INTRODUCTION TO GENERAL EMBRYOLOGY

GAMETOGENESIS, FERTILISATION, CLEAVAGE

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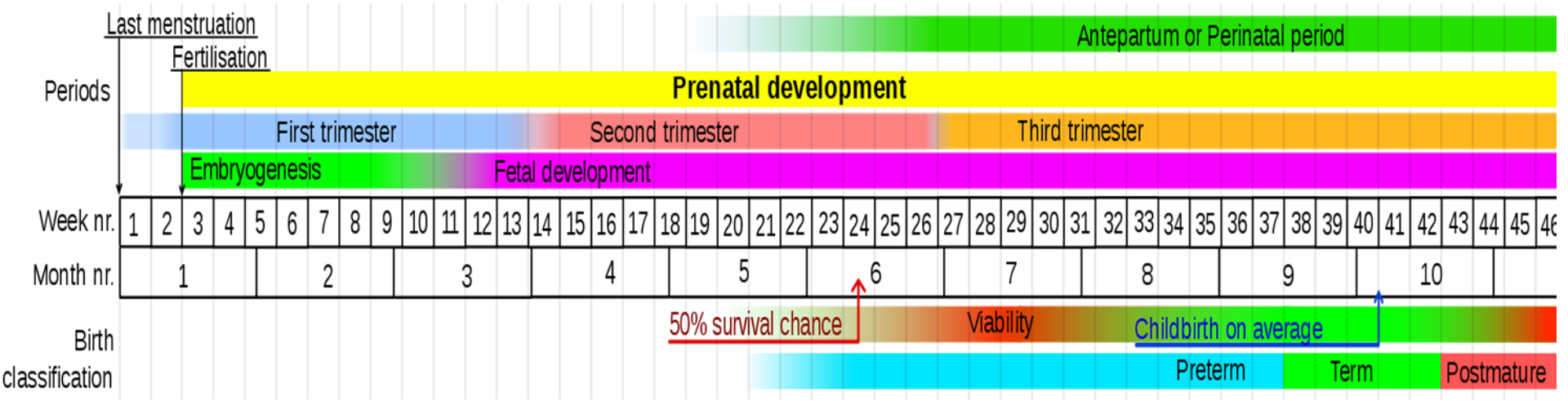
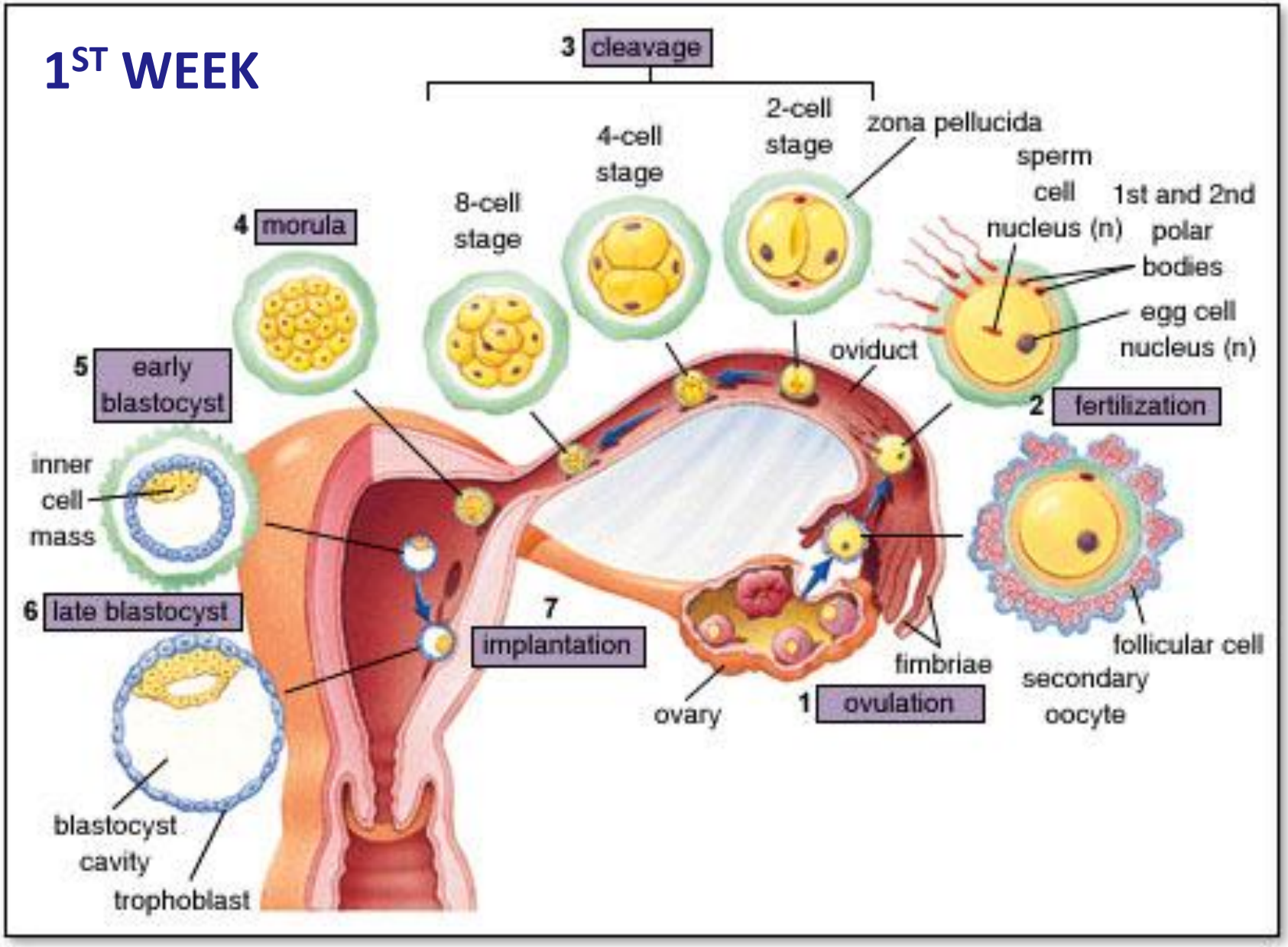
EMBRYOLOGY

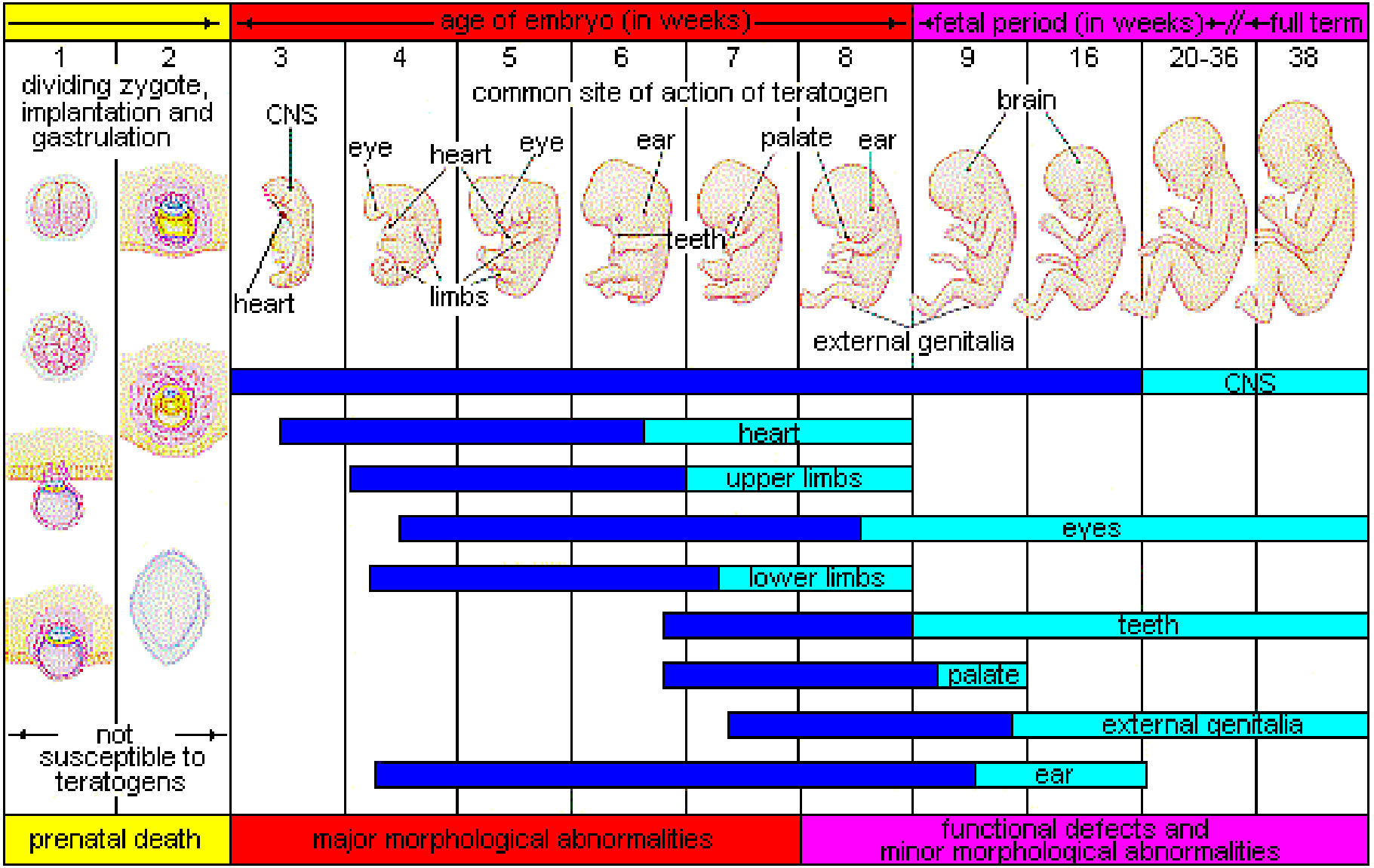
- Definition: the study of the origin and development of an organism
- Prenatal period: before birth
  - **38 weeks** from conception to birth (average) "fetal" age
  - Gynecologic timing has been from LMP therefore refers to **40 weeks "*gestational*" age**
    - Date of conception has been difficult to time
    - LMP is on average two weeks before ovulation



EVENTS IN THE FEMALE GENITAL TRACT

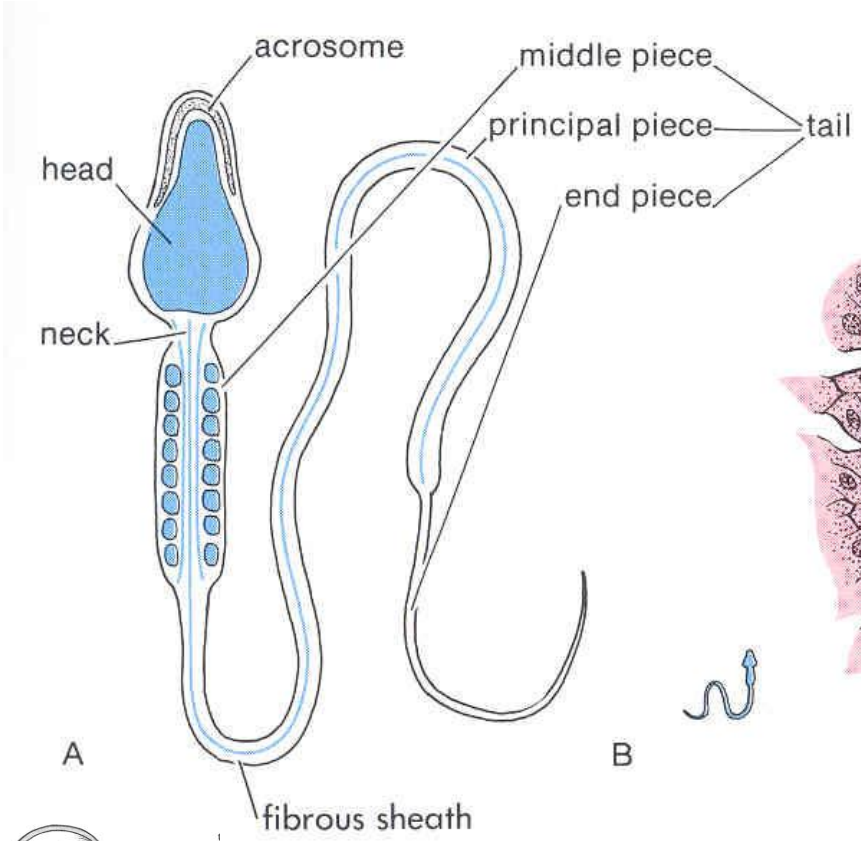


HUMAN DEVELOPMENT TIMELINE

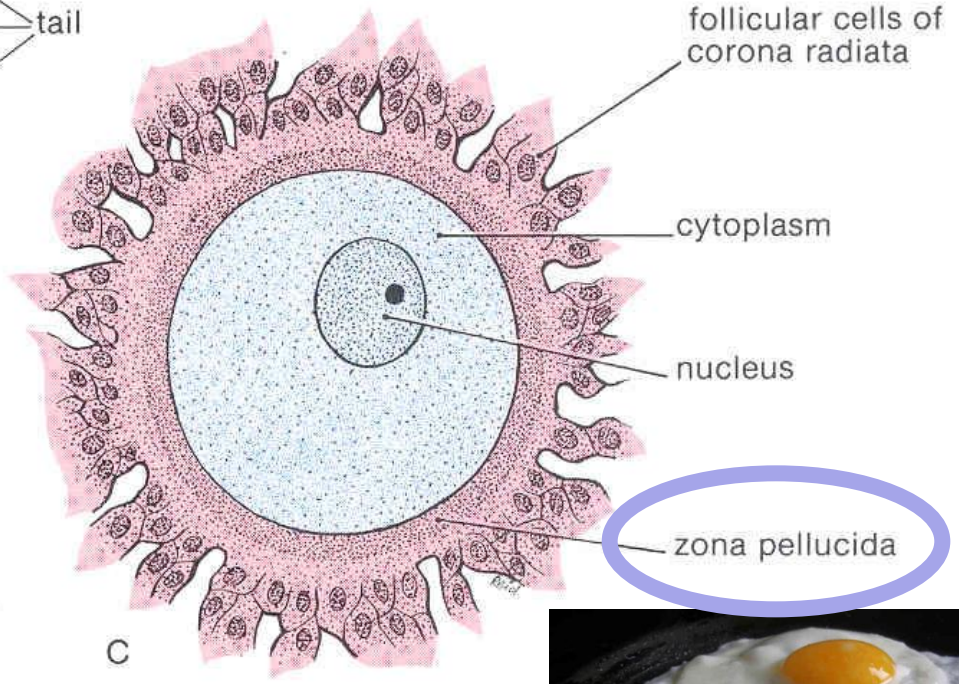


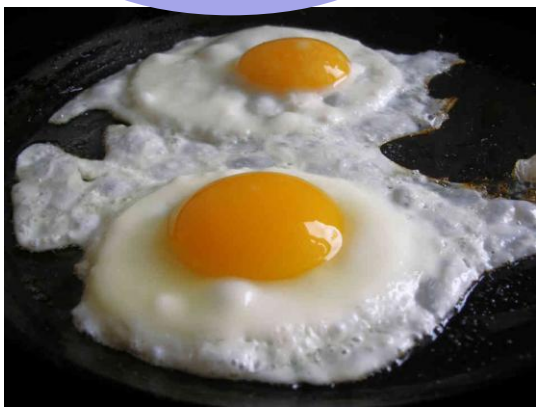
HUMAN GAMETES

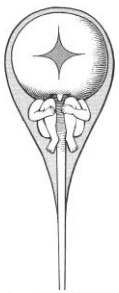
SPERMS or *SPERMATOZOA*



OVUM or OOCYTE







Historical idea of spermatic components

1–6. Copy of a seventeenth century drawing of a sperm by eker. The miniature human being within it was thought to e after the sperm entered an ovum. Other embryologists at

GAMETOGENESIS - MEIOTIC DIVISION

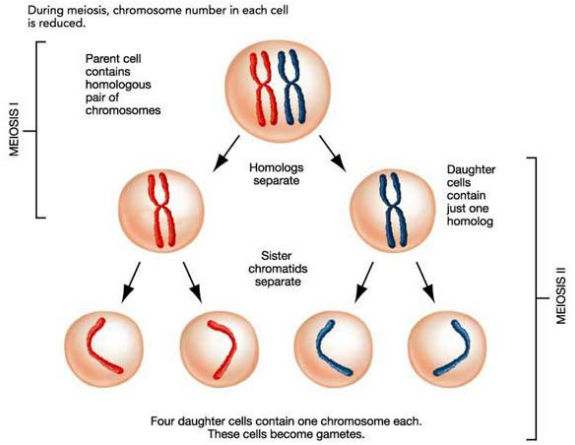
- takes place in germ cells to generate male and female gametes
- reduces the number of chromosomes to the haploid number of 23

meiosis I

- spermatocytes and primary oocytes replicate their DNA (**duplication)**
- homologous chromosomes align themselves in pairs (synapsis)
- the pairs separate into two haploid daughter cells

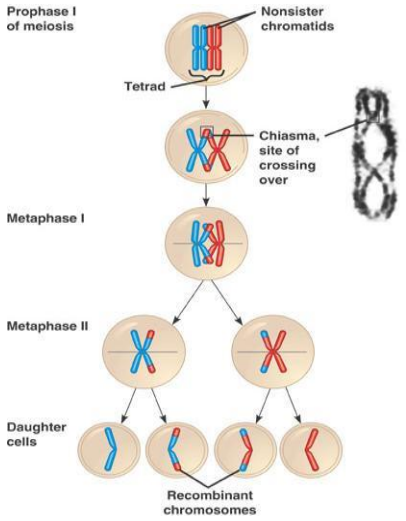
meiosis II

- sister chromatids separate (23 single chromosomes)



„Crossing over" **(**meiosis I)

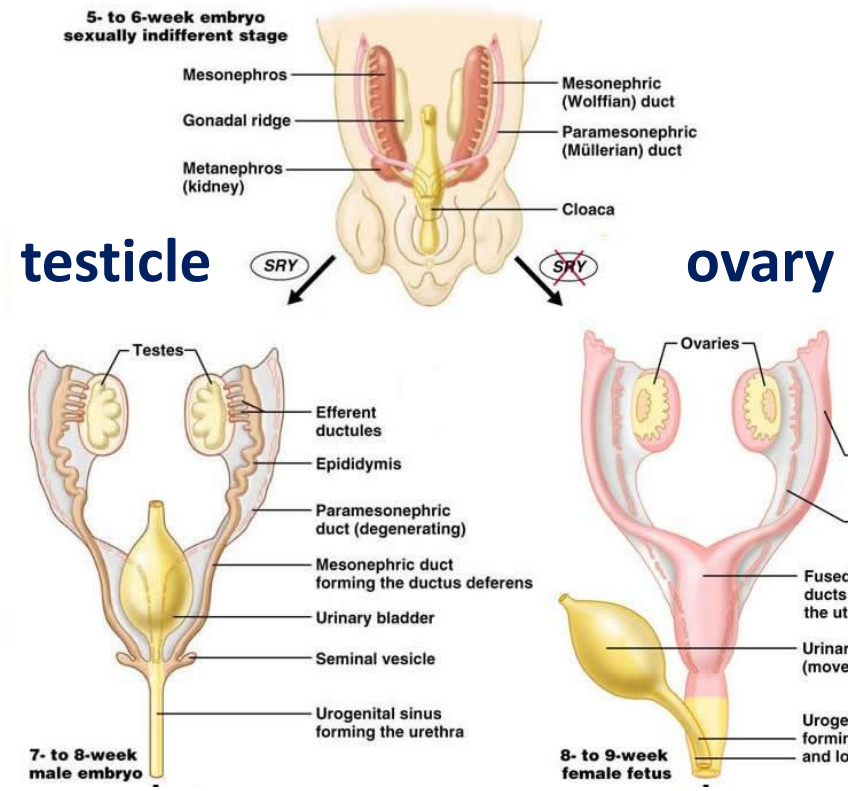
interchange of chromatid segments between
paired homologous chromosomes
Temporary unification between chromosomes (chiasma)



Genetic variability is enhanced through

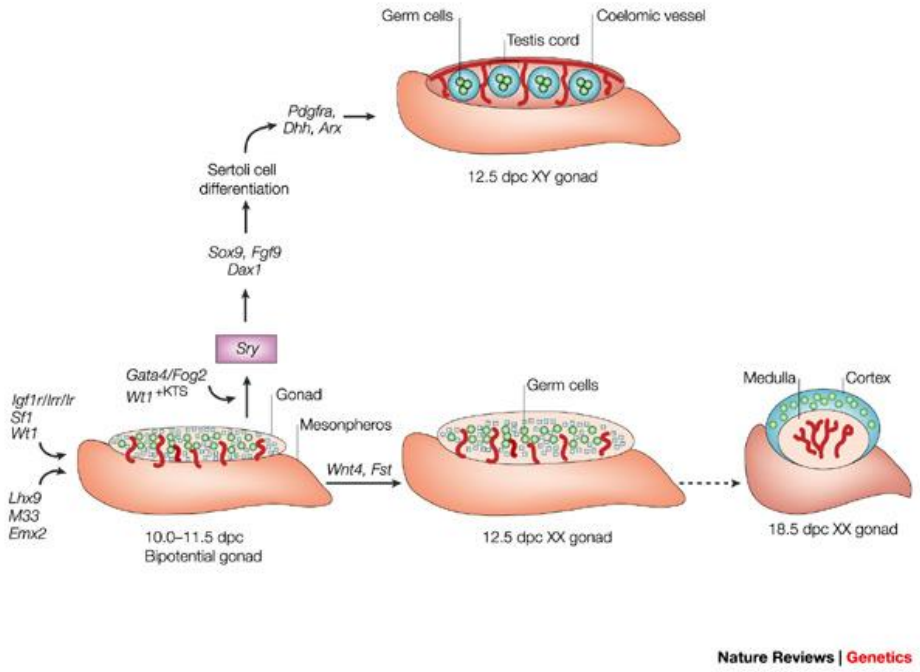
- crossover, which redistributes genetic material
- random distribution of homologous chromosomes to the daughter cells
- Each germ cell contains a haploid number of chromosomes, so that at fertilization the diploid number of 46 is restored.

GAMETES ARE PRODUCED IN GONADS



GONADS develop from an
- indifferent framework
- primordial germ cells

SRY



It has all been decided well before birth

SPERMATO- AND SPERMIOGENESIS

SPERMATOGENESIS

2 STAGES

SPAGHETTI

PRENATAL

migration of stem cells into the mesodermal primordium from celom

MACARONI

POSTNATAL

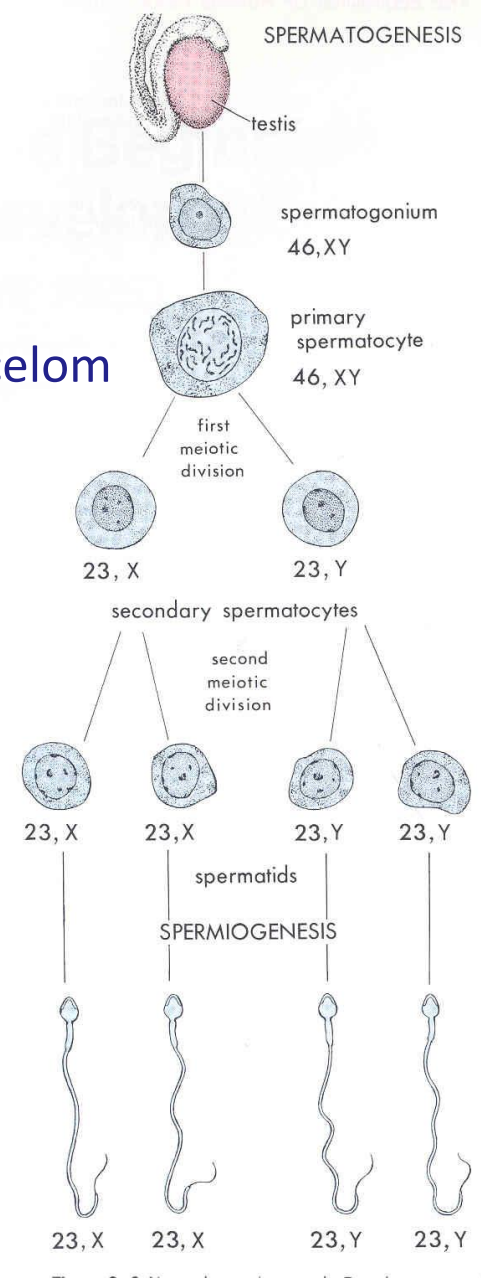
begins with the onset of puberty

-*spermatogonia* – mitotic divisions

-enlargement – *primary spermatocyte*

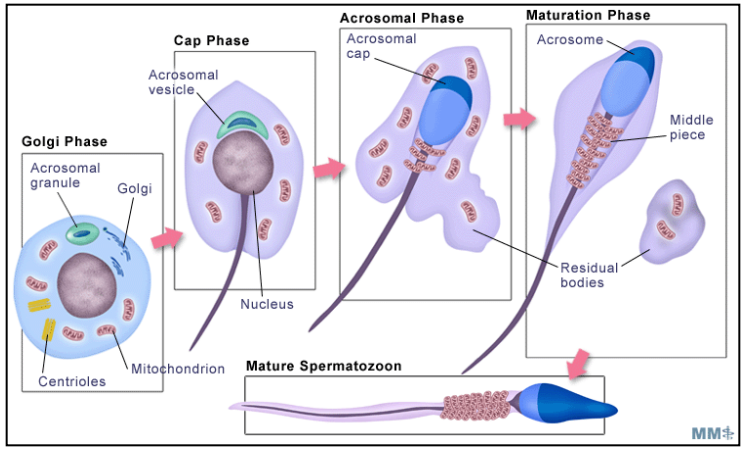
-1st meiotic division – *secondary spermatocyte*

-2nd meiotic division – *spermatids*

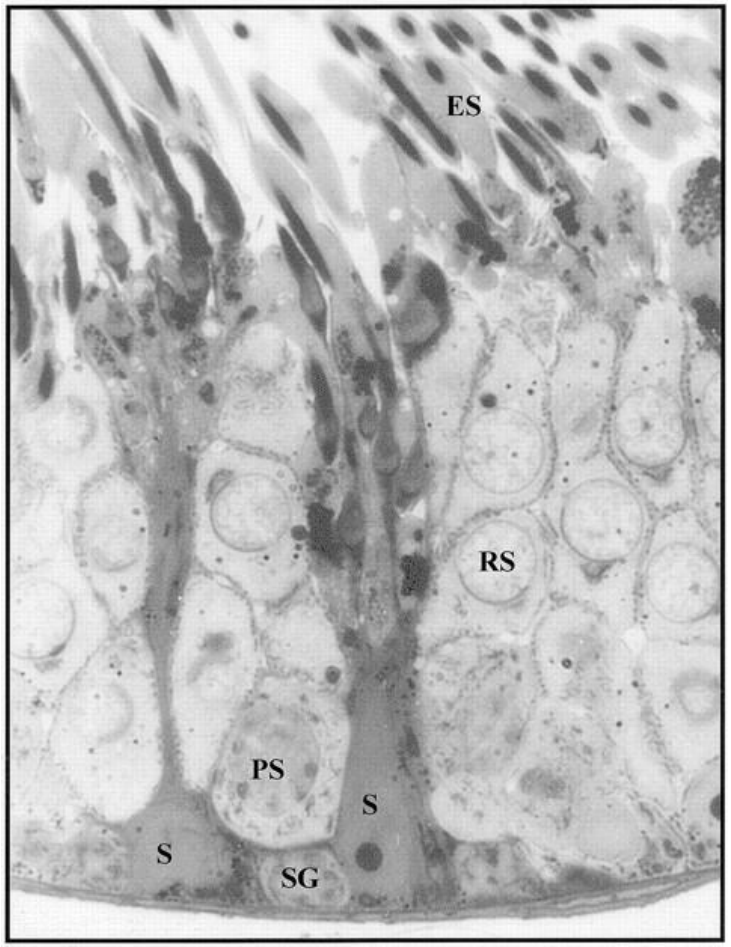


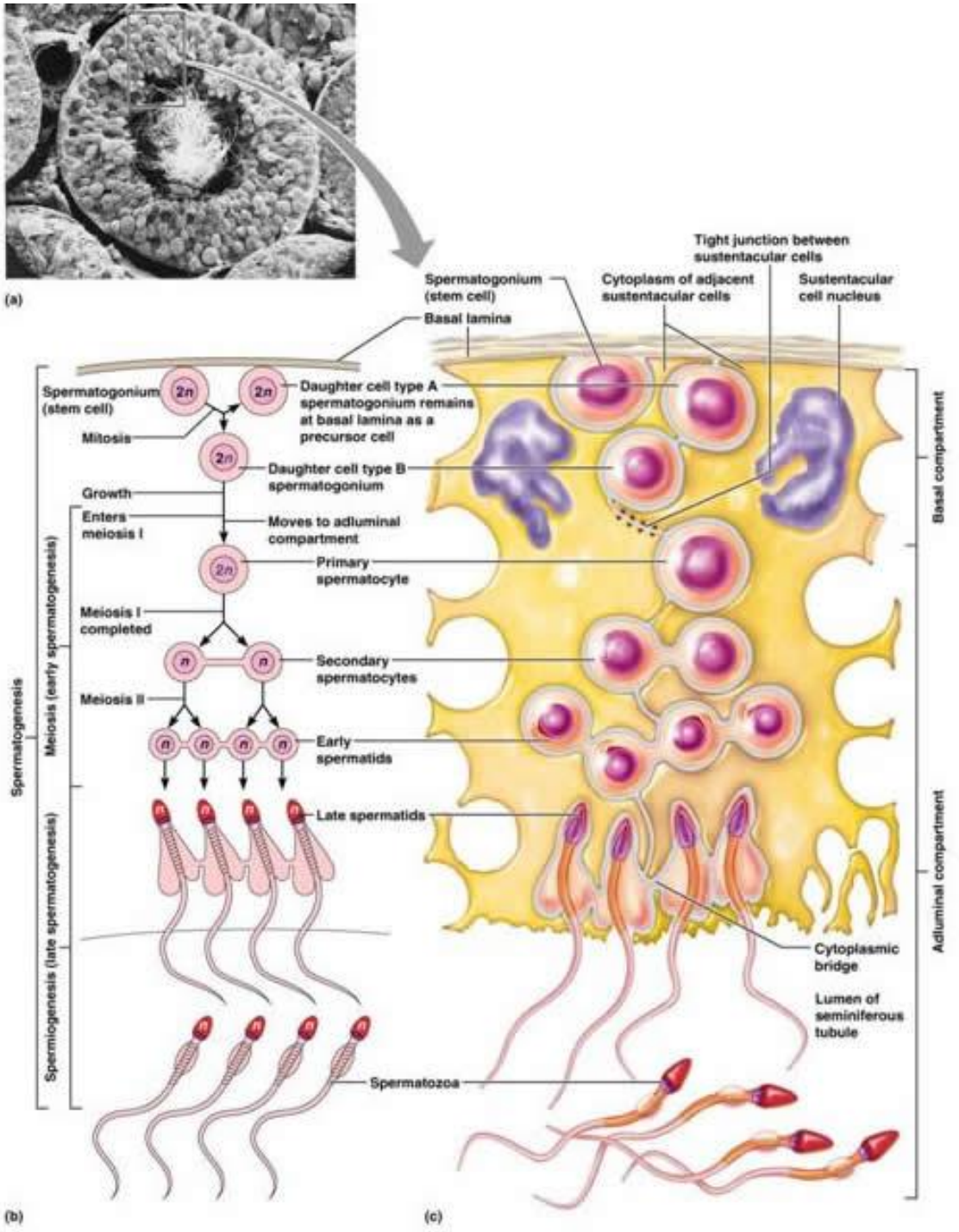
SPERMIOGENESIS

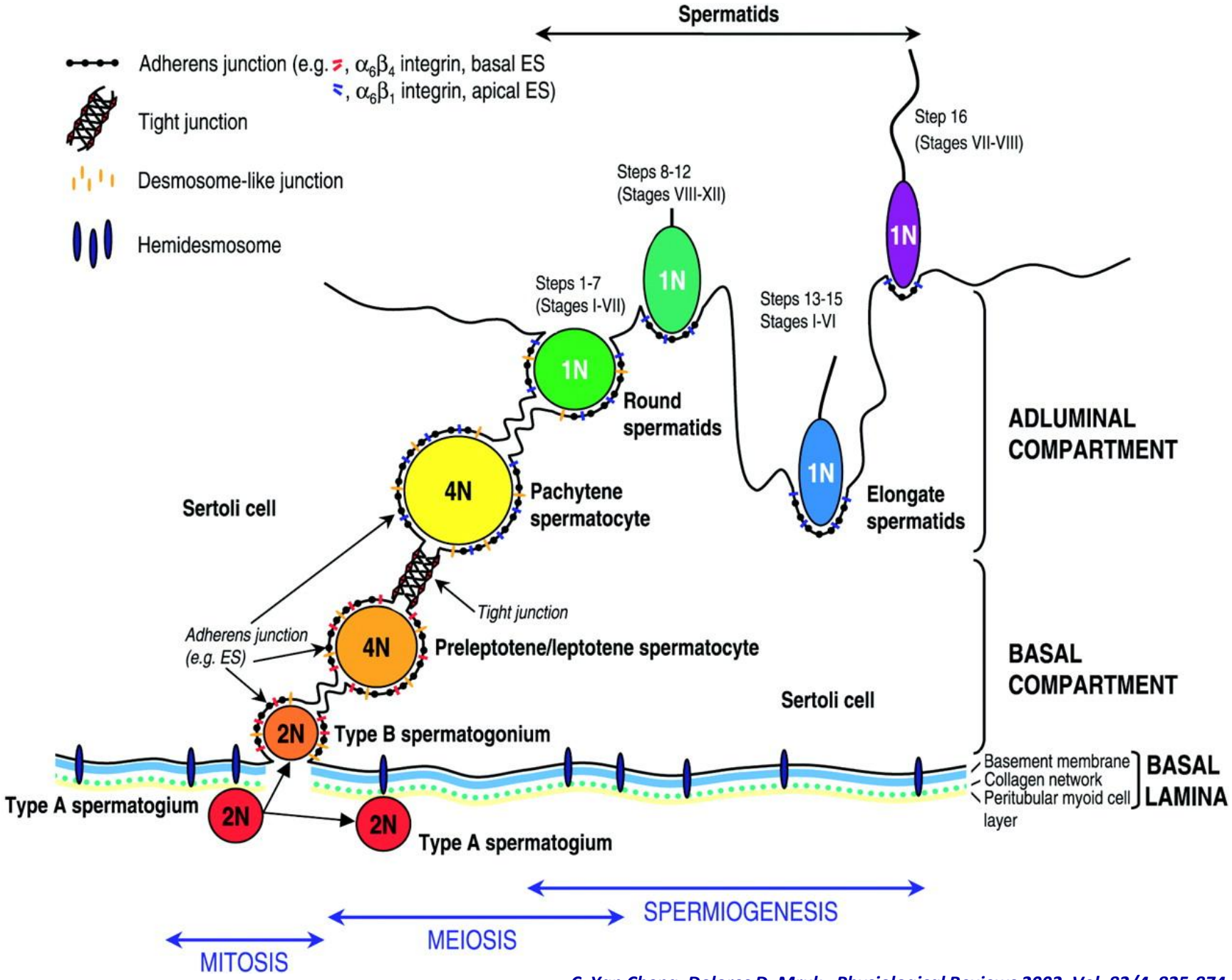
maturation of spermatids to form sperms „within” the **SERTOLI** cells of the testicle



SPERMATO-AND SPERMIOGENESIS

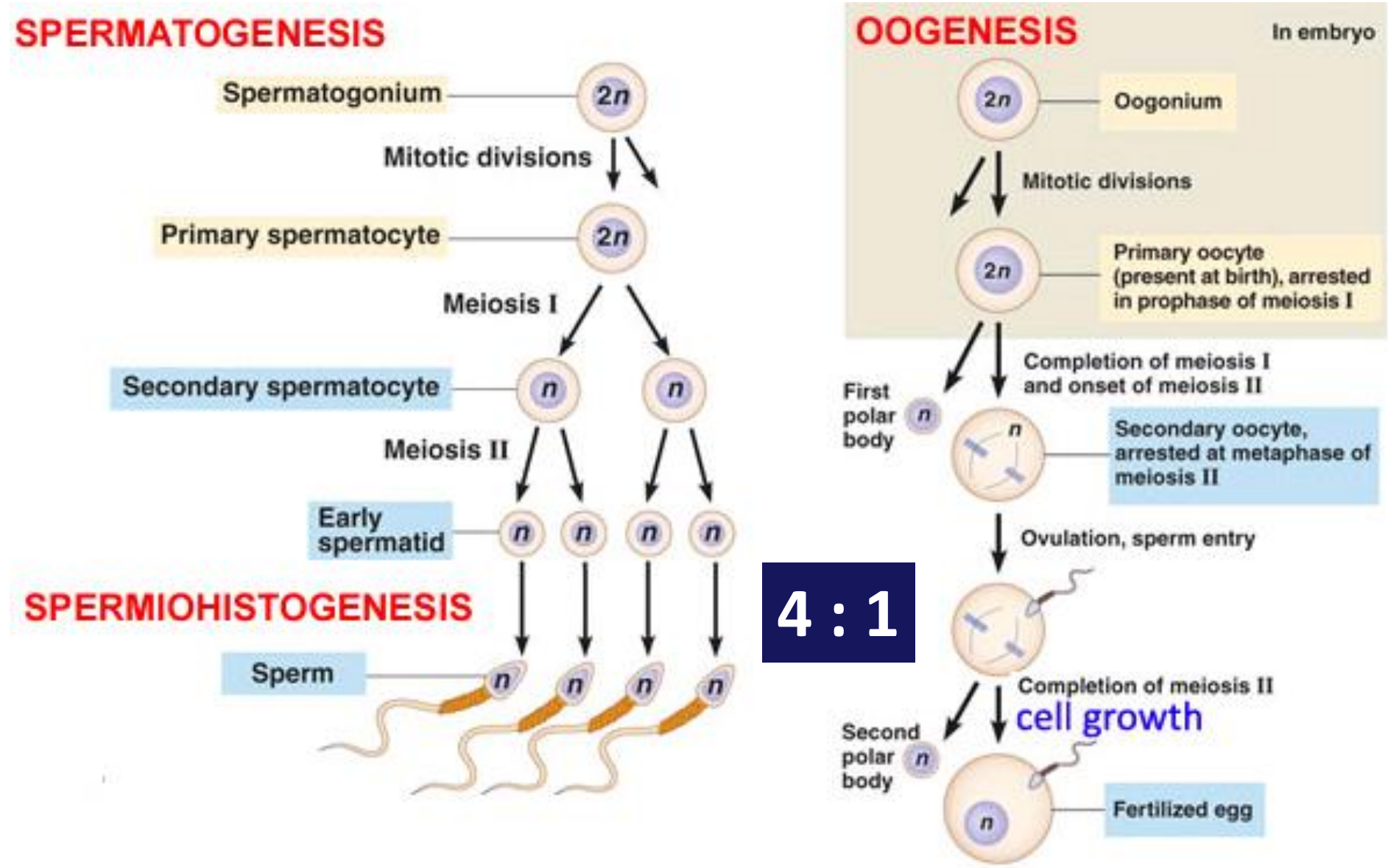






C. Yan Cheng, Dolores D. Mruk , Physiological Reviews 2002. Vol. 82/4, 825-874

MAJOR DIFFERENCE IN THE NUMBER OF ACTIVE DAUGHTER CELLS



STAGES OF OOGENESIS

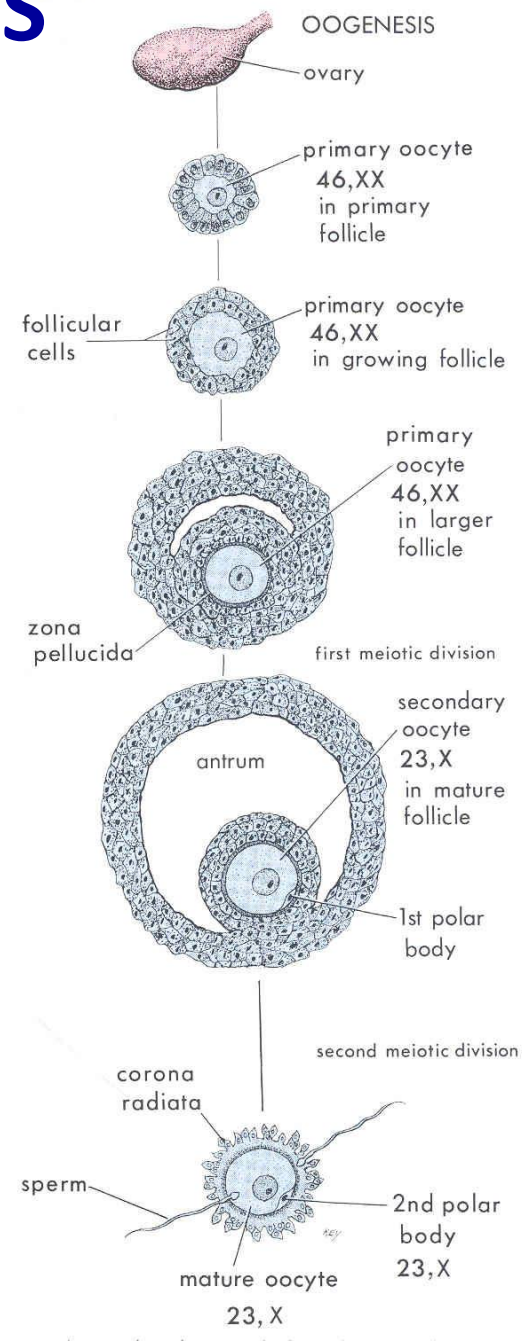
PRENATAL

- serial mitotic divisions
- enlargement (*primary oocyte in a primordial follicle*)
- 1st meiotic division interrupted in the ***prophase***

POSTNATAL

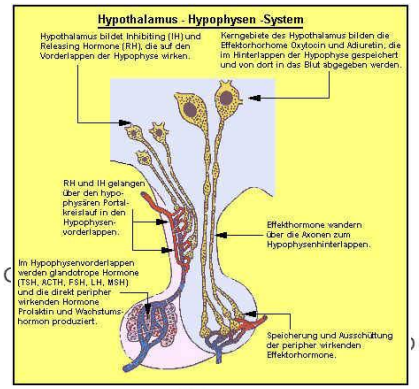
-in puberty starts the further ripening of the oocytes (*primary oocyte in primary, then secondary follicle*)
-completion of the 1st meiotic division
-formation of a ***polar body*** (soon degenerates)
(*secondary oocyte in a tertiary **GRAAFIAN** follicle*)

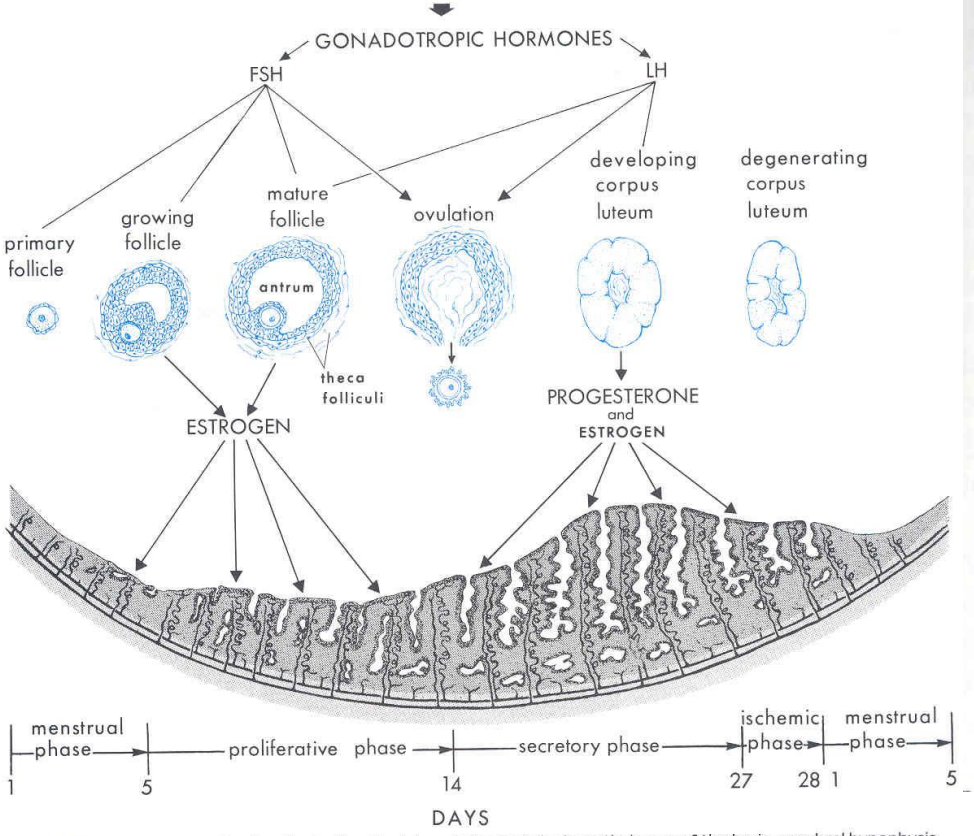
-**OVULATION** – 2nd meiotic division stops in ***prophase***
-fertilization - penetration of the sperm
-completion of the 2nd meiotic division
(formation of a ***polar body*** (degenerates)



THE FEMALE MENSTRUAL CYCLE

HORMONES





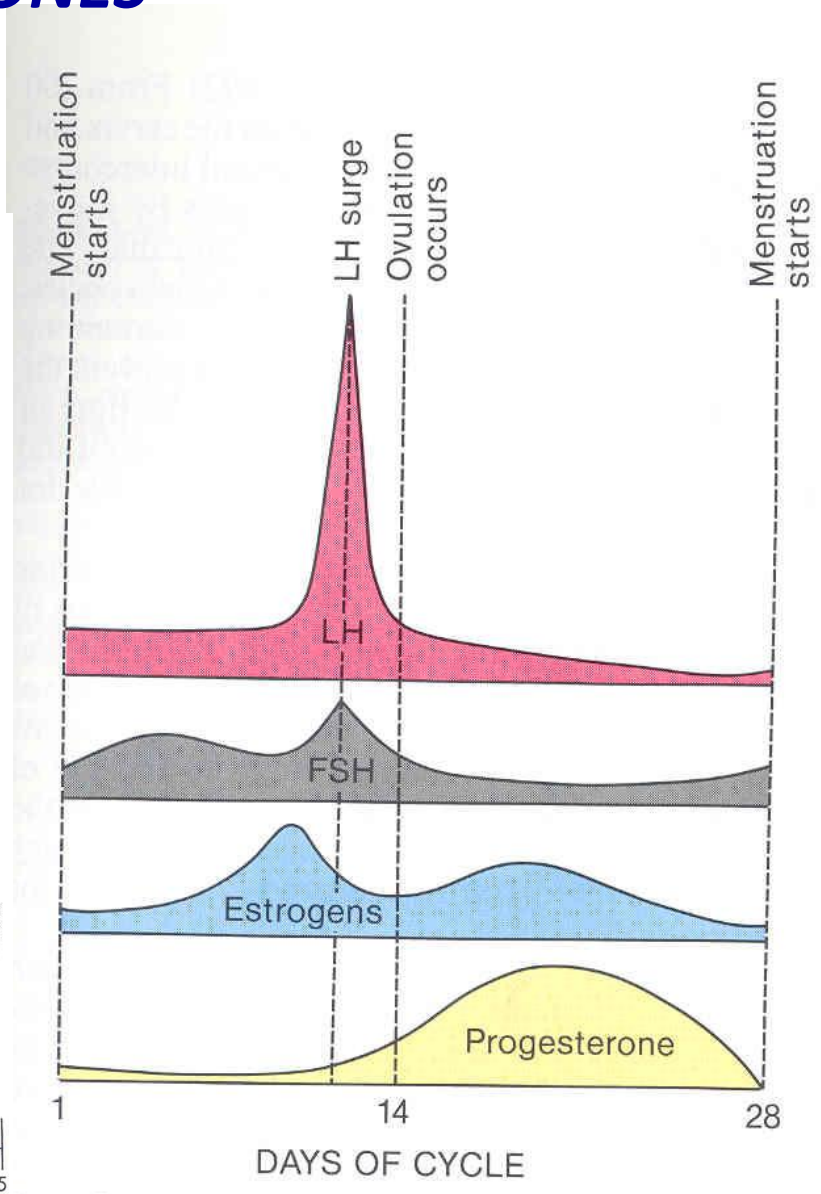
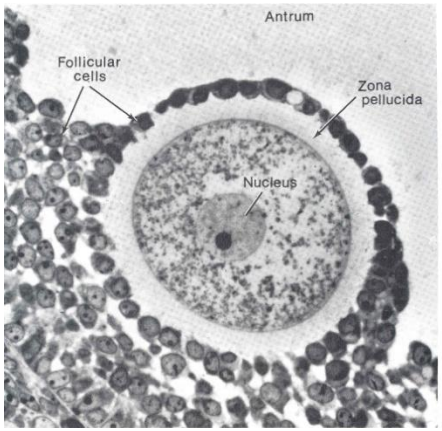
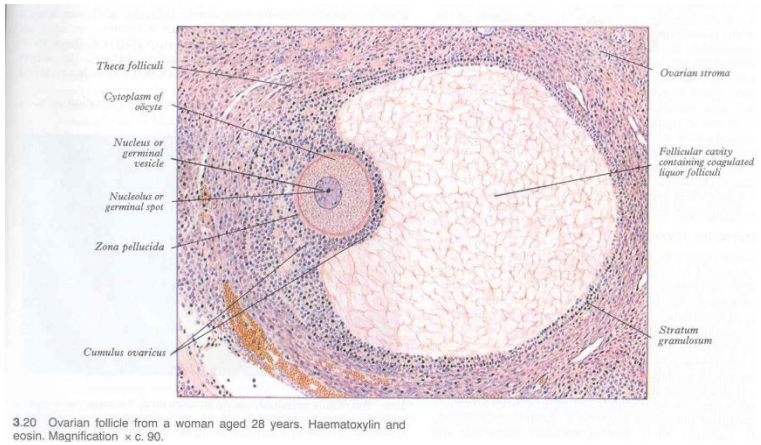


Figure 2–6 Schematic drawing illustrating the interrelations of the hypothalamus of the brain, cerebral hypophysis

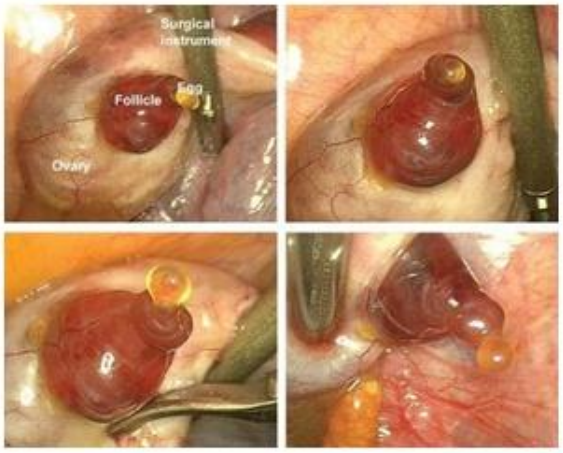
THE FEMALE MENSTRUAL CYCLE

OVULATION

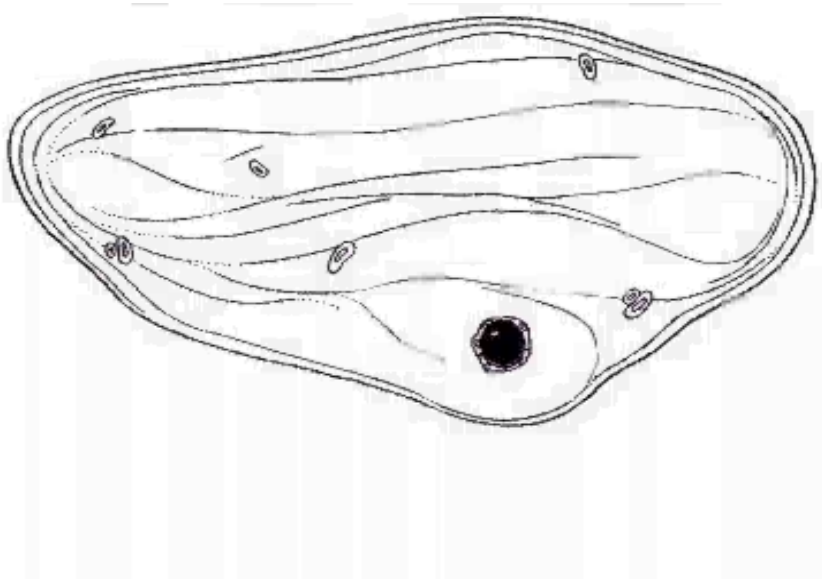


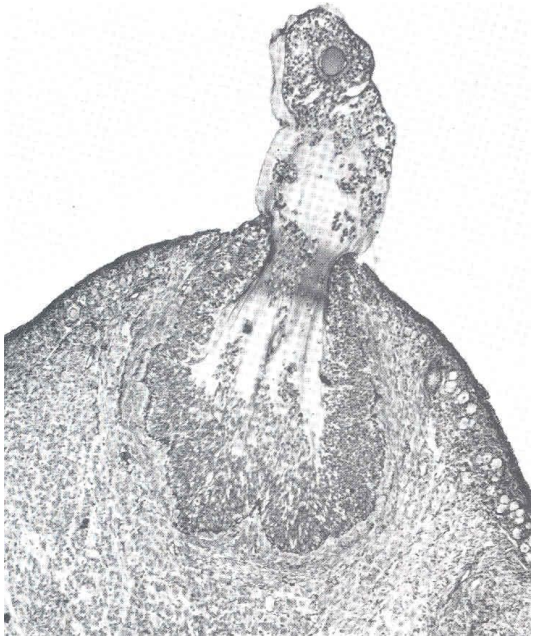


3.20 Ovarian follicle from a woman aged 28 years. Haematoxylin and eosin. Magnification × c. 90.



Picture of Egg being ovulated from an ovary





12-24 hours from now on

CONCEPTION (*FERTILISATION*)

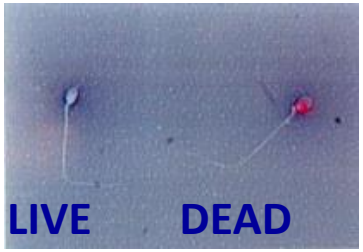
the fusion of gametes to produce

a new organism

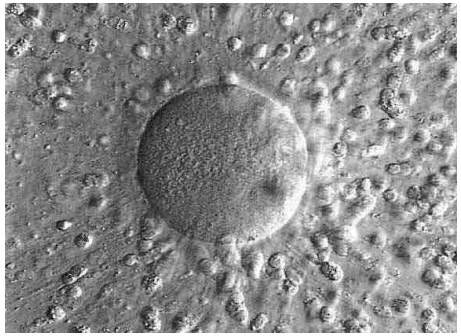
of the same species

FERTILISATION

EJACULATE (3.5 ml)
10% sperms (200-600 M/ml)
50% seminal fluid (*coagulation*)
30% prostatic secrete (*fructose*)
10% Cowper's (*rinsing, lubrication*)



MATURE EGG



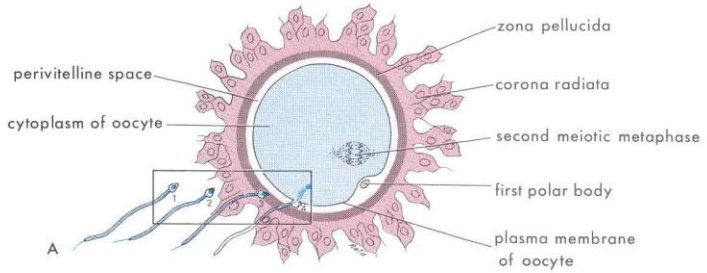
IMMATURE EGG

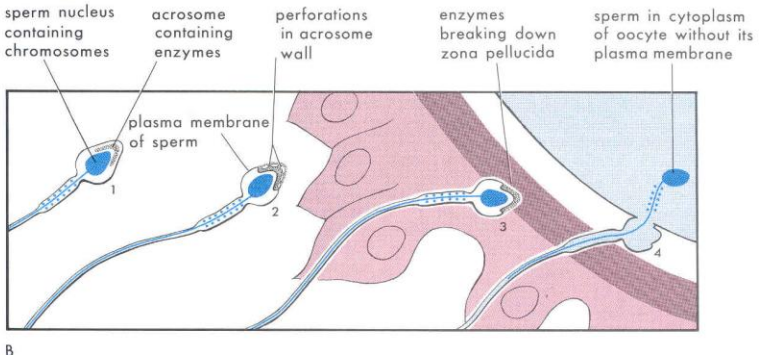
FERTILIZATION begins when a sperm penetrates an oocyte, the entire process takes cca 24 hours

- sperm survival: up to 5 days in FERTILE MUCUS

- only 1% (3 million) enter the uterine cavity, even less will reach the tubes

-takes about ten hours to reach the *fallopian tube*





1 oocyte VERSUS 300 million sperm cells

STAGES OF FERTILISATION

Capacitation (takes about 7 hours, the acrosome is denuded – conditioning)

Penetration of the Corona radiata

Acrosomal reaction (lytic enzymes are secreted in reponse to contacting the corona radiata)

Binding – specific binding between the membranes

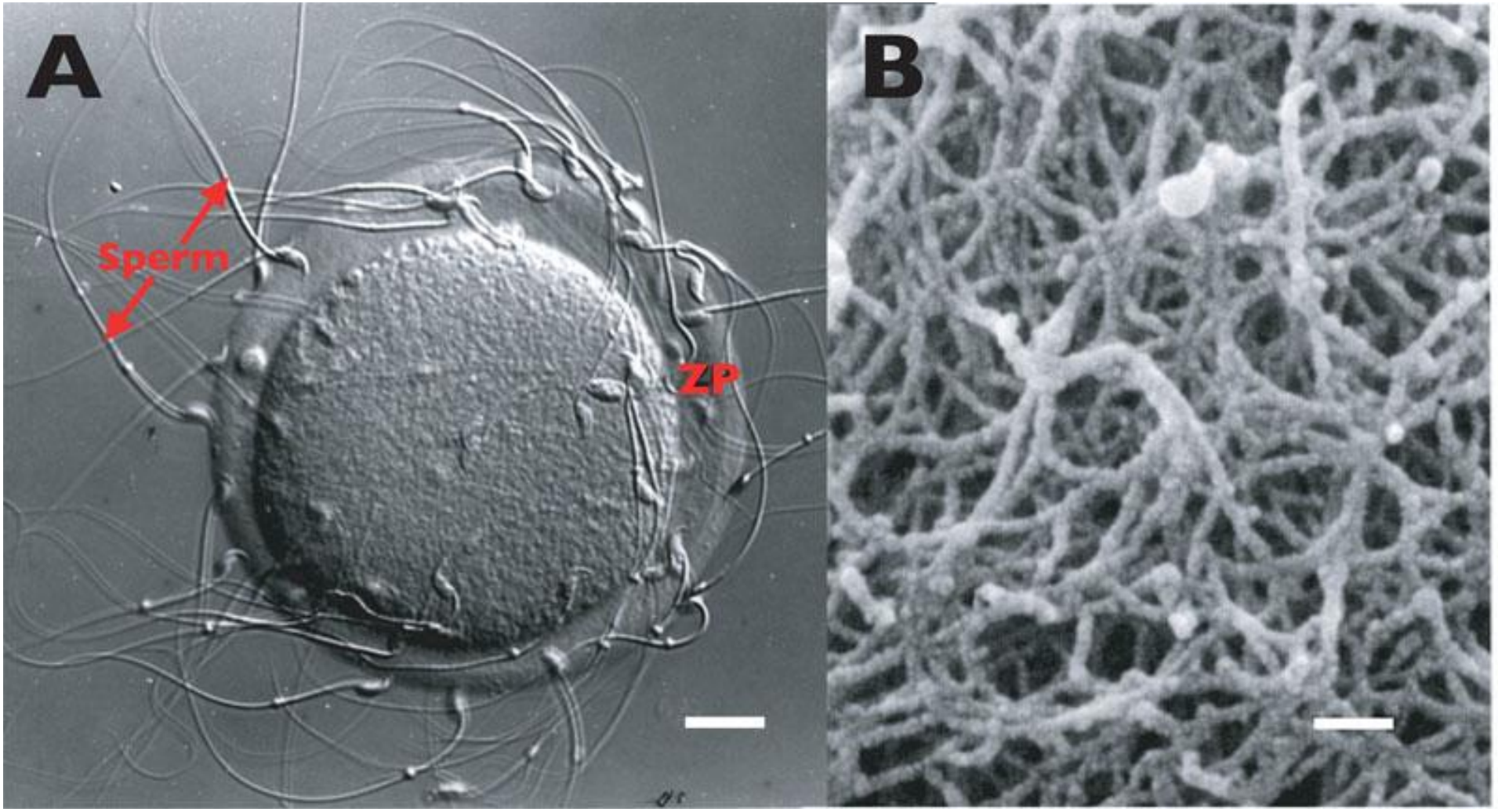
Penetration of zona pellucida and the vitelline membrane (takes about twenty minutes)
-enzyme reaction triggered

Zona reaction (retraction of the vitelline membrane from the zona pellucida)

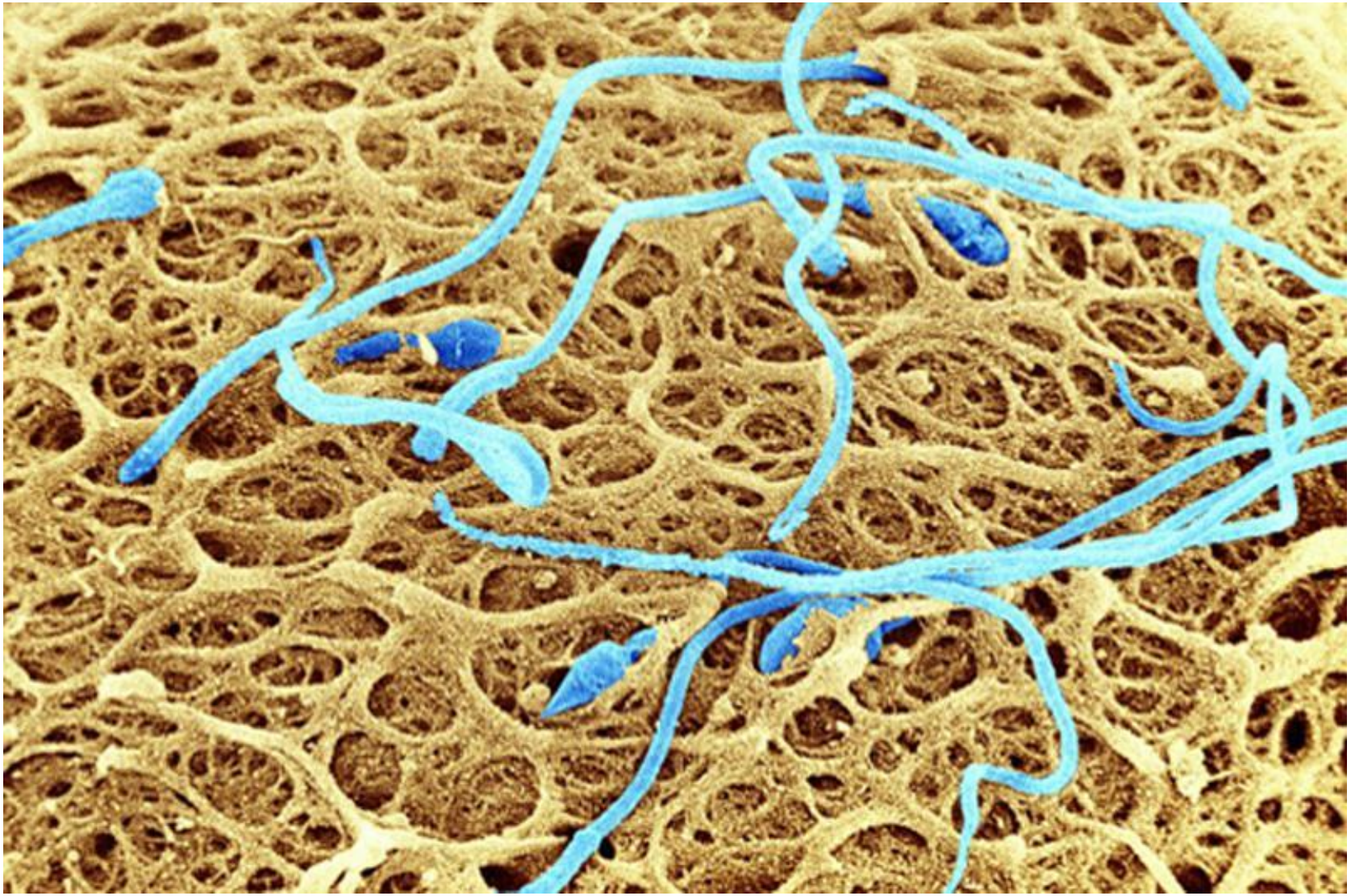
Within 11 hours a polar body is formed

Preembryo formation
-fusion of the nuclei:
-creation of the zygote

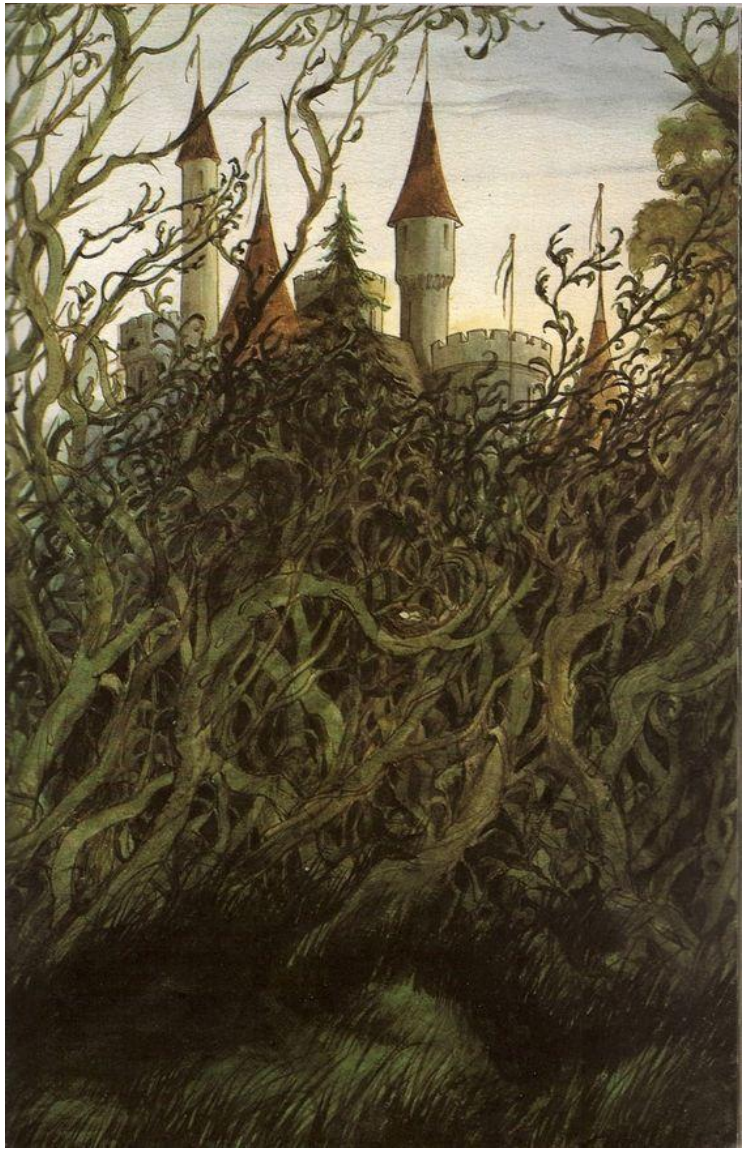
WHAT IS THE ZONA PELLUCIDA?

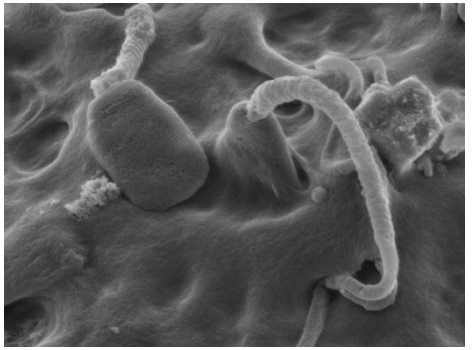


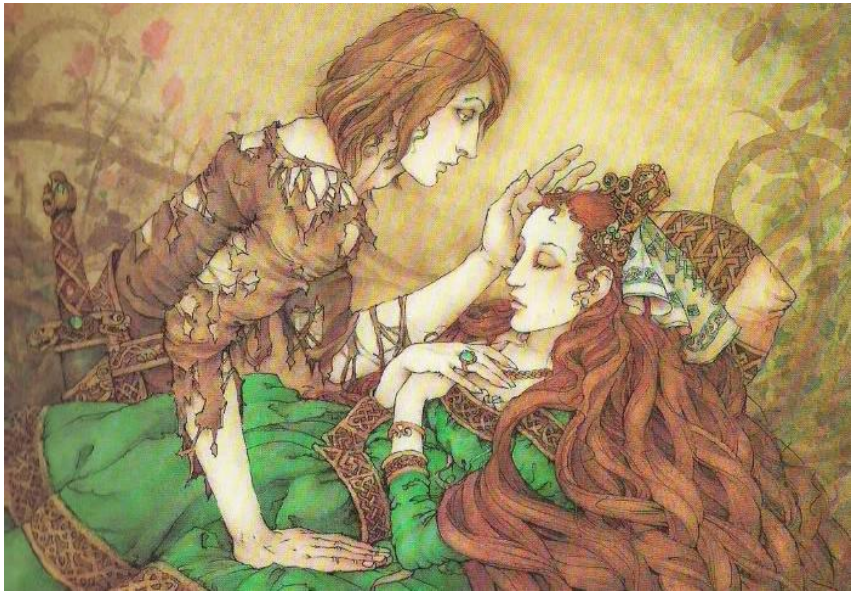
THE ZONA PELLUCIDA PREVENTS POLYSPERMIA



THE „*SLEEPING BEAUTY*” EFFECT







ZONA PELLUCIDA

A glycoprotein meshwork surrounding the plasma membrane of mammalian oocytes.

- first appears in unilaminar primary oocytes
- secreted by both the oocyte and the follicular cells

Electron microscopic studies have shown a variety of appearances from a porous, net-like structure to a nearly smooth and compact structure.

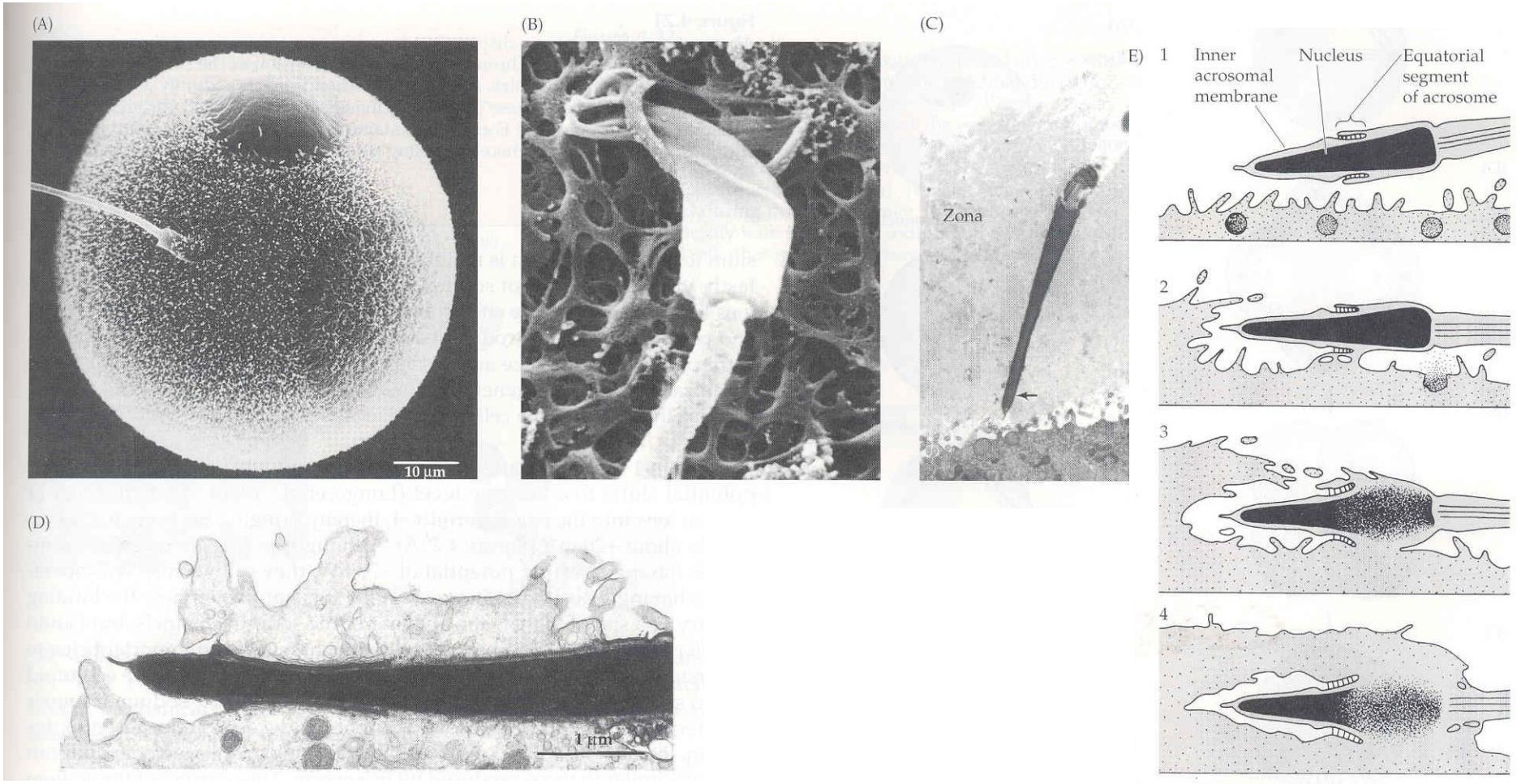
A scanning electron microscopic review of zona pellucida structures at ovulation show: spongy ZP appearance well correlates with mature oocytes.

- a delicate **meshwork** of thin interconnected filaments
- a **regular alternating pattern** of wide and tight meshes.
  - ***wide*** meshes correspond to "pores" of the "spongy" ZP.
  - ***tight*** meshes correspond to the compact parts of the ZP surrounding the pores.

ZONA PELLUCIDA GLYCOPROTEINS *bind to capacitated spermatozoa and induce the acrosome reaction. Successful fertilization depends on the ability of sperm to penetrate the extracellular matrix that surrounds the egg.*

ZP3 allows species-specific sperm binding
ZP2 mediates subsequent sperm binding
ZP1 cross-links ZP2 and ZP3.

THE ELECTRON MICROSCOPY OF FERTILISATION



PHASES OF FERTILIZATION

Capacitation

the spermatic membrane is rigid (***cholesterol***) and contains glycoproteins, e.g. **DF-R** (*decapacitating factor receptor*) binding to membrane stabilizing factors.

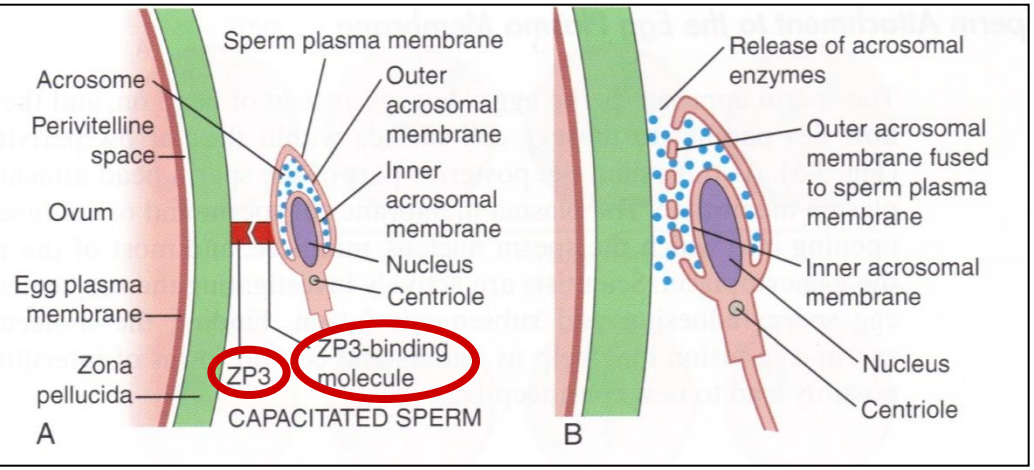
FPP (*fertilization promoting factor in prostatic discharge*) and **heparin** (from ***endometrium***) breaks down the rigid membrane compartments, thus leads to more flexibility at the acrosome, as well as inducing a higher **Ca++ influx** (necessary for motility and the acrosomal reaction)

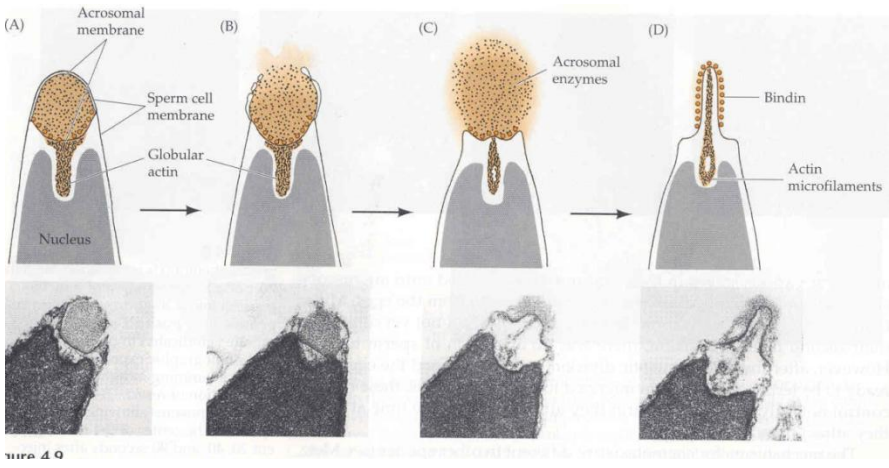
Corona penetration

The cells of the corona radiata are embedded in a **hyaluronic acid rich ECM**
Hyluronidase and **CPE** (enzymes) are mainly responsible for penetration

Acrosomal reaction

(lytic enzymes are secreted in response to contacting the corona radiata)

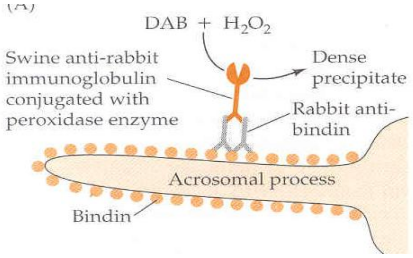


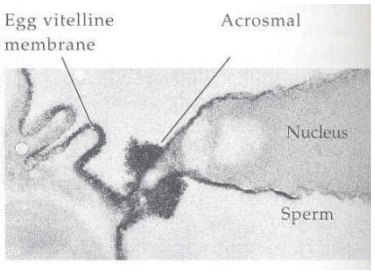


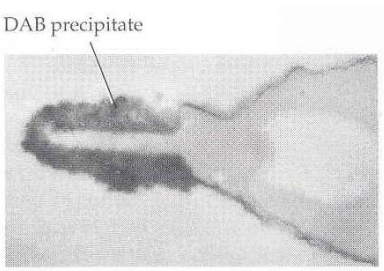
PHASES OF FERTILIZATION

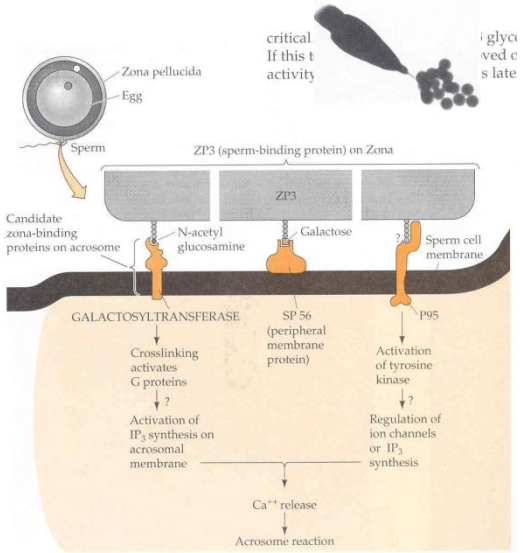
Binding

between *sperm* and the *zona pellucida*, then the acrosome fuses with the vitelline membrane)









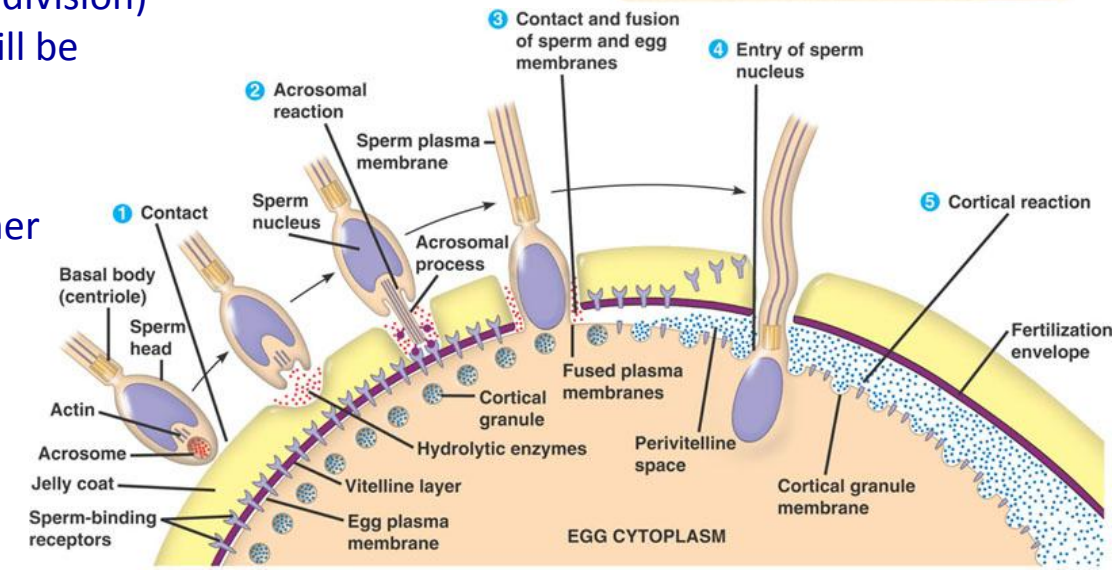
Fusion and Zona reaction

The membrane of the sperm (***fertilin***) fuses to the ***integrins*** of the vitelline membrane - male centriole enters the oocyte (regulates further cell division) Mitochondria may enter too but soon will be sequestered in a phagosome

exocytosis of cortical granules
(to break down **ZP3** and to prevent further sperms from docking)

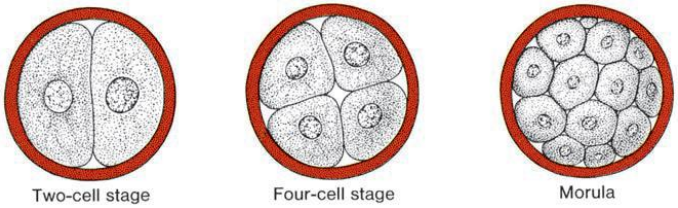
osmotic pressure rises and the perivitelline space fills with water

membrane composition changes to ***prevent polyspermia*** (docking)

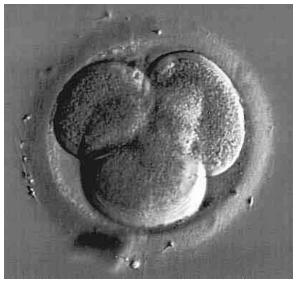


CLEAVAGE (*SEGMENTATION*)

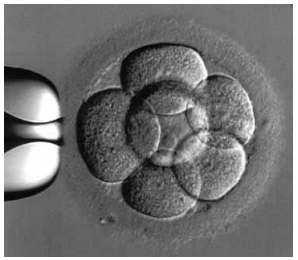
1.5 - 3 days post-ovulation
mitotic divisions
0.1 - 0.2 mm

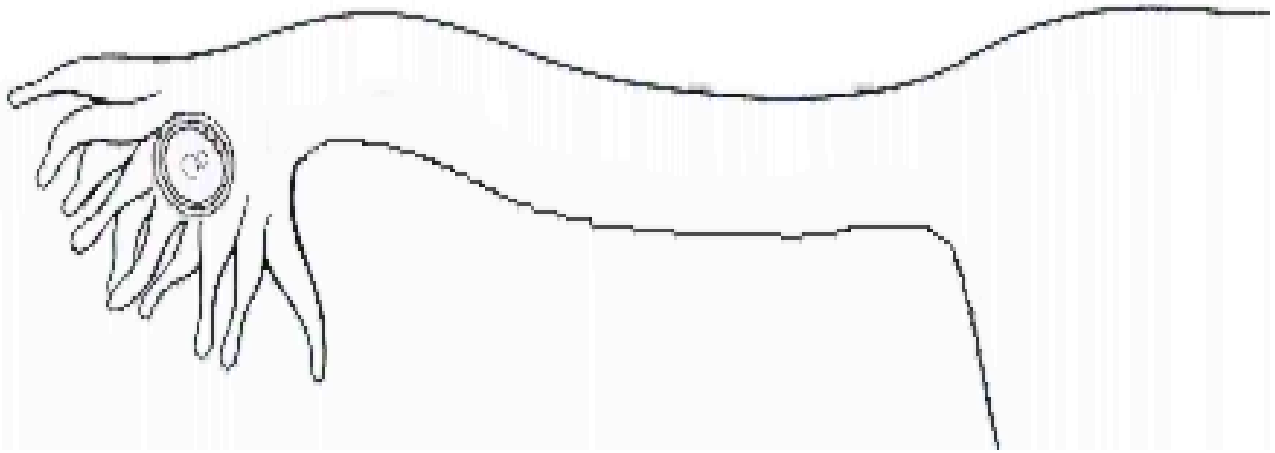


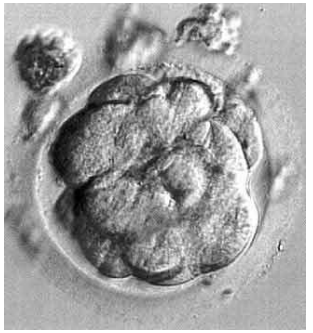
ZYGOTE - begins to cleave, with each division producing twice as many cells (*blastomeres*) approximately every twenty hours



MORULA - sixteen cells, it leaves the fallopian tube and enters the uterine cavity on day 3-4.



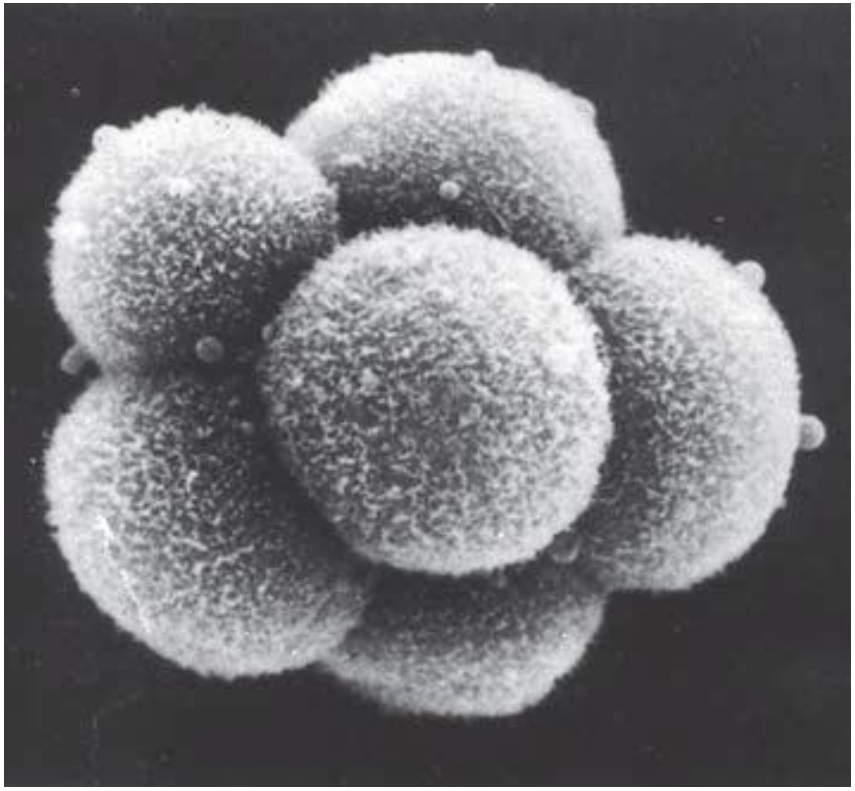


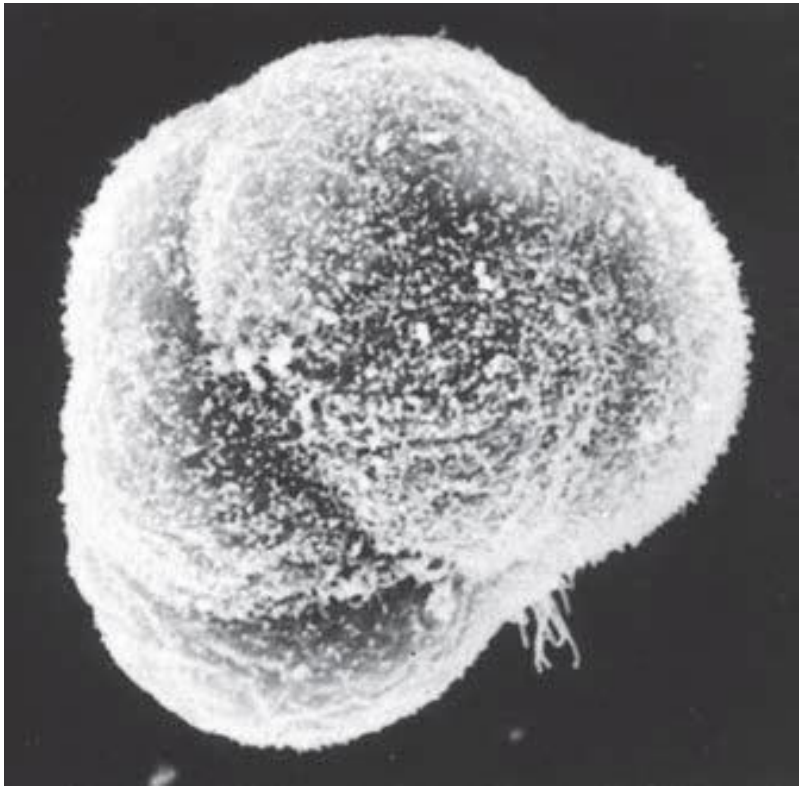


MORULA

BEFORE AND AFTER

COMPACTING

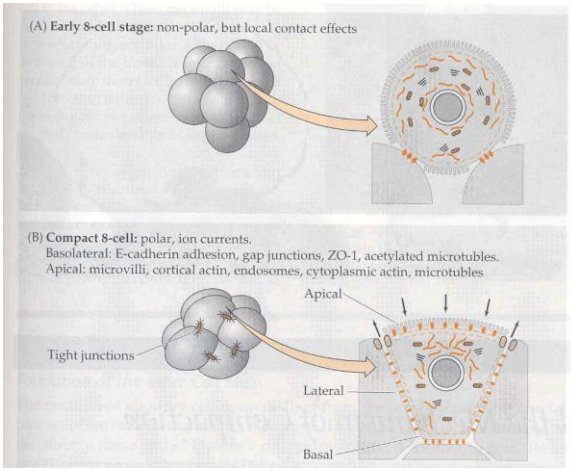


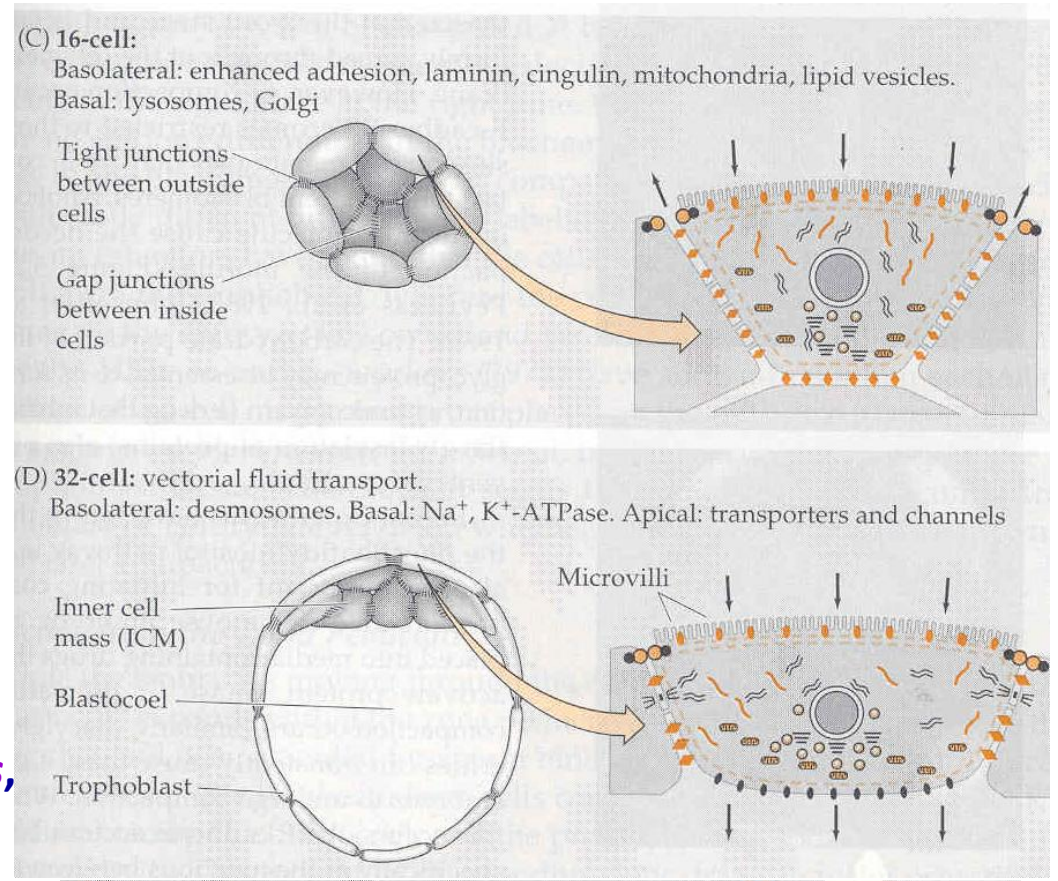


WHAT HAVE WE LEARNED SO FAR?

- **Capacitation** (it is imperative for fertilization – „maturation" – happens in the isthmus of the oviduct)
- **Chemotaxis** (ovulation releases certain substances: progesteron and atrial natriuretic factors in the follicular fluid – hyperactivity of sperms)
- **Recognition of the oocyte** (first at the **zona pellucida**, galactosyl transfrease in the sperm head binds to ZP3)
- **Acrosomal reaction** (fertilin is located in the back part of the sperm head so it is not removed douring the reaction)
- **Docking** (2nd recognition to follow : binding between ***tetraspannin*** (oocyte) and ***Izumo*** (sperm) proteins)
- **Inhibition of polispermia** (cortical degranulation: glycosidases and ovoperoxidases harden the zona pellucida + immediate Ca flux)
- **Paternal chromatin and centriole** may only enter the oocyte
- **Cleavage** = serial mitotic divisions (1 – 2 – 4 – 8)
- **Differentiation** (compacting etc.)

ADHESION MOLECULES





The blastomeres are bound to each other by *outer* and *inner* layers of structures.

The *outer* layer contains *Na ion pumps*, whereas *internally* liquid will accumulate and aids the cavity (blastocoel) formation).

„Pumping" blastocyst

CAVITATION WITHIN THE MORULA

4 days post-ovulation
early blastocyst formation
0.1 - 0.2 mm

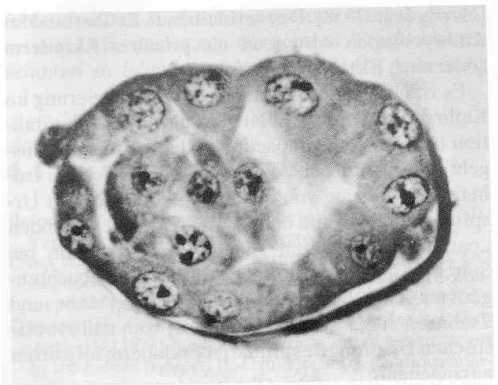


Abb. 4-8. Frühe Blastocyste im lichtmikroskopischen Schnittbild (Carnegie 8794). Beginnende Konfluenz der Interzellularräume
(Mit freundlicher Genehmigung von Prof. O'Rahilly, Carnegie Collection of Embryology, Davis)

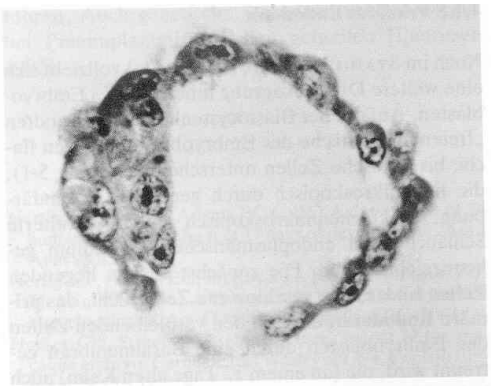


Abb. 4-9. Schnitt durch eine noch freie 107-Zellen-Blastocyste (Carnegie 8663). Embryoblastzellen mit etwas größeren Zellkernen werden vom polaren Trophoblasten, die Blastocystenhöhle ist vom muralen Trophoblast umschlossen.
(Mit freundlicher Genehmigung von Prof. O'Rahilly, Carnegie Collection of Embryology, Davis)

MORULA

- reaches the uterine cavity
- cell division continues

BLASTOCYST

-cavity (blastocoel) formation
-cells flatten and compact

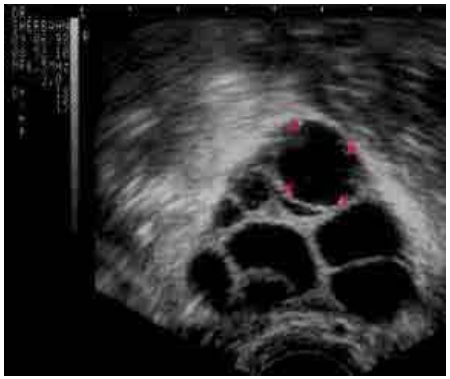
-zona pellucida remains the same size

-two cell types

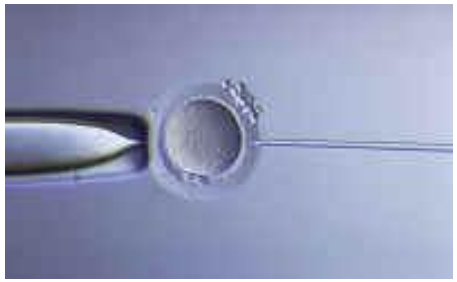
- embryoblast (inner cell mass)
- trophoblast (outer layer)

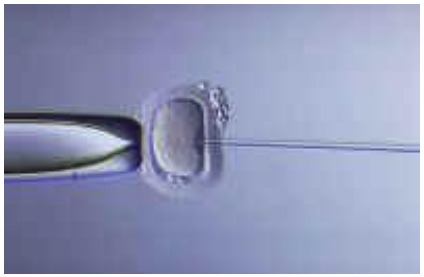
‘*IN VITRO*’ FERTILIZATION

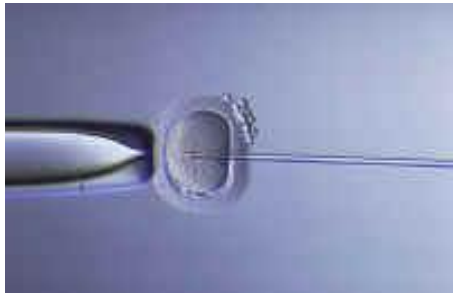
ovarian stimulation



ICSI – intrcytoplasmatic sperm injection







embryo biopsy

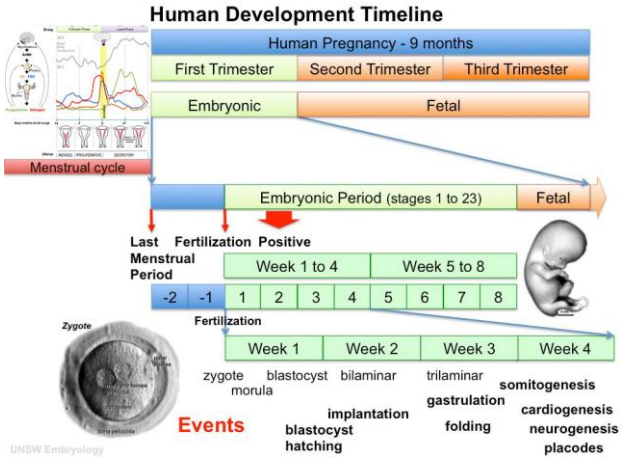
assisted hatching

oocyte retrieval

How did it happen?

A Washington couple took a paternity test after their son's blood type didn't match that of either parent.

THANK YOU
FOR YOUR ATTENTION



http://www.visembryo.com/baby/index.html
http://embryo.soad.umich.edu/
www.advancedfertility.com

http://classes.midlandstech.edu/carterp/Courses/bio211/Chap27/Reproductive_System.html

http://physrev.physiology.org/content/82/4/825