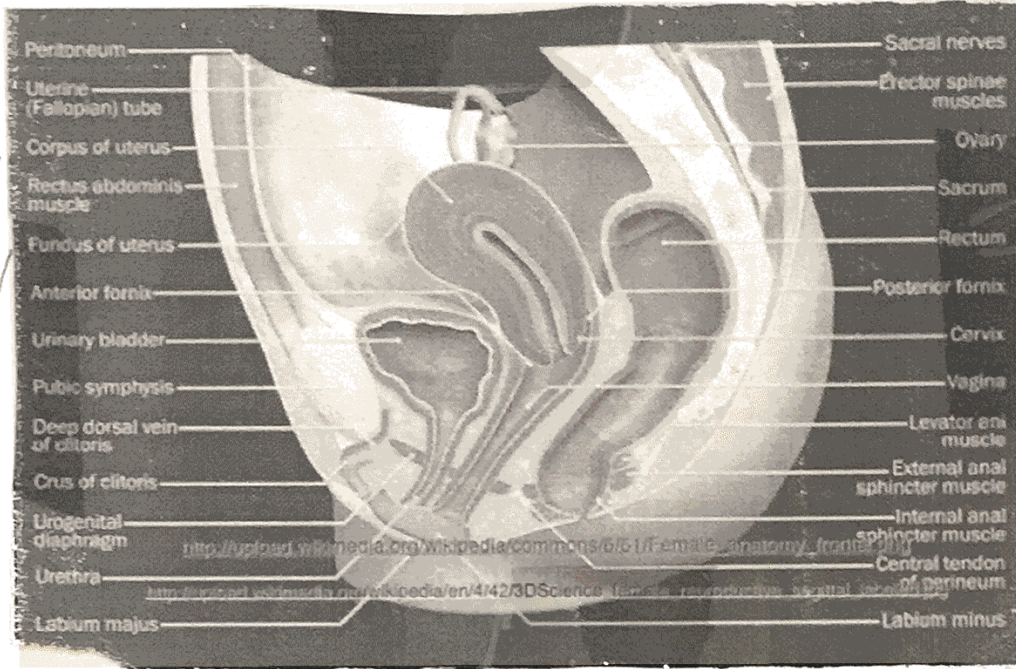
process of sperm creation



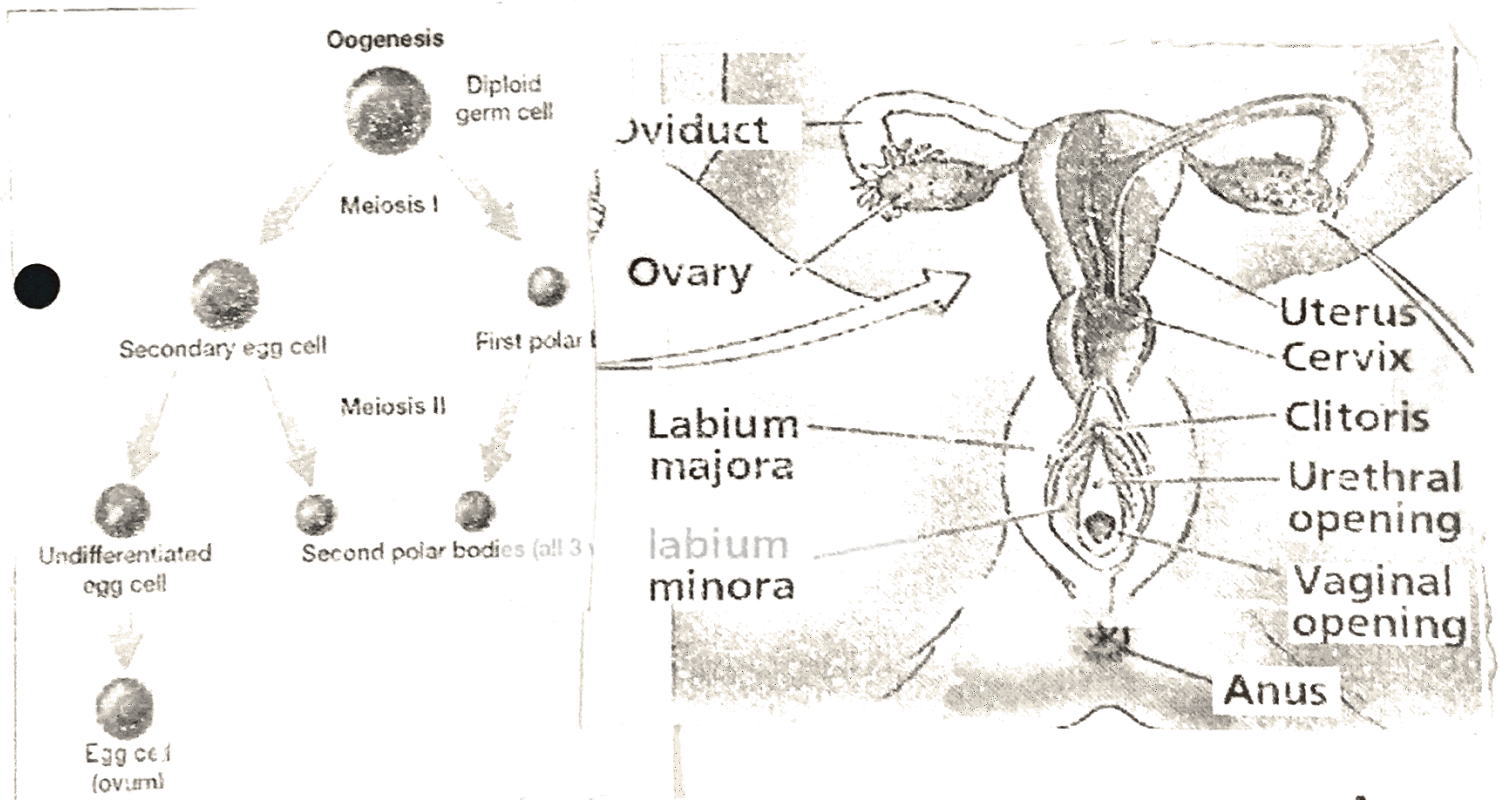
sperm structure



# REPRO. SYSTEM: FEMALE



functions:-  
 produces female egg cells for reproduction  
 designed to transport ova to fertilisation site  
 uterus = safe enviro. for fetus to dev.  
 produces female sex hormones



process of  
egg creation

system structure



# SPERM V. OVUM

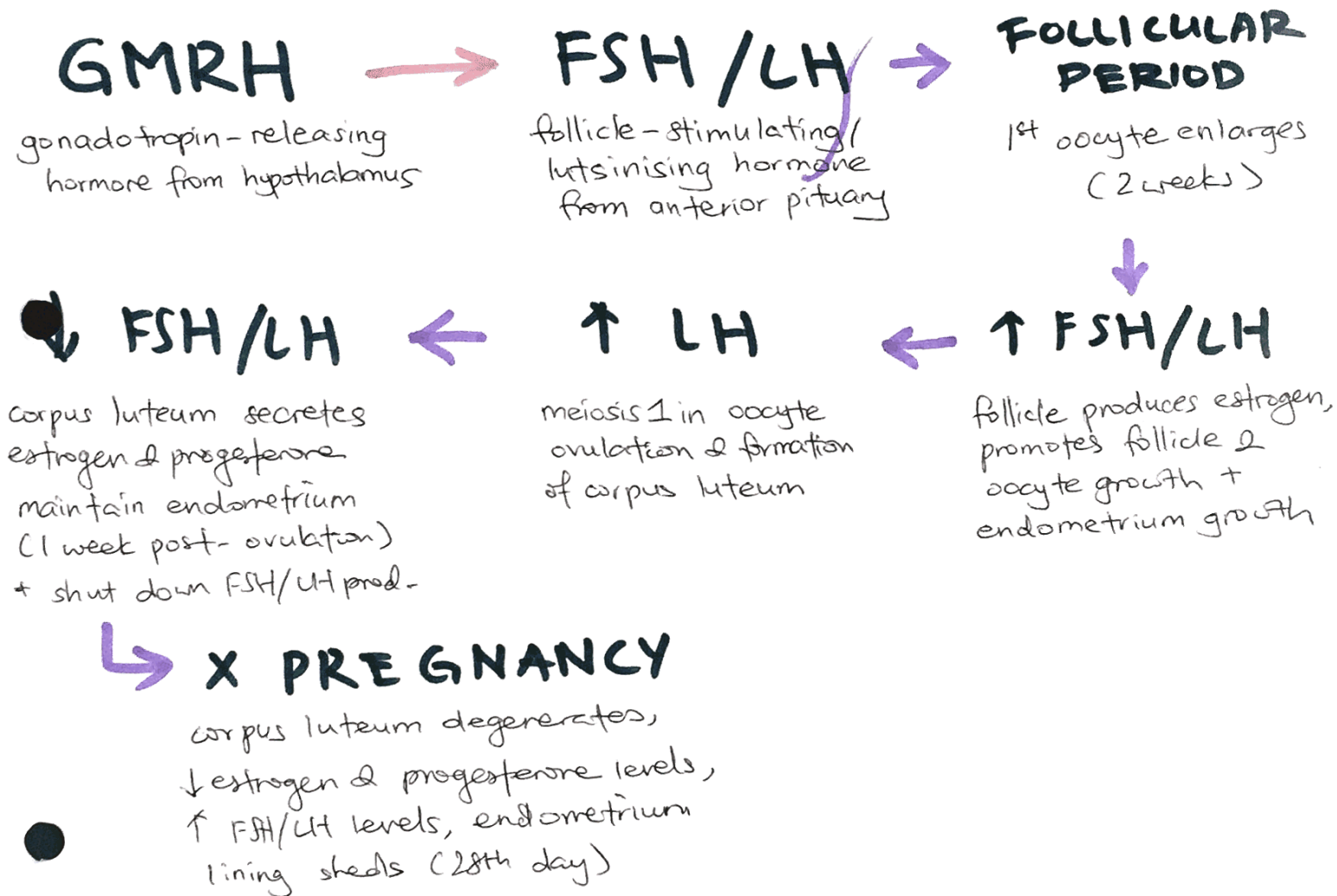
sperm	ovum
smaller elongated/narrow + distinct structure motile	larger spherical  moves by external agents
X/Y chromosome cytoplasm x yolk	X chromosome cytoplasm = yolk granules

spermatogenesis	oogenesis
number of gametes	
principle: continuous production. sperm continuously engendered but quantity/quality subject to extreme fluctuations	principle: use up pre-birth generated oocytes. continual decrease beginning w/ fetal period, ending w/ menopause
meiotic output	
4 functioning, small, motile spermatozooids	1 large immobile oocyte, 3 shriveled polar bodies
fetal period	
no meiotic divisions or germ cell productions	entering into meiosis prod. of entire supply of germ cells

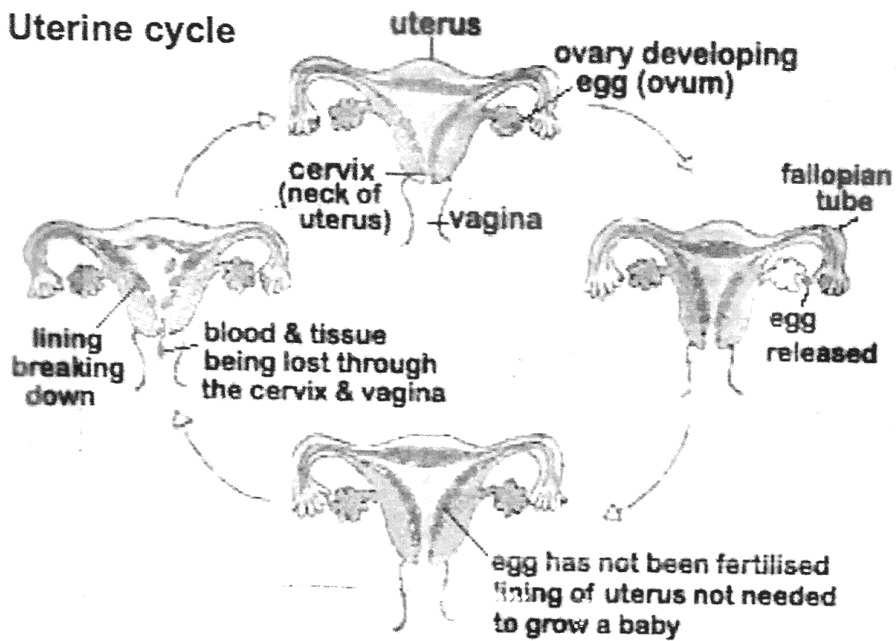
# MENSTRUAL CYCLE

function: provides a favourable enviro. for devt. of fetus

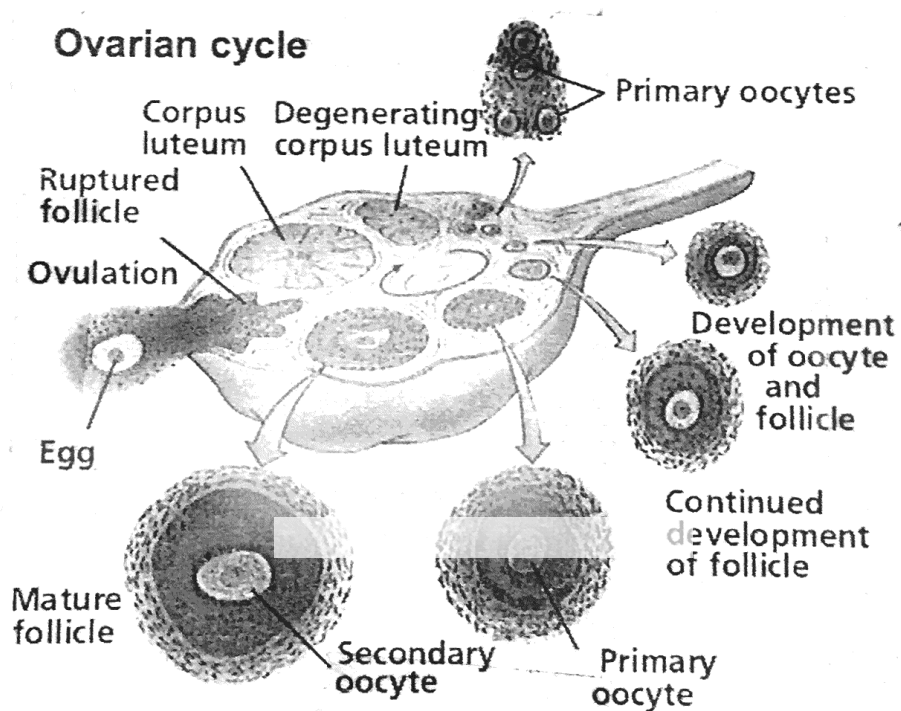
process: synchronised recurring sequence of changes in endometrium of non-pregnant female (uterine cycle)  
linked to sequence of changes in ovaries (ovarian cycle)

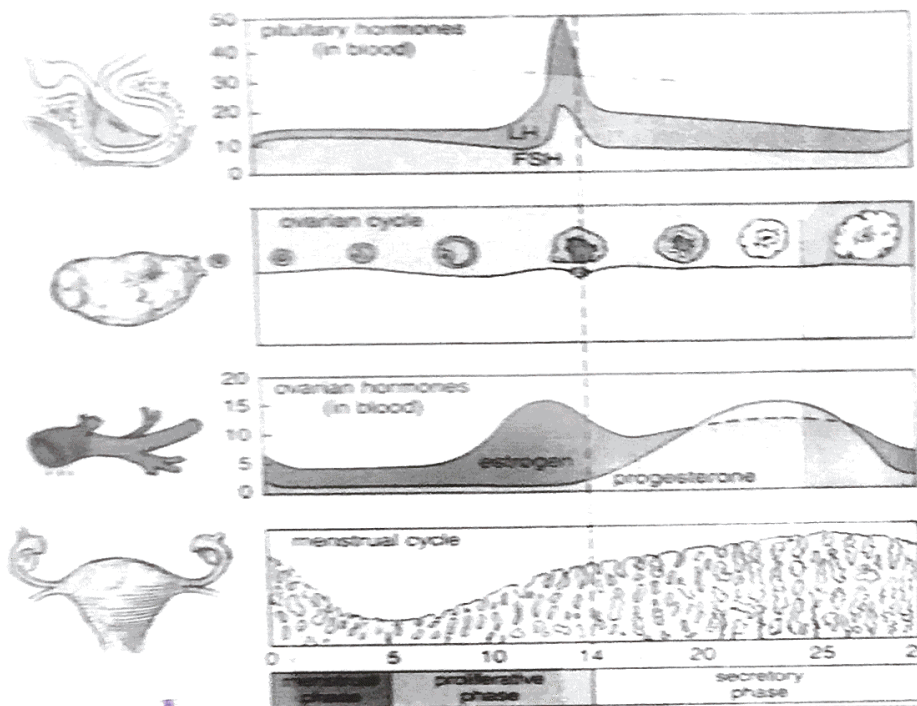


## Uterine cycle



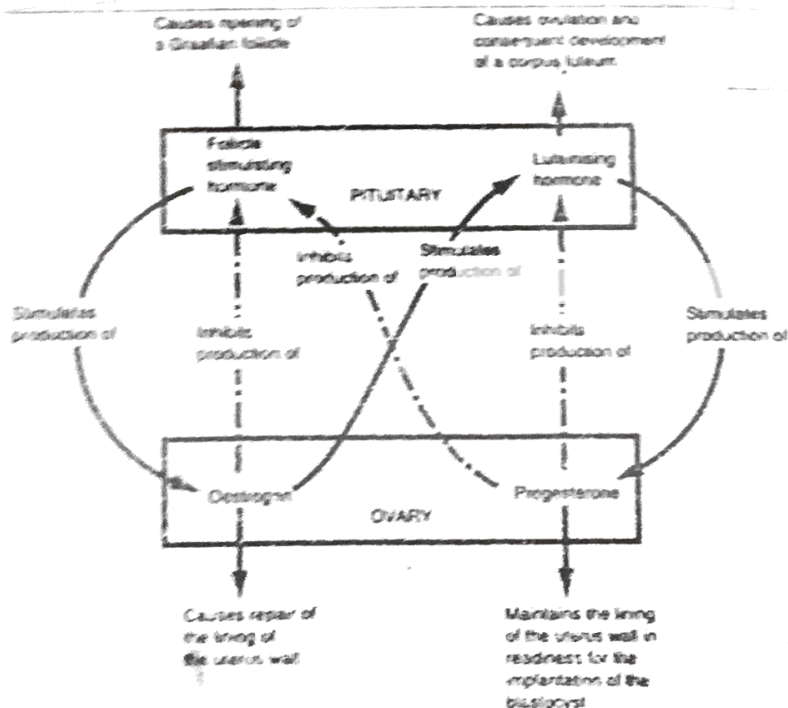
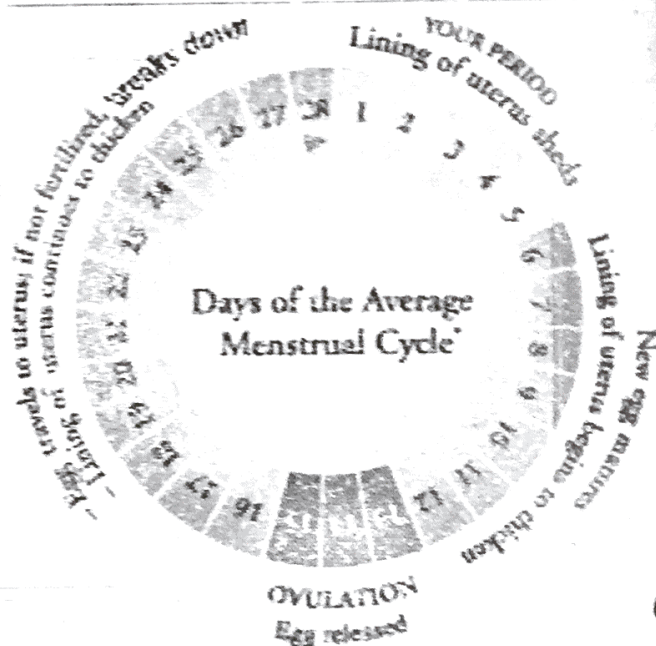
## Ovarian cycle





production  
& secretion  
periods

menstrual cycle  
days →



production  
cycle



# FERTILISATION

FERTILISATION



CLEAVAGE



GASTRULATION



NEURULATION



● ORGANOGENESIS

egg & sperm fuse

zygote subdivides into ball of many cells

cells rearrange, produce embryos w/3 cell layers

neural tube forms

body organs form

## GASTRULATION

process: formation of 3 body layers

● **OUTER:** ECTODERM

skin, hair, sweat glands, epithelium, brain, nervous system

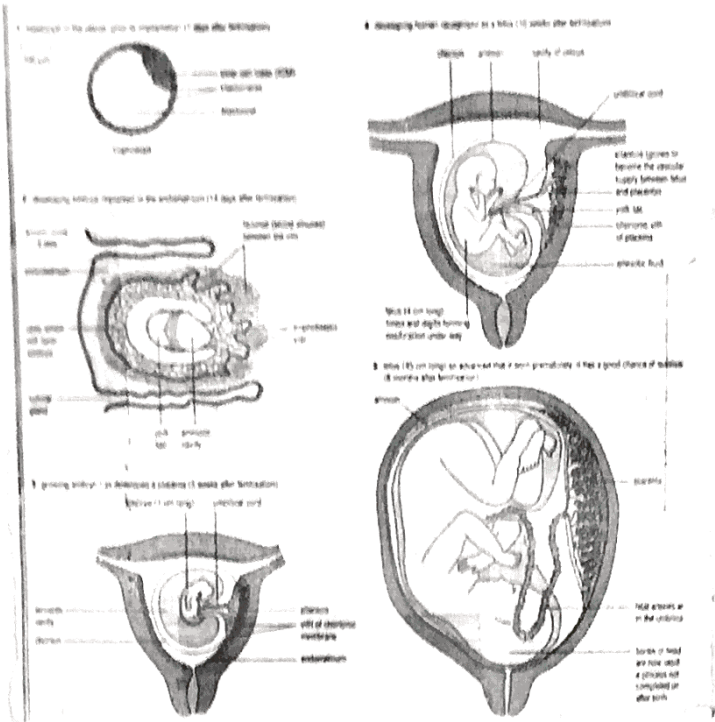
● **MIDDLE:** MESODERM

muscles, cartilage, bone, blood, connective tissue, kidneys, reproductive system

**INNER:** ENDODERM

digestive system, respiratory system, endocrine structures, liver, pancreas, gall bladder

# PREGNANCY

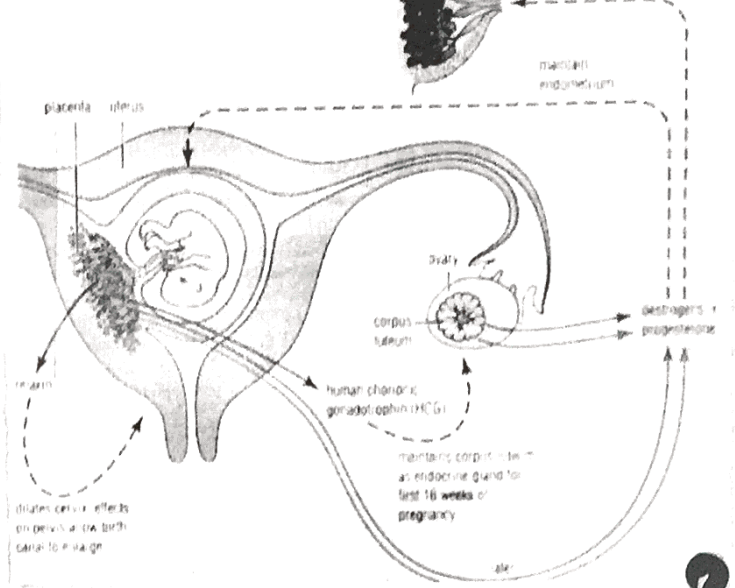


process & structures ↗

## Hormones of Pregnancy

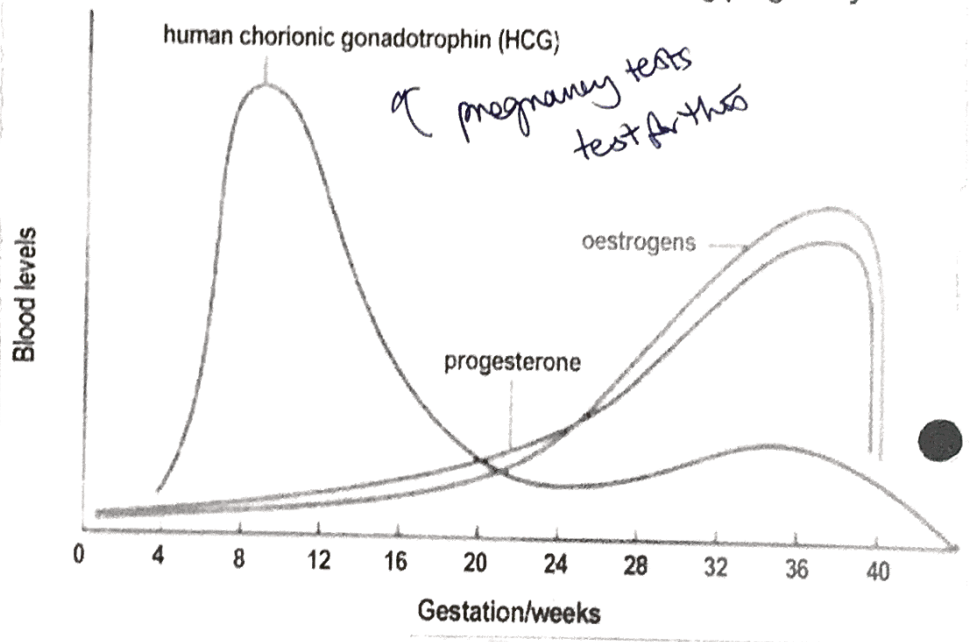
the chief hormones of pregnancy and their spheres of activity

secretion → actions



↖ hormone production

## blood levels of these hormones during pregnancy



## 1<sup>ST</sup> TRIMESTER

- 3 embryonic layers formed
- cellular differentiation forms organs (week 3)
- embryo 5mm long, mostly paired somites (month 1)
- embryo → fetus (week 6)
- sex determination process begins (week 7)

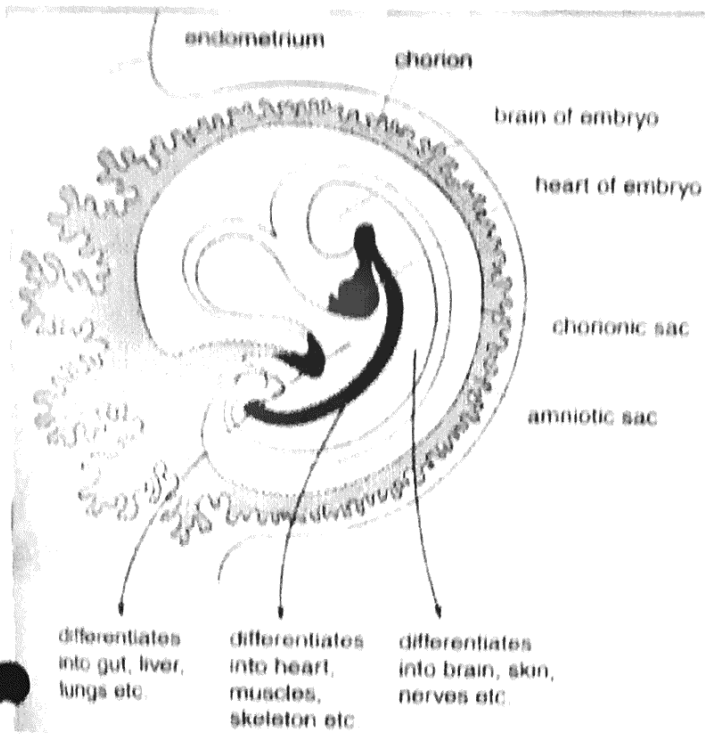
## 2<sup>ND</sup> TRIMESTER

- fetus size ↑
- bony parts of skeleton start to form

## 3<sup>RD</sup> TRIMESTER

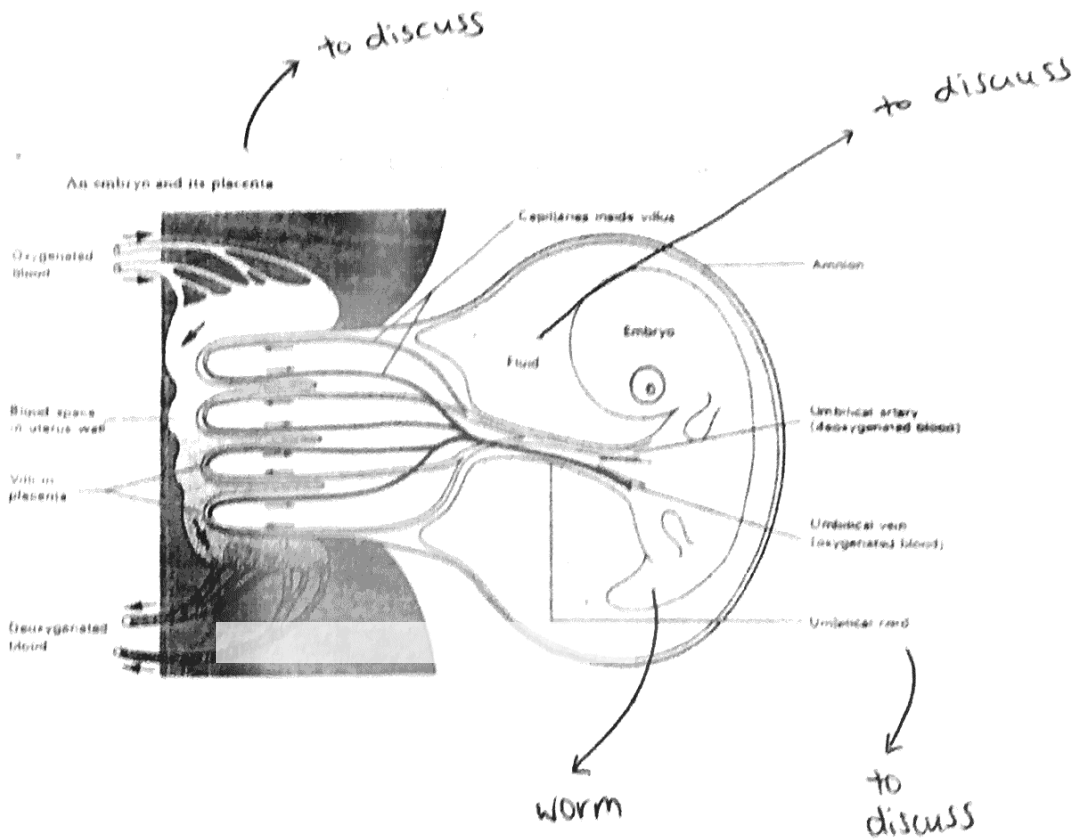
- circulatory & respiratory systems mature

# DIFFERENTIATION



process where  
unspecialised cells  
become altered & adapted  
to perform specific functions  
as part of permanent tissues

## WORM PARTS



## AMNIOTIC FLUID

- supports & cushions fetus pre-birth
- shock absorber
- incompressible  $\therefore$  protects fetus against mech. injury
- higher temp. than mother's body  $\therefore$  keeps fetus warm/cozy

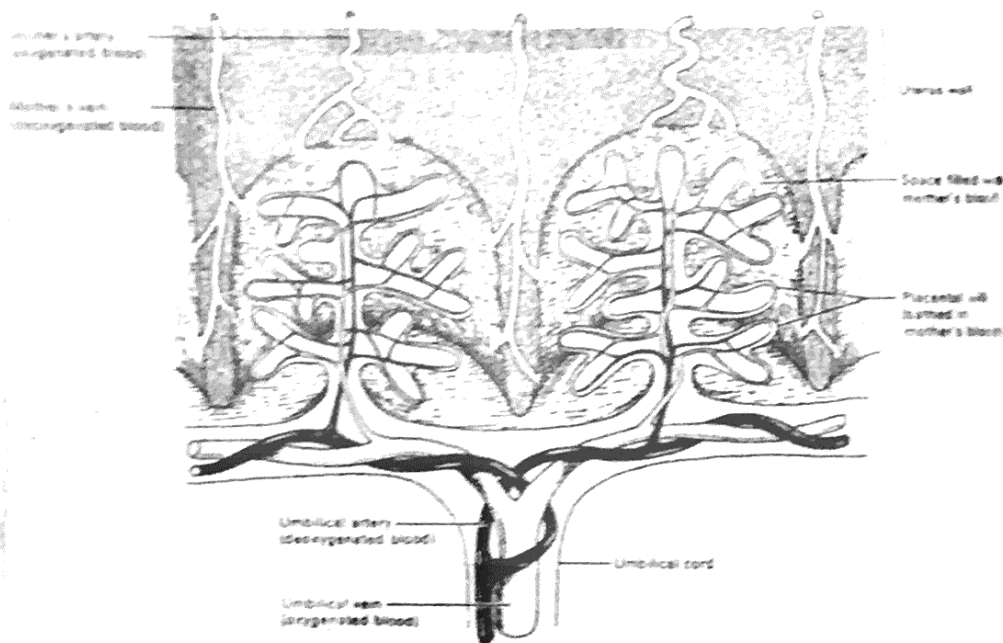
## UMBILICAL CORD

- attaches placenta to fetus
- made out of 2 arteries  $\rightarrow$  carries deoxygenated blood; fetus  $\rightarrow$  mother  
larger vein  $\rightarrow$  carries oxygenated blood; mother  $\rightarrow$  fetus

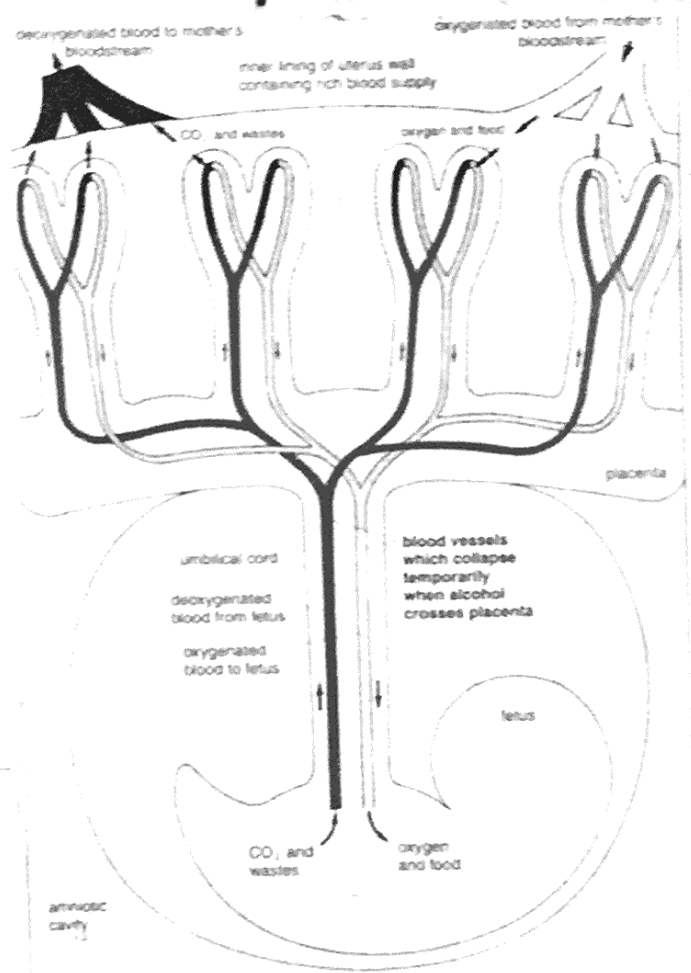
## PLACENTA

- allows exchange of materials between fetus & mother w/o blood mixing
- diffusion of food substances from mother to fetus
- rids metabolic waste products from fetus
- diffusion of maternal antibodies to fetus = immunity
- prevents pathogens/toxins from reaching fetus
- barrier to hormones/other chemicals in mother's blood
- permits 2 blood systems to operate @ different pressures
- produces pregnancy related hormones
- produces progesterone preventing menstruation

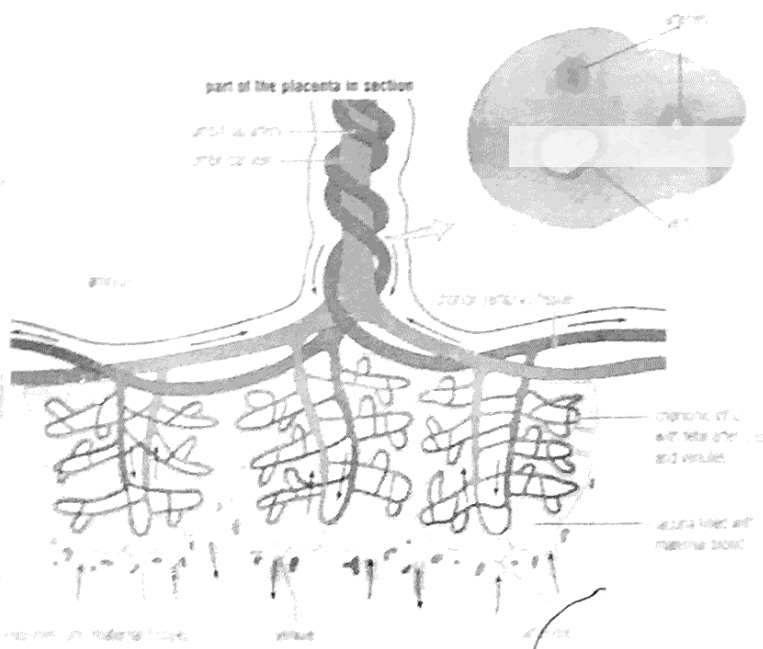




blood transfer



TS of the umbilical cord (x2)



umbilical cord structure

# DNA & RNA

## DNA

Stats: 46 chromosomes, 23 pairs

3bil subunits (AT/CG)


30K genes (code for proteins that perform most life functions)

double helix

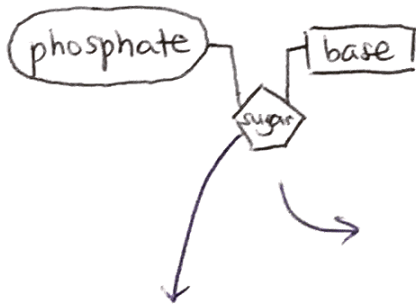
located in nucleus

made of sugar phosphate backbone + hydrogen bonds + ATCG bases

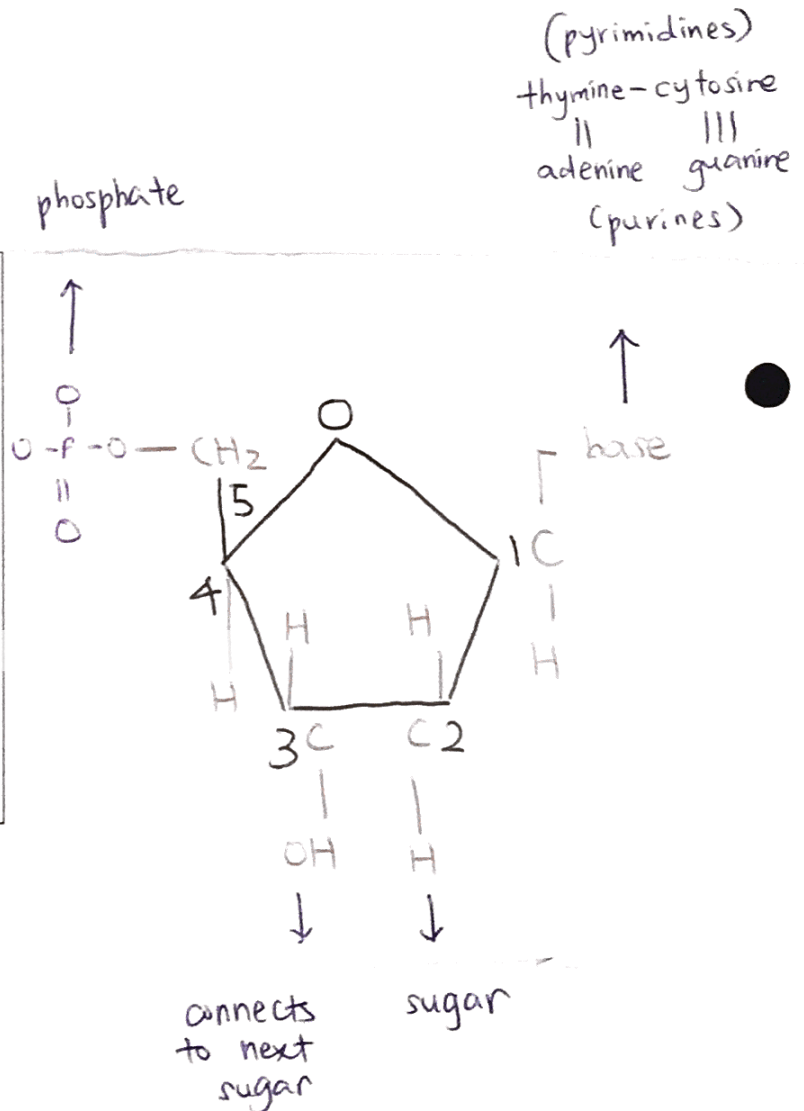
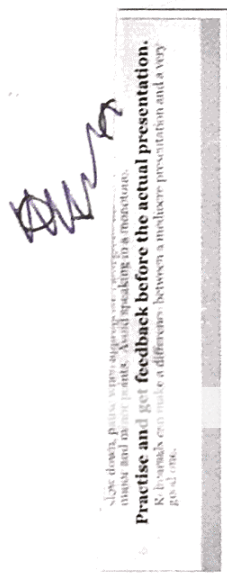
## NUCLEIC ACIDS

 → singular polynucleotide  
(nucleotide polymer chain)

## NUCLEOTIDE



5-carbon  
DNA: deoxy  
RNA: ribose



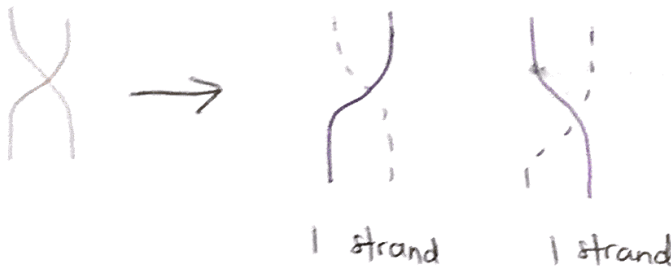
~~BA~~ DNA = ACGT  $\rightarrow$  thymine

RNA = ACGU  $\rightarrow$  uracil

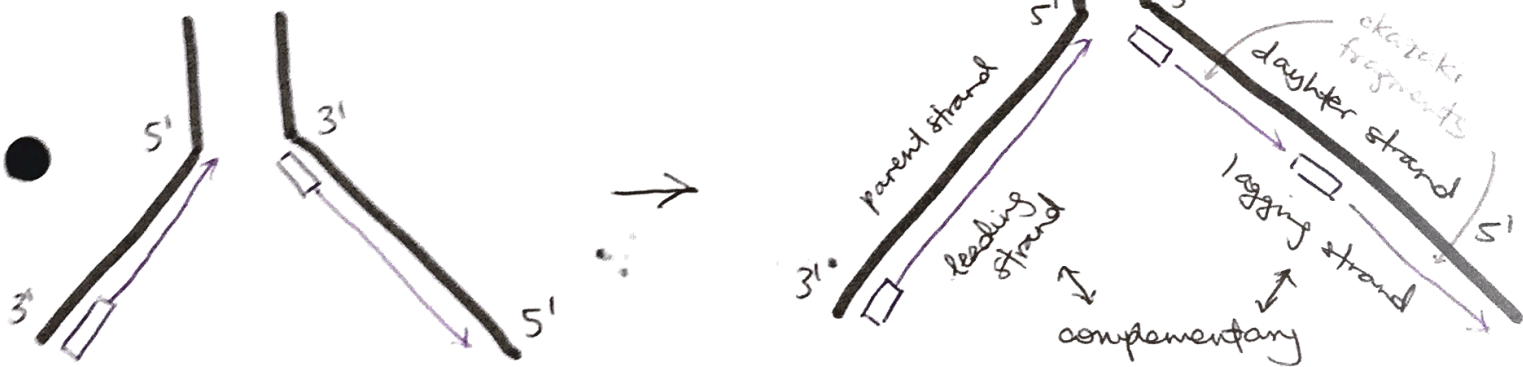
## CONDENSATION

Sugar + Base  $\rightarrow$  Nucleoside +  $H_2O$

Strands held together by hydrogen bonds between the nitrogenous bases on both strands.



after division, each parent strand acts as a template for the synthesis of the other strand thru complementary base pairings



rna moves in relation to direction of other strand

(primase only moves 5'  $\rightarrow$  3')

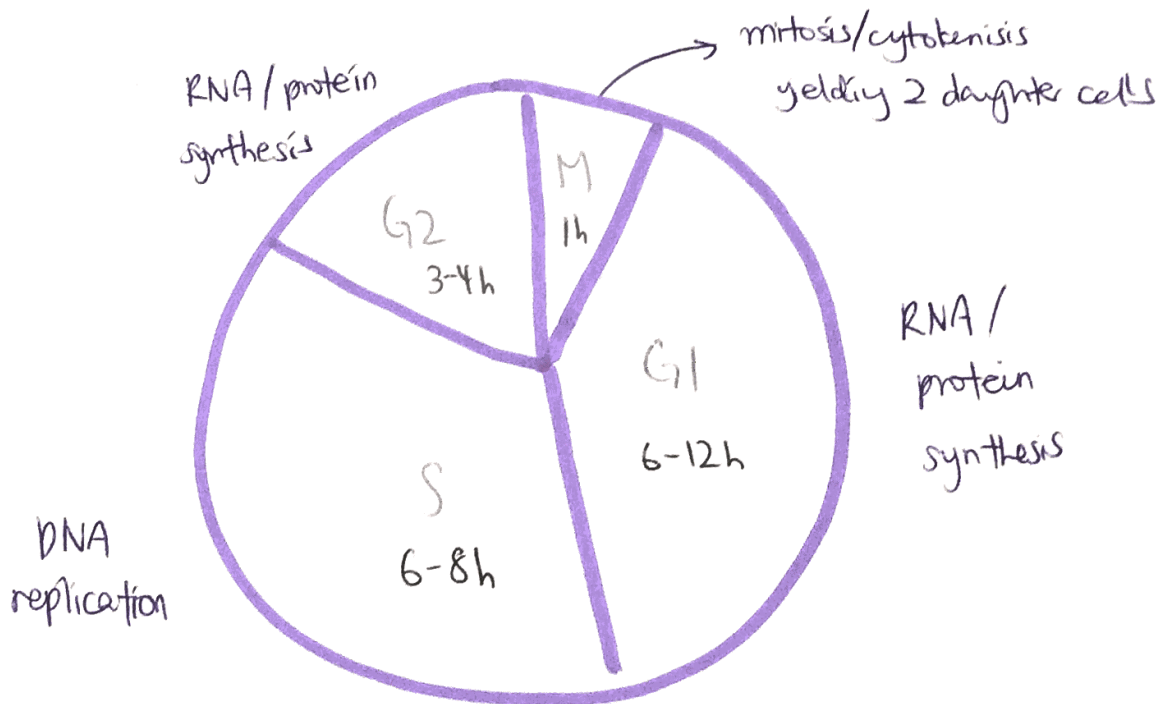
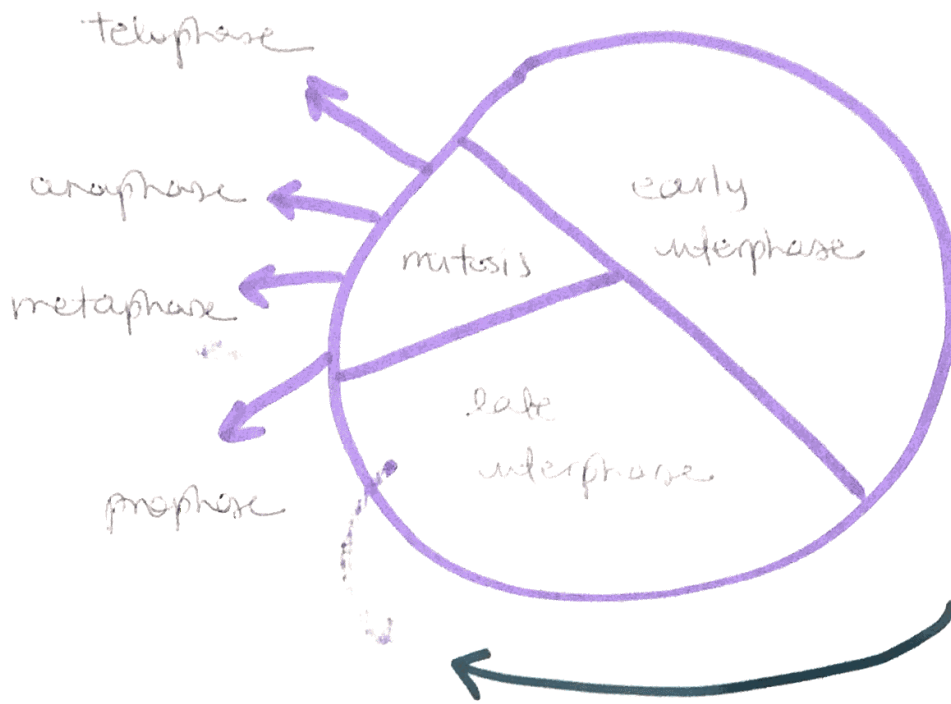
□ primer RNA

→ DNA

SEMI-CONSERVATIVE	CONSERVATIVE	DISPERSIVE
<p>gen 0</p> <p>gen 1</p> <p>gen 2</p>	<p>gen 0</p> <p>gen 1</p> <p>gen 2</p>	<p>gen 0</p> <p>gen 1</p> <p>gen 2</p>



# CELL CYCLE



# LEGEND

$2n$  = diploid  
(set of chromosomes)

$n$  = haploid

Centromeres =  
no. of chromosomes

Chromatids =  
dna content

**ORDER**

early interphase

↓  
late interphase

↓  
prophase

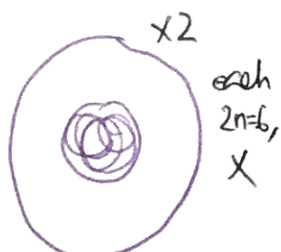
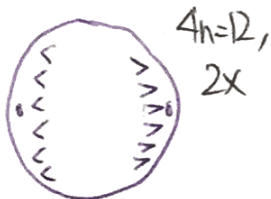
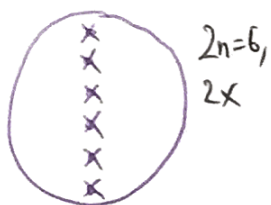
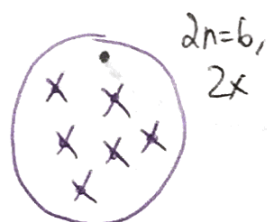
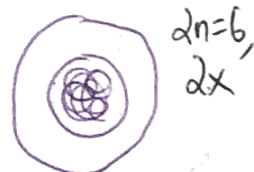
↓  
metaphase

↓  
anaphase

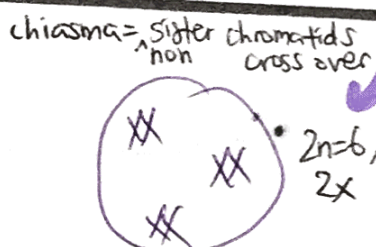
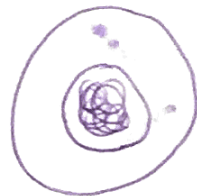
↓  
telophase

↓  
cytokinesis

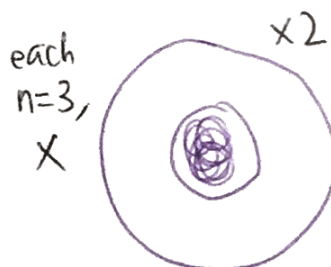
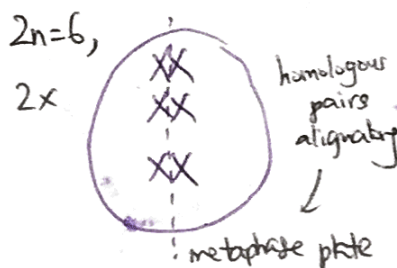
## MITOSIS



## MEIOSIS I



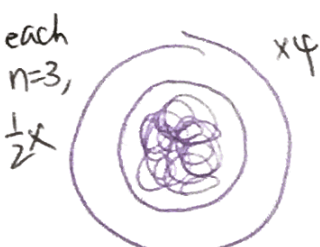
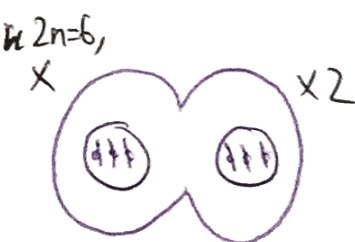
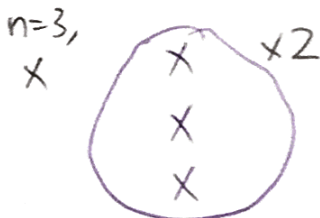
synapsis = homologous pairing



## MEIOSIS II

after meiosis I,  
meiosis II

as → occurs,  
- condense chromatin threads to chromosomes  
- nuclear membrane disintegrates  
- spindle fibres form from centrioles  
- centrioles move to poles



# DNA V RNA

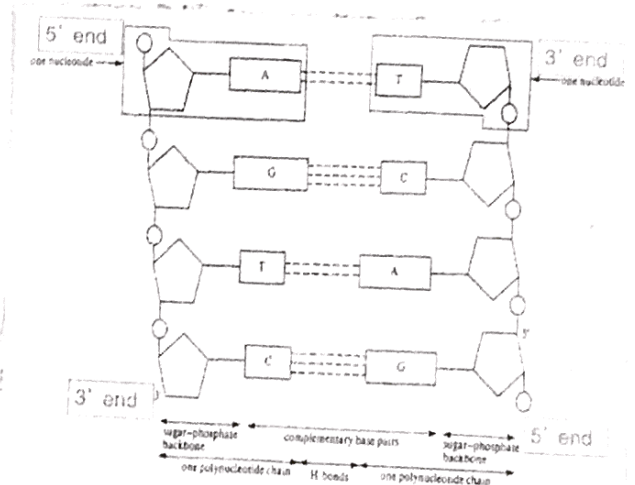
**DNA:** capable of carrying coded info (base sequence) to direct cell activity & accurate replication bc rule of base pairing

**RNA:** found in both nucleus & cytoplasm of eukaryotic cells + single stranded macromolecules, made of many nucleotides joined by 3'→5' phosphodiester bonds; more created thru transcription

mRNA	tRNA	rRNA
<ul style="list-style-type: none"> <li>- messenger</li> <li>- long single stranded polynucleotide</li> <li>- 300 nucleotides, smallest molecule</li> <li>- manufactured in nucleus</li> <li>- base sequence complementary copy of template 2 strand</li> </ul>	<ul style="list-style-type: none"> <li>- transfer</li> <li>- 5'-3' coding</li> <li>- 3'-5' template</li> <li>- any tRNA in coding → mRNA</li> </ul>	<ul style="list-style-type: none"> <li>- ribosomal</li> <li>- ribosome component</li> <li>- synthesised in nucleus</li> </ul>

## WATSON CRICK MODEL

- 2 polynucleotide chains coiled around each other forming double helix
- each chain made up of a sugar phosphate backbone
- chains run in opposite direction (3' - 5')
- hydrogen bonds formed between corresponding bases of 2 chains
- each base pair = 1 purine 1 pyrimidine



# DNA REPLICATION

BEFORE: free deoxyribonucleotides synthesised in cytoplasm, replication begins

UNWINDING OF  $\times 2$  HELIX: ATP-dependent enzyme (helicase) causes DNA molecule to unzip @ origin of replication

hydrogen bonds between complementary bases break

DNA strands separate. each strand acts as a template

complementary deoxyribonucleotides assemble alongside partners

sugar phosphate backbone formed thru condensation reactions between nucleotides

FORMATION OF RNA PRIMER STRAND: primase attaches to unwound chains behind replication fork, catalyses formation of short RNA chain complementary to DNA template strand  
primer initiates polynucleotide synthesis, consists of  $\approx 10$  ribonucleotides

DNA polymerase III adds deoxyribonucleotides to preexisting strand

SYNTHESIS OF NEW STRAND: DNA polymerase III synthesises new DNA continuous from RNA primer strand & fits free deoxyribonucleotides complementary to those on parent strand

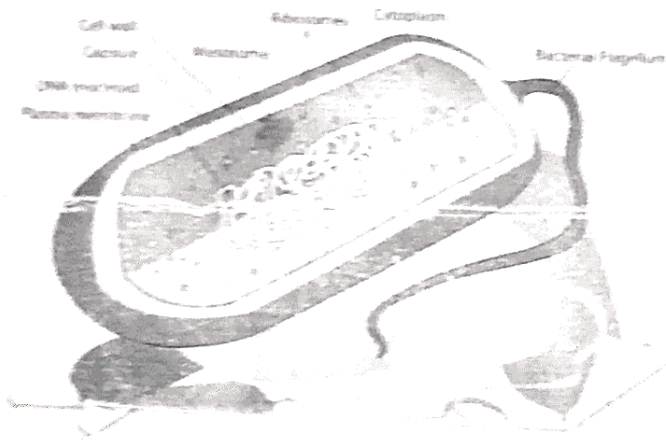
REMOVAL OF PRIMER: gaps filled w/ complementary deoxyribonucleotides  
DNA ligase joins okazaki fragments thru phosphodiester bonds + hydroxyl group of 3' end to other phosphate group of 5' end

AT THE END: 2 DNA MOLECULES FORMED

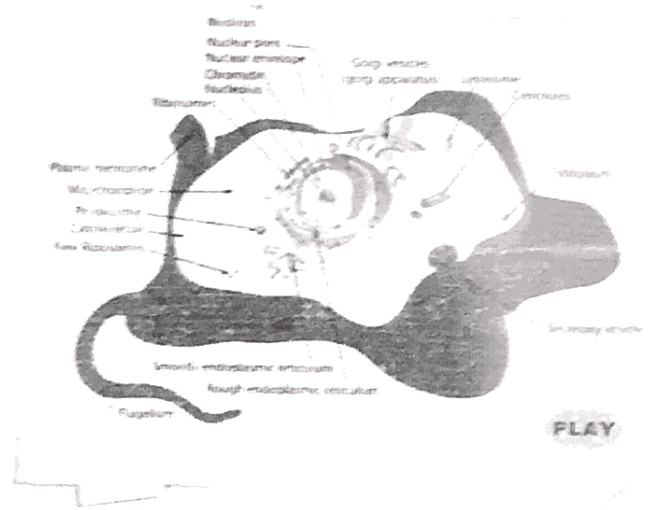
each contains a newly synthesised + parent strand in double helix



# CELLS



PR



## PROKARYOTES

cellular organisms w/o nucleus or membrane bound organelles

- smaller genome cell
- simple
- plasmids present
- continuous gene coding sequence
- small amt of non-coding DNA
- DNA in nucleoid
- 2 lvs of packing - supercoils/looped domains
- single, doublestranded closed circular DNA
- cell wall present, chemically complex
- no cytoskeleton or cytoplasmic streaming
- cell division by binary fission
- ribosomes smaller

## EUKARYOTES

cellular organisms with nucleus & membrane bound organelles

- larger genome size
- complex
- plasmids absent
- intron-interrupted gene coding sequence
- large amt of non-coding DNA
- DNA in nucleus
- 4 lvs of packing - nucleosomes, solenoid, looped domains, metaphase chromosome
- 2/more doublestranded linear DNA
- Cell wall only in plant cells, cellulose
- cytoskeleton or cytoplasmic streaming
- cell division by mitosis
- ribosomes larger

# MITOSIS SIG FIG.

## GENETIC STABILITY

- 2 nuclei of same number/kind of chromosomes as parent cell, preserving chromosome constitution
  - ↳ replication (interphase)
  - ↳ arrangement (metaphase)
  - ↳ separation (anaphase)

## ASEXUAL REPRO.

- genetically similar, offspring = same advantages

## GROWTH & DVPMT

- ↑ in cell number

## REGEN/REPLACEMENT

- regen. missing parts
- wound healing, tissue healing

# MITOSIS V MEIOSIS

## CROSSING OVER (prophase I)

- synapsis leads to genetic xchange btwn homologous chromosomes

## PAIRING & INDIE. ASSORTMENT (metaphase I)

- homologous chromosomes line up in pairs @ equatorial plane not individually

## POLES (anaphase)

one homologous ~~pair~~ chromosome from each pair goes to opposite pole instead of separating.

MITOSIS → 1 div, 2 daughter cells, genetically identical

MEIOSIS → 2 div, 4 daughter cells, genetically diff.

# PCR

## ADVANTAGES

Speed - 30 min to 3h

Specificity - can amplify specific DNA fragment from mixture

Sensitivity - can amplify DNA from 1 cell / poor quality DNA

## RESTRICTION ENZYMES

- special class of naturally occurring proteins
- cut specific palindromic sequences @ recognition sites
- cleavage occurs thru breaking of phosphodiester bonds, reversible through DNA ligase
- generate ends (sticky or blunt)
  - ↳ if cut closer to 5' end, 5' overhang
  - ↳ if cut closer to 3' end, 3' overhang
  - ↳ if no overhang, blunt

## PROCESS

denaturation ( $94^{\circ}\text{C} / 30\text{sec}$ ) → separates DNA strands

annealing ( $64^{\circ}\text{C} / 45\text{sec}$ ) → primers target regions within gene + bond

extension ( $72^{\circ}\text{C} / 45\text{sec}$ ) → DNA polymerase amplifies target region

## STUFF

gel electrophoresis: separates DNA by size

glycerol: renders DNA samples denser than buffer so they sink into well

DNA ladder: helps determine DNA BP

IF SUCCESSFUL → one band only

# MENDELIAN GENETICS

## DEFINITIONS

gene: segments of DNA coding for protein

gene locus: physical location of gene as found in specific chromosome

ALLELES: any of gene's alt. forms, found in pairs  
each on 1 pair of homologous chromosomes

### DOMINANT

heterozygous organism  
expresses trait from  
allele  $E$

$Ee$  hetero  
 $EE$  homo

### RECESSIVE

heterozygous organism  
does not express trait from  
allele  $e$

genotype: determined by alleles, determines phenotype  
phenotype: expressed, what you see

## PUNNET SQ.

### STEPS

① parental phen/genotype

② punnet sq.

③ F1 gen phen/genotype ratios

Parental gen  $Aa \times Aa$

Parental phen: Hetero Trait  $\times$  Hetero Trait  
or Hetero or Hetero

F1 gen:  $1:2:1$   $1AA, 2Aa, 1aa$   
1 homo dom 2 hetero  
1 homo rec (1 traits)

④ conclude

(replace underlined w/cont)

Let letter be ALLELE for trait.

Gametes	(A)	(a)
(A)	AA	Aa
(a)	Aa	aa



# CROSSES

MONOHYBRID = 1 gene involved eg. Rr

DIHYBRID = 2 genes involved eg. AaBb

LAW OF SEGREGATION = 2 alleles for a heritable character separate during gamete formation ending up in diff gametes

LAW OF INDEP. ASSORTMENT = gene pairs on diff chromosomes assort independently @ meiosis in 9:3:3:1 ratio

INCOMPLETE DOMINANCE = hybrid where both traits disappear by combining into smth new  
eg. red flower x white flower = pink flower

CODOMINANCE = hetero where end product exhibits phenotype of both traits  
eg. bloodtype A + bloodtype B = bloodtype AB

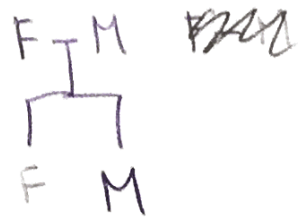
## SEX-LINKED

where gene is on X-chromosome (recessive)

♀ express trait  
requires 2 copies of allele

♂ express trait  
requires 1 copy of allele  
because males only have 1 X chromosome

affected  
carrier  
nil



∴ males ↑ susceptible to sex-linked diseases

# NON-DISJUNCTION

when sister chromatids fail to separate @ meiosis II.

= abnormal chromosome number

when chromosomes in a cell fail to separate properly, some gametes end up normal & others have no chromosomes.

↳ TRISOMY: 3 copies of chromosome instead of 2

↳ eg. Down's = chromosome 21 trisomy (1 in 800)

eg. Klinefelter's = sex chromosome trisomy (boys)

eg. Turner's = no Y chromosome (girls)

## CHROMOSOMAL MUTATIONS

changes in DNA sequence OR chromosomal number  
structural modification of chromosome

## DNA REPLICATION ERRORS

### BASE SUBSTITUTION

(point mutation)

single base swapped w/ another  
usually tolerable re: gene function

- TRANSITION: pyrimidine subs pyrimidine  
purine subs purine

- TRANSVERSION: pyrimidine subs purine  
purine subs pyrimidine

- MISSENSE: codon altered  
alters for ~~amino~~ new amino acid

- NONSENSE: stop codon altered ∴ transition  
of messenger RNA stops prematurely  
truncated nonfunctional protein

- SILENT: trans- error occurs but same amino acid  
produced ∴ no change in product &  
cannot be detected w/ gene sequencing

### INSERTION/ DELETION

(frameshift mutation)

base inserted/ deleted from sequence  
alters ALL subsequent codons  
downstream from mutation site  
= nonfunctional protein product

# CANCER & CHECKPOINTS

definition: uncontrolled cell division  
apoptosis doesn't occur  
clones descended from one cell  
begins as primary tumour  
establishes metastases over body

causes: anything that damages DNA  
mutagens  
anything that stimulates mitosis rate

checkpoints:-

G1 (end of G1 phase)

decides if cell should divide/delay division/rest

G2 (end of G2 phase)

triggers mitosis

Metaphase

all chromosomes have aligned

# STEM CELLS

## FEATURES

- capable of continually dividing & reproducing themselves over long periods (proliferation)
- unspecialised cell (no tissue-specific structures)
- differentiate into specific cells under specific conditions

## TYPES

**TOTIPOTENT:** found in zygotes, can convert to all cell types formed thru fusion of 2 haploid nuclei

**PLURIPOTENT:** found in embryos; can convert to almost all cell types hollow ball of cells (blastocyst embryonic) 4 days after fertilisation + inner cell mass gives rise to specialised cells

**MULTIPOTENT:** found in umbilical cord; genetically identical to body a variety of conversions

**IN ADULTS:** tiny amounts to repair & maintain tissue can remain as stem cells OR develop



# GENE MUTATION

the ultimate source of ALL genetic variation, because there is NO other source for ENTIRELY NEW alleles.

## MUTAGEN TYPES

Chemical: alter DNA of cell

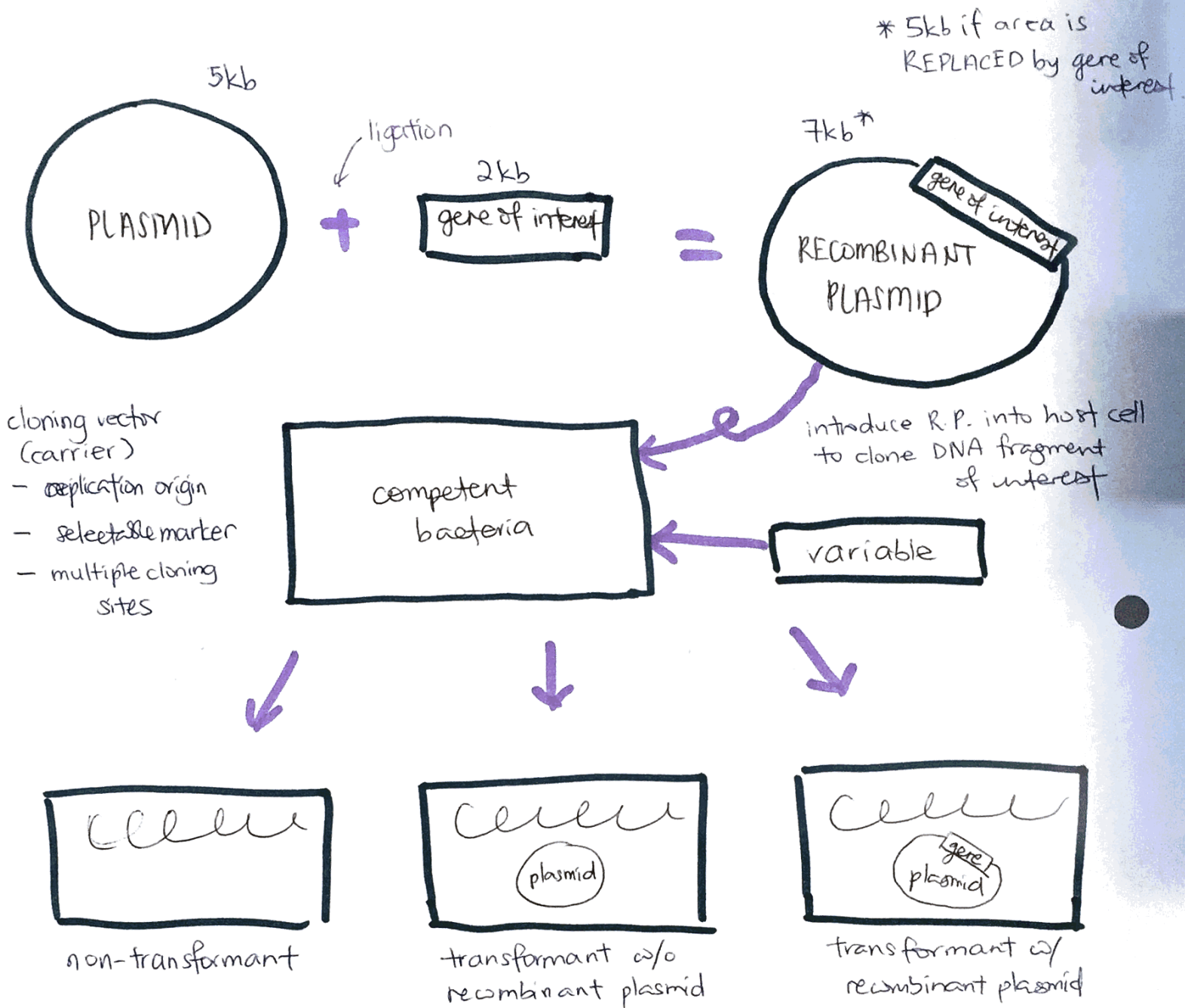
Biological: viruses insert into genome

Radiation: ~~to~~ DNA damage thru radioactivity

# MOLECULAR CLONING

PROCESS (replace underlined w/ context)

- ① RESTRICTION DIGESTION of plasmid & gene of interest w/ same restriction enzyme
- ② LIGATION of digested plasmid & digested gene of interest with DNA ligase to produce recombinant plasmid
- ③ TRANSFORMATION of recombinant plasmid into calcium chloride treated competent antibiotic-sensitive bacteria to produce transformant
- ④ SELECTION of transformants with recombinant plasmid on LB agar selection media containing (see question).



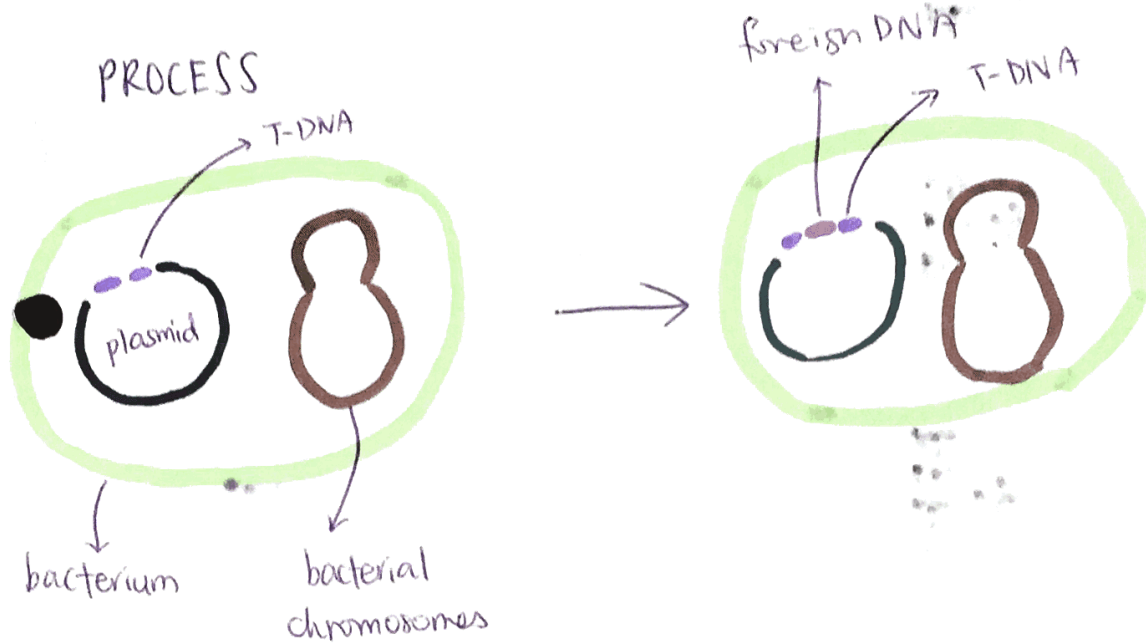
## MULTIPLE CLONING SITES

engineered to contain recog. sequences for numerous restriction enzymes. no 2 restriction enzymes in MCS are the same, so when you cut abt a restriction enzyme within MCS, you know for sure that gene of interest will stick in MCS & won't inactivate essential genes. single cuts occurring in enzyme prevent insertions into essential regions of plasmid.

# GMOs

organisms w/ artificially altered DNA thru deliberate transfer of genes between species (which need not be closely related)

goal is to induce new host to create 1 or more protein that is not normally produced



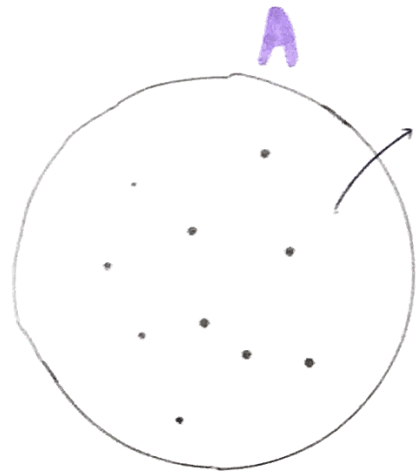
plasmid removed  
T-DNA cut by  
restriction enzyme

foreign DNA cut  
w/ same enzyme

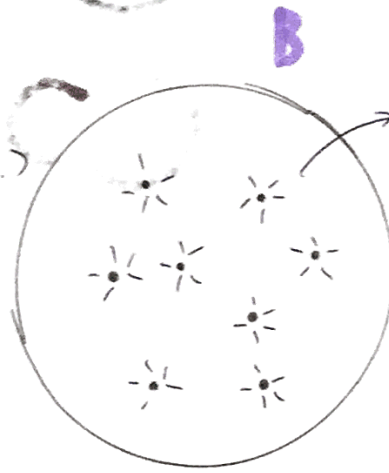
plasmid reintegrated  
w/ DNA

integration - foreign  
DNA inserted into T-DNA

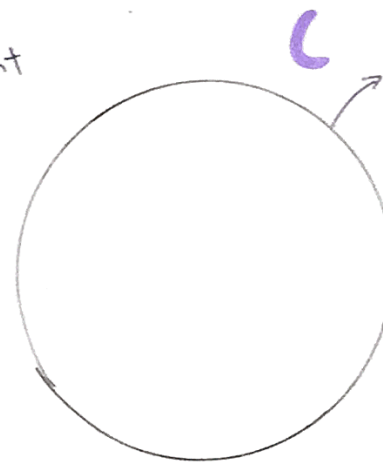
# EXPERIMENT RESULTS if they still do this LOL



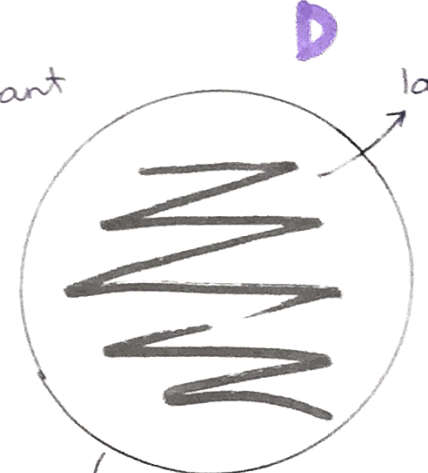
transf no RP  
+ plasmid & ampicillin



transf RP  
+ plasmid, arabinose, ampicillin  
fluorescent



transf no RP  
+ ampicillin  
-ve constant



lawn  
+ve constant nontransf

**PLASMID = BLA**  
↓  
inactivates ampicillin  
+ transformed bacteria has this

**GFP**  
↓  
if ARAc on, activated (green flour. protein)  
+ useless unless expressed; once expressed gives you a protein.

**ARA<sub>c</sub>**  
↓  
if arabinose, ARA<sub>c</sub> switched on

**pGLO**  
↓  
transformant

## ACCOUNT FOR DIFFERENCES

- A v B: presence/absence of arabinose
- A v C: presence/absence of pGLO
- A v D: transformant efficiency
- C v D = presence/absence of ampicillin





# REPROGRAMMING

generation of pluripotent stem cells from patients' own somatic cells

NUCLEAR TRANSFER: transfer of nucleus from patients' somatic cells into enucleated egg

FUSION: fuse patients' somatic cells with ES cells  
derived pluripotent cells = used for transplantation  
= tetraploid  
= rejection possible

● DEFINED FACTORS: intro. pluripotency factors into somatic cells  
leads to IPS cells (induced pluripotent stem cells)

## DERIVATION OF IPS CELLS

- pluripotency factors intro. into skin fibroblasts
- only cells harbouring antibiotic resistance gene survive after selection
- colonies resembling ES cells are isolated & expanded in culture to give IPS cell lines
- - IPS cells injected into blastocysts of different strain
- IPS cells that give rise to germ line form chimeras

## BIO SPA if still relevant

DILUTION FACTOR:  $\frac{\text{sample} + \text{diluent}}{\text{sample}}$

DNA CONCENTRATION:  $(50 \times A_{260} \times \text{dilution factor}) \mu\text{g/ml}$

PURITY:  $\frac{\text{Absorbance at 260}}{A_{280}}$  (pure if 1.65-1.85)

YIELD: concentration  $\times$  vol of sample & diluent

eg. if  $289 \mu\text{g/ml}$ ,  $\therefore 289 \mu\text{g}/1000 \text{ ml}$   
for  $50 \mu\text{l}$ ,  $\frac{289}{1000} \times 50$ .

- nucleic acids absorb light in the UV region of the electromagnetic spectrum, can be used for quantitation & purity determination thru spectrophotometer.
- DNA's net -ve charge = it migrates towards anode (+ve)  
larger molecules slower than smaller molecules  
gel electrophoresis separates for size
- loading dye tracks how fast DNA migrates
- glycerol allows samples to sink into wells
- PTC gene amplified in PCR  $\rightarrow$  DNA replication in tube cuts at specific site & amplifies; primer targets replication site

\*