WHO Collaborating Center for Research in Human Reproduction
Clinic for Infertility and Gynecological Endocrinology
University Hospital, Geneva, Switzerland

Gametogenesis

Dr Corinne de Vantéry Arrighi
Dr Hervé Lucas

Definition of gametogenesis

Stem cells =

Primordial Germ Cells (PGC)

↓

Gametes =

Spermatozoa = spermatogenesis

or

Oocytes = oogenesis

Sexual differentiation

Genesis of the two sexes depends on genetic differences

The genetic determinant of sex is on the Y chromosome

YES ← Y chromosome → NO

differentiation ↓

Male gonad = testis

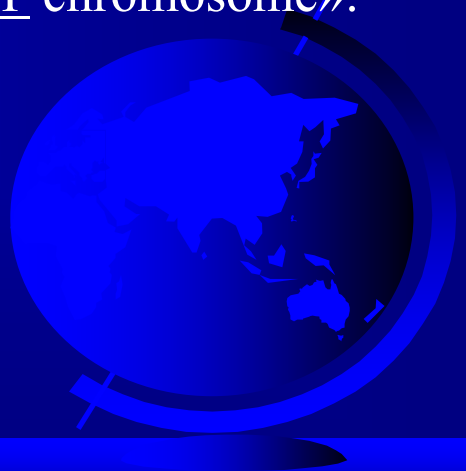
Female gonad = ovaries

Y chromosome says «make a testis»

Testis-determining gene = SRY «Sex-determining Region of the Y chromosome»:

- located on the short arm of the Y chromosome
- switching or controller gene regulating the expression of other downstream genes (= transcription factor)

Clinical cases: XX men (translocation)
XY women (deletion)



Developement of gonads

Gonads are made up:

- Somatic mesenchymal tissues = matrix of the gonad
- Primordial Germ Cells (PGC)

↓

3 weeks embryo: PGC are identifiable in the epithelium of the yolk sac

4 weeks embryo: PGC proliferation and migration from the yolk sac to the genital ridge

Indifferent gonads (not possible to differentiate them)

6 weeks embryo: PGC colonization is completed

6-8 weeks embryo: In male: Testicular pathway: expression of SRY

- Initial decision as to whether to make a testis or an ovary
- Initiation of gonad formation

    are dependent on the presence or absence of SRY activity

- Completion of normal gonad development

    is dependent upon the presence of a population of normal germ cells

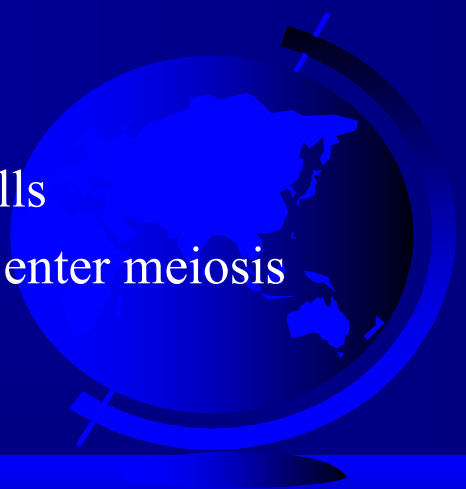
    2X for ovary

    1X for testis

Clinical cases:

Turner's syndrome (XO): ovary but death of oocytes and follicle cells

Klinefelter's syndrome (XXY): testis but germ cells die when they enter meiosis



Testis

- Testis
  - Interstitial tissue
    - Leydig cells → Androgens «Testosterone»
  - Tubular
    - Seminiferous cords/tubules
      - Sertoli cells → MIS/AMH «Müllerian-Inhibiting Substance» «Anti-Müllerian Hormone»
      - PGC → Spermatogonia → → → → Spermatozoa
    - Myoid cells

Ovary

Ovary

- Interstitial tissue = interstitial glands set in stroma
- Follicles
  - Theca cells → androgens
  - Granulosa cells → estrogens
- PGC → Oogonia → Oocytes

Spermatogenesis

Definition

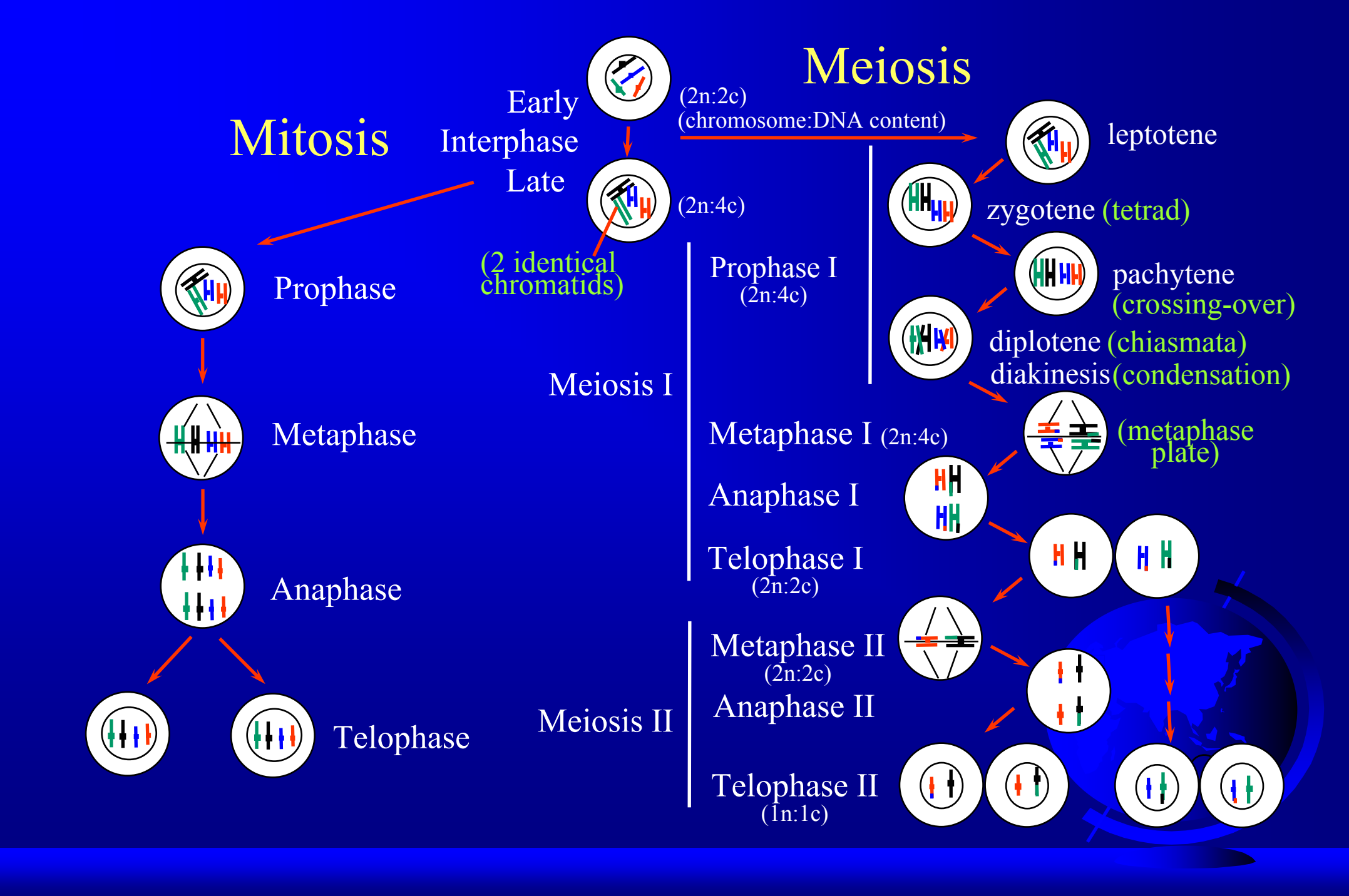
Starts with the division of spermatogonia and ends with the formation of spermatozoa
Begins at puberty and then goes on continously

Localisation and time for completion

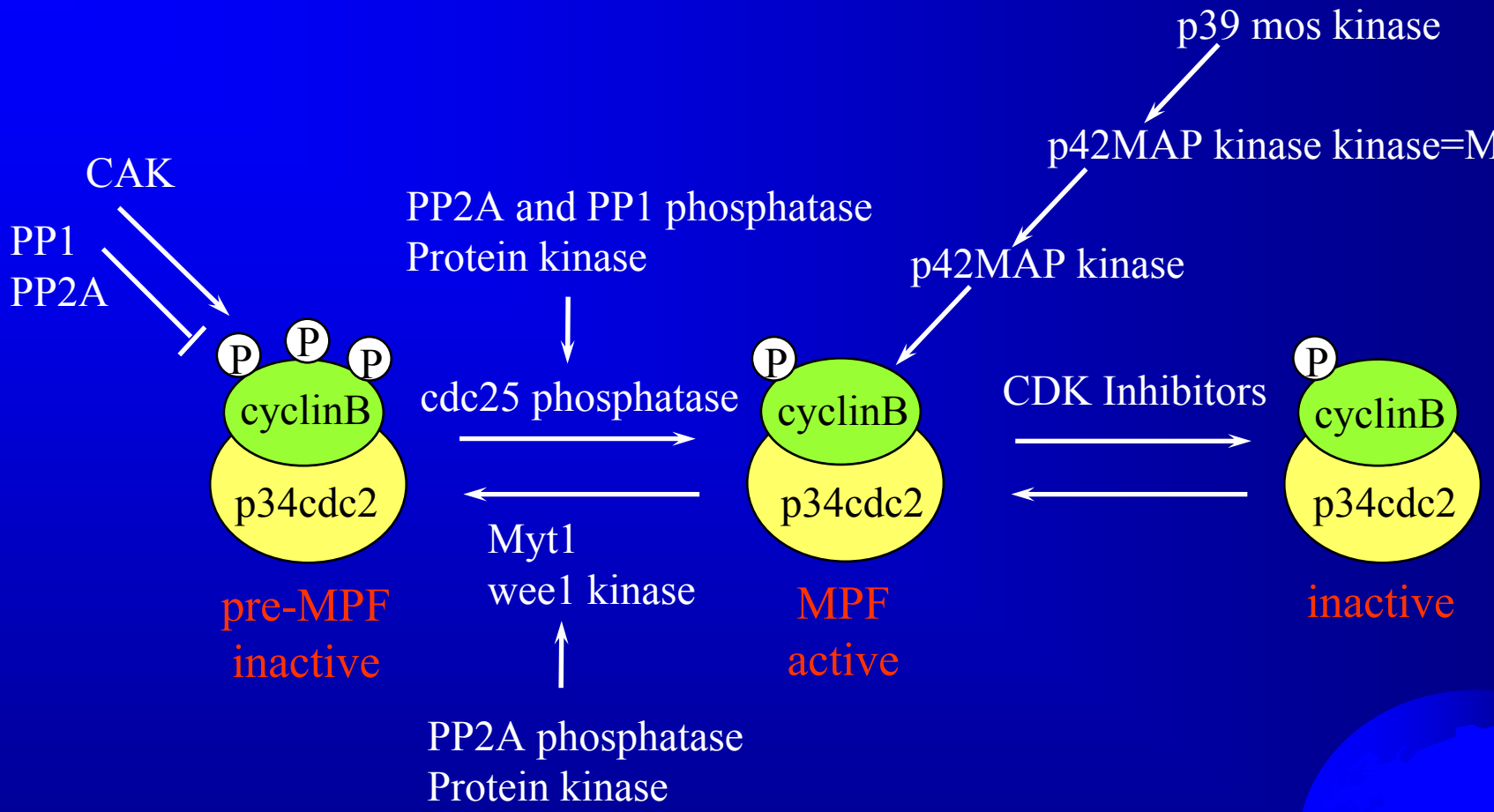
Takes place in seminiferous tubules of the testis
Lasts 64 days in men

Three main phases

- Mitotic proliferative phase: increases cell number
  (spermatogonia)
- Meiotic phase: halves the chromosome number and generates genetic diversity
  (spermatocytes I to II and spermatocytes II to spermatids)
- Differentiation phase = spermiogenesis: «packages the chromosomes for delivery»
  (spermatids to spermatozoa)


Mitosis
Early Interphase
Late
(2n:2c)
(chromosome:DNA content)
(2n:4c)
(2 identical chromatids)
Prophase
Metaphase
Anaphase
Telophase
Meiosis
Meiosis I
Meiosis II
Prophase I (2n:4c)
Metaphase I (2n:4c)
Anaphase I
Telophase I (2n:2c)
Metaphase II (2n:2c)
Anaphase II
Telophase II (1n:1c)
leptotene
zygotene (tetrad)
pachytene (crossing-over)
diplotene (chiasmata)
diakinesis (condensation)
(metaphase plate)

Control of meiosis



A cascade of activation of kinases and phosphatases leads to the initiation of meiosis

Spermatogenesis

in seminiferous tubules

PGC

Mitosis

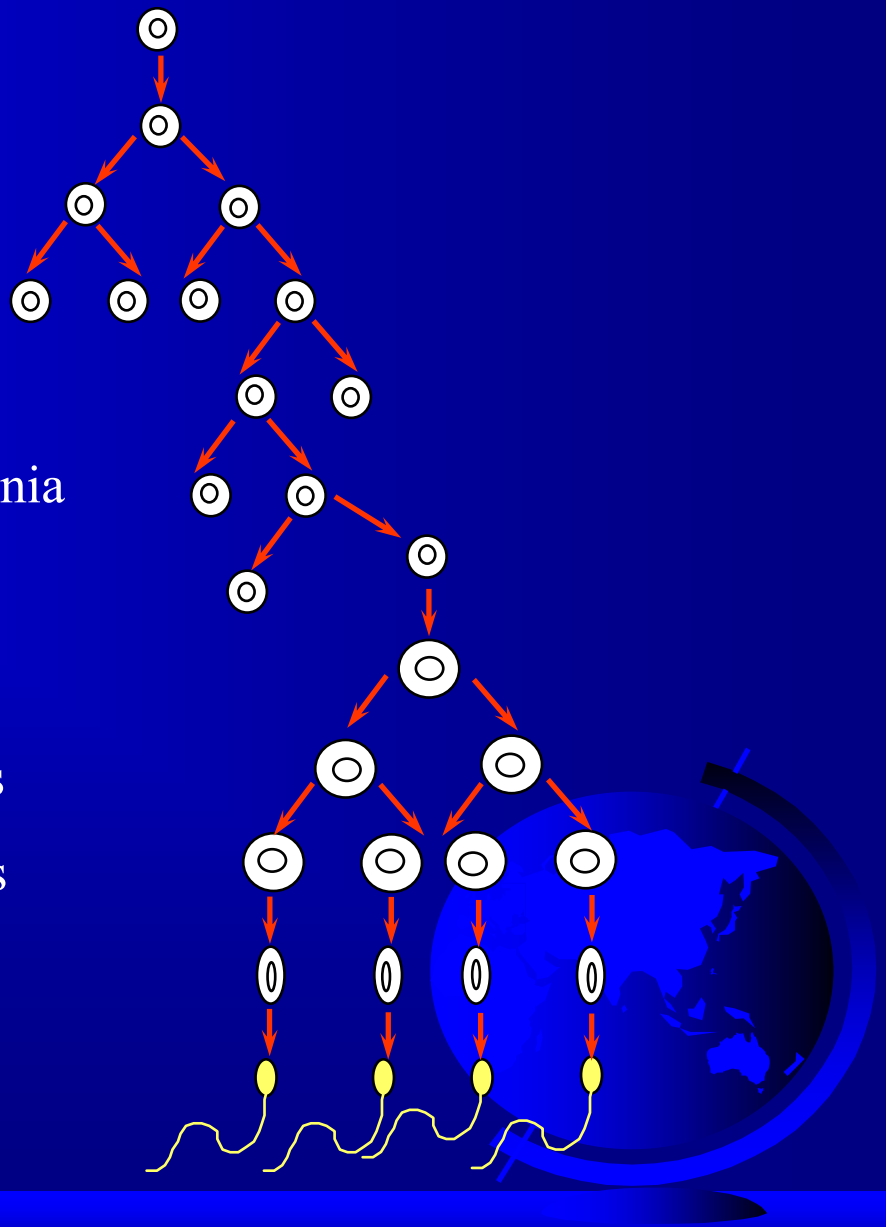
- Spermatogonia A1
- Spermatogonia A2
- Spermatogonia A3
- Spermatogonia A4
- Intermediate spermatogonia
- Spermatogonia B

Meiosis I and II

- Primary spermatocytes
- Secondary spermatocytes
- Young/Round spermatids

Spermiogenesis

- Elongated spermatids
- Spermatozoa



Genetic factors affecting male fertility

AZF = Azoospermic Factor
DAZ = Deleted in Azoospermia

2-21% of men with severe oligozoospermia or azoospermia

↓

Deletions in the AZF region on the long arm of the Y chromosome where the DAZ genes are located

↓

Spermatogenic disorder

Temporal and spatial organization of spermatogenesis

Goal: continuous spermatozoa production

Spermatogenic rate

Spermatogenesis proceeds at a constant and characteristic rate

Time for completion of spermatogenesis is in men 64 days

(From entry into first mitosis of a spermatogonia A to the release of its descendant spermatozoa)

Spermatogenic cycle (occurs in time)

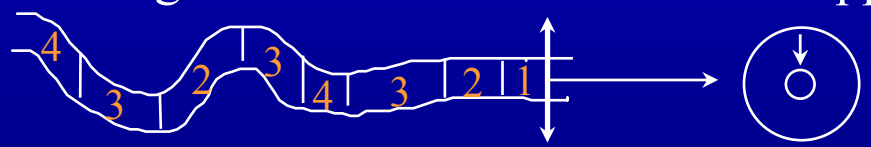
Cyclic initiation of spermatogenesis at a particular point in the tubule

Rounds of spermatogenesis are initiated at time intervals that are constant

This cycle lasts oin men 16 days and is made up 6 stages, which occurs at the same time, displaced progressively from the periphery to the lumen of the seminiferous tubule

Spermatogenic wave (occurs in space)

Spermatogenesis in adjacent regions of a seminiferous tubule appears to be phase advanced or retarded



Spermiation, release of spermatozoa into the lumen of the seminiferous tubules and travel to the cauda of the epididymis: 10 days

Sertoli cells

might be implicated in the organization of the spermatogenic rates, cycles and waves

- Adjacent Sertoli cells are in continuity via extensive gap junctions through which communication and synchronization might occur

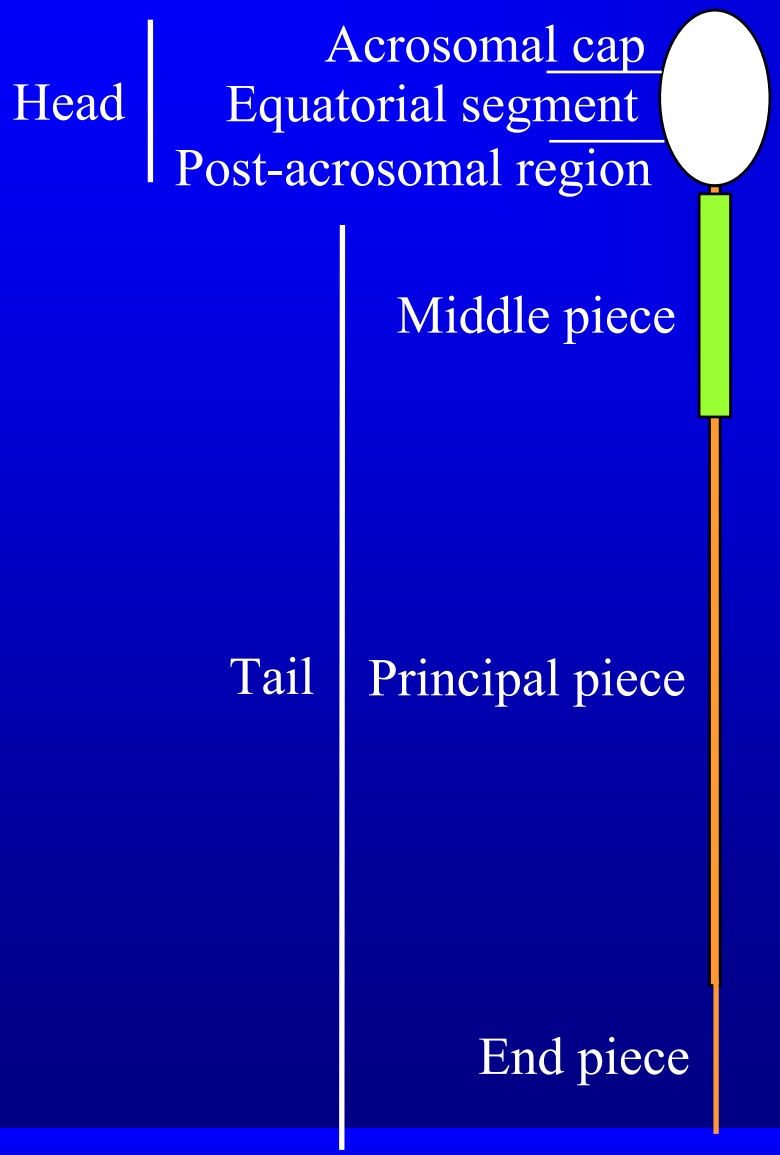
- Sertoli cells are associated with the cells of the spermatogenic lineage:
  - Sertoli cells-spermatocytes (gap junctions)
  - Spermatocytes-spermatids (ectoplasmic specializations)
  - Sertoli cells-elongating spermatids (tubulobulbar complexes)

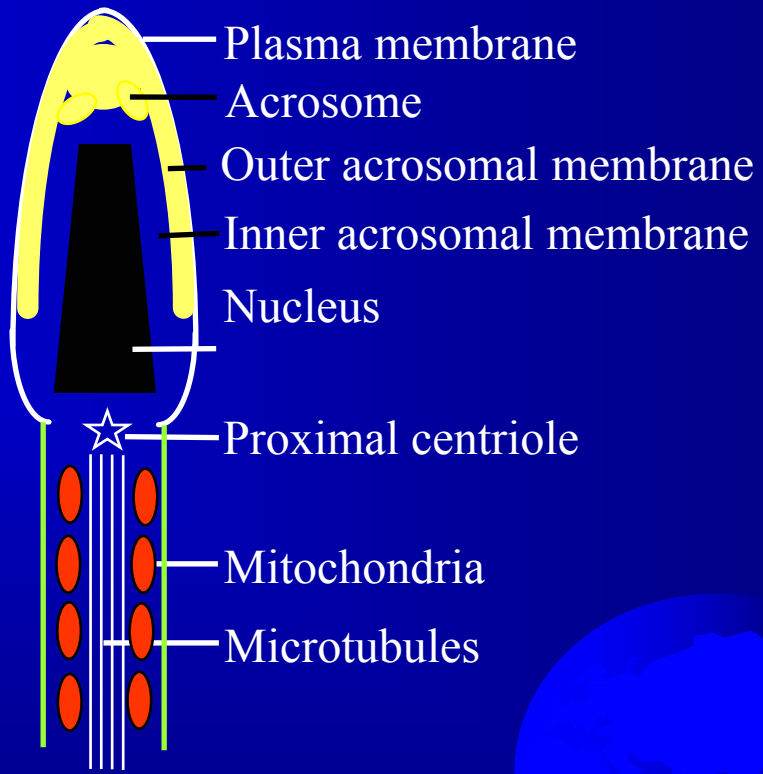
Spermiogenesis

Differentiation phase of spermatogenesis
Passage from Spermatids to Spermatozoa

- Morphological remodelling:
  - change in shape: from a round to an elongated form
  - elimination of cytoplasm
- Chromatin condensation (protamines)
- Generation of a tail for forward propulsion
- Formation of a midpiece containing the mitochondria (energy)
- Formation of the equatorial and postacrosomal cap
- Development of the acrosome (enzymes)

Spermatozoa





Role of hormones in spermatogenesis

At puberty androgens levels rise and spermatogenesis starts

LH binds to LH receptors on Leydig cells to stimulates the production of testosterone

Testosterone passes into the tubules, binds to androgen receptors within Sertoli cells

FSH binds to FSH receptors on Sertoli cells to stimulate:

- RNA and protein synthesis
- mobilization of energy sources
- production of testicular fluid
- output of Sertoli cell proteins, ABP and inhibin
- production of intracellular androgen receptors

Testosterone and FSH act synergistically on the Sertoli cells to allow spermatogenesis to go to completion

Spermatogenesis and apoptosis

Apoptosis or programmed cell death

- Spermatogonia, spermatocytes and spermatids

Role of apoptosis during spermatogenesis

- Regulation of germ cell numbers

(maintain an appropriate number of cells that can be supported and matured by Sertoli cells)

- Removal of aberrant cells (failure of DNA repair or presence of chromosomic anomalies)

Inductors of apoptosis

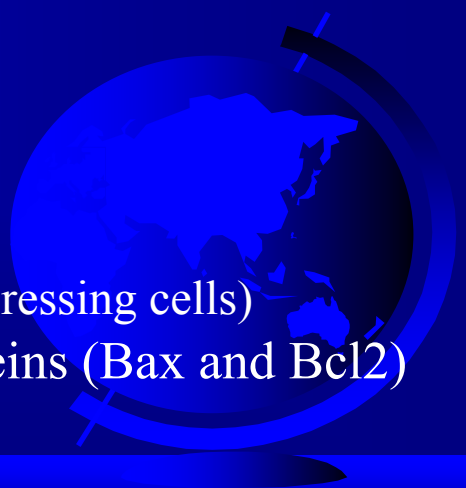
- Genetic defects
- Hormonal depletion
- Increased temperature
- Toxic compounds, radiations

Control of apoptosis in spermatogenic cells

- Fas/FasL system: Fas and Fas Ligand transmembrane proteins

(Fas Ligand expressed by Sertoli cells can induce apoptosis in FAS-expressing cells)

- Balance between apoptosis-inducing (Fas) and -inhibiting proteins (Bax and Bcl2)



Oogenesis

Definition

Starts with the division of oogonia and ends with the formation of an oocyte II

Localisation and time for completion

Takes place in the ovary

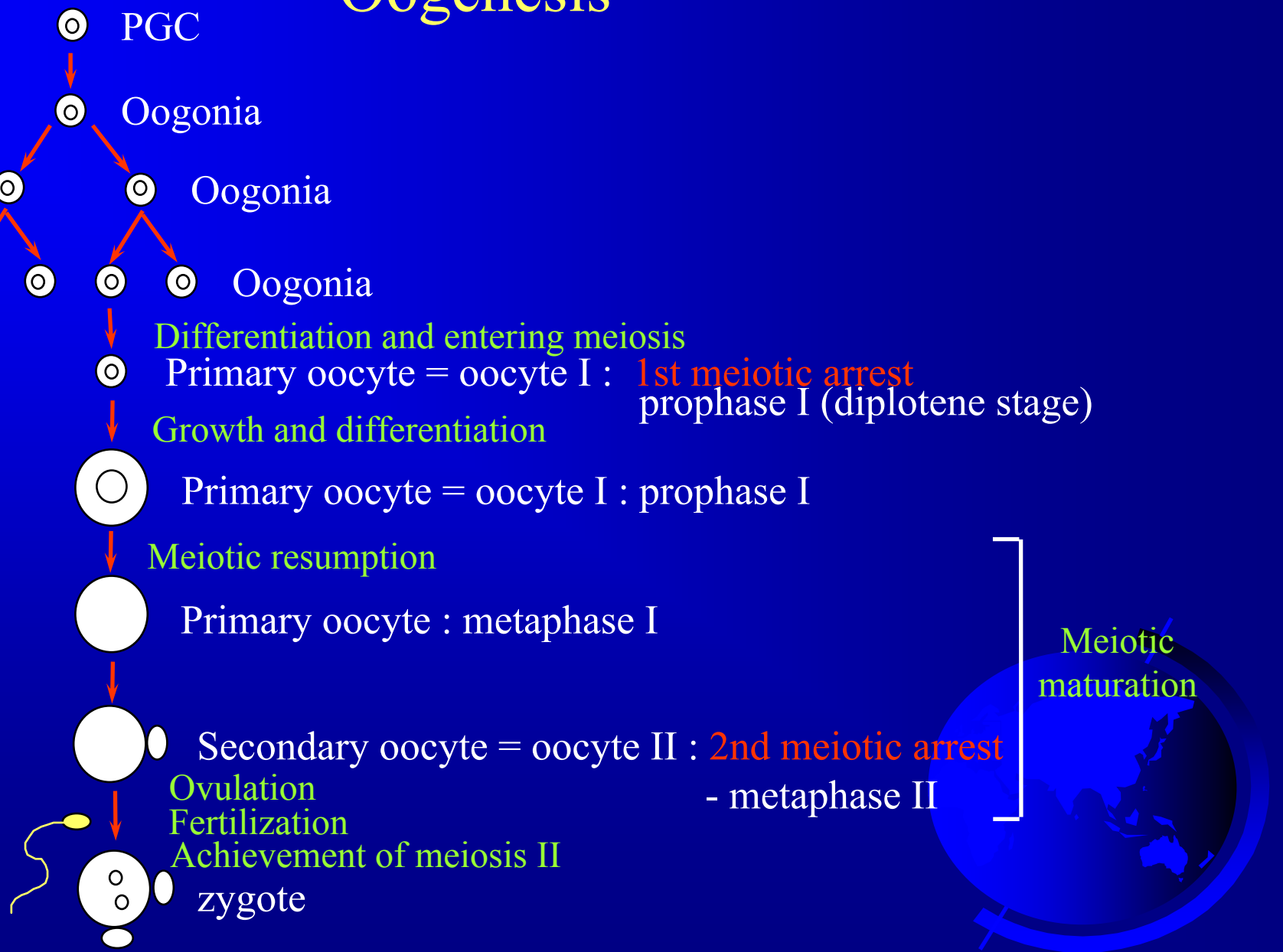
Starts during fetal life and is arrested 2 times : - diplotene stage (prophase I) of meiosis I
- metaphase II of meiosis II

13 to 50 years (puberty-menopause)

Three main phases

- Mitotic proliferative phase: increases cell number (oogonia)
- Meiotic phase: halves the chromosome number and generates genetic diversity (oocyte I to oocyte II)
- Differentiation phase during the arrest in prophase I (oocyte I)

Oogenesis

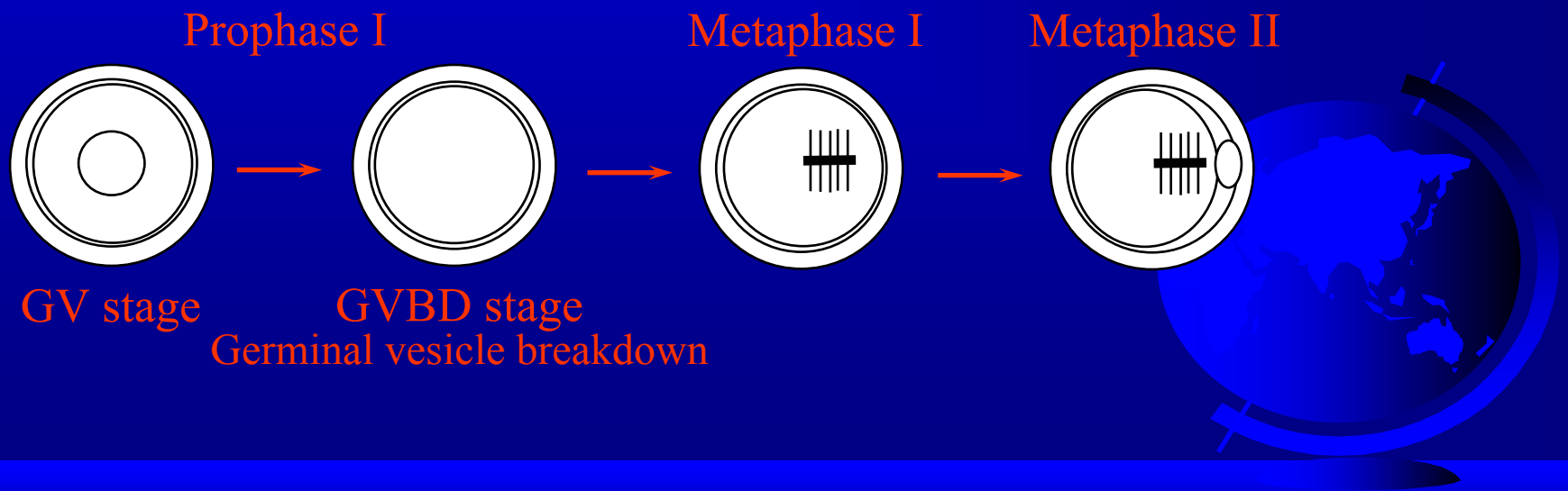

PGC
Oogonia
Oogonia
Oogonia
Mitosis
Differentiation and entering meiosis
Primary oocyte = oocyte I : 1st meiotic arrest
prophase I (diplotene stage)
Meiosis I
Growth and differentiation
Primary oocyte = oocyte I : prophase I
Meiotic resumption
Primary oocyte : metaphase I
Meiotic maturation
Secondary oocyte = oocyte II : 2nd meiotic arrest
- metaphase II
Meiosis II
Ovulation
Fertilization
Achievement of meiosis II
zygote

Meiotic maturation

The oocyte I arrested at the diplotene stage (prophase I) of meiosis I resume meiosis and progress to metaphase II.

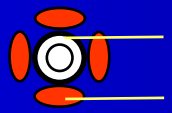
Meotic maturation occurs under 2 conditions:

- in antral follicules after the endogenous preovulatory gonadotrophin surge or the administration of exogenous gonadotrophins
- in vitro: spontaneously in competent oocytes retrieved from their follicles



Follicular development

Primordial follicles
= functional unit of the ovary
may stay in this arrested state for up to 50 years



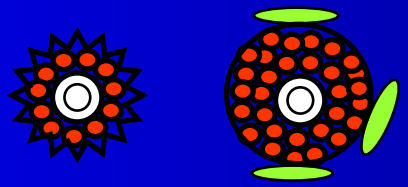
oocyte I blocked in diplotene stage of meiosis I

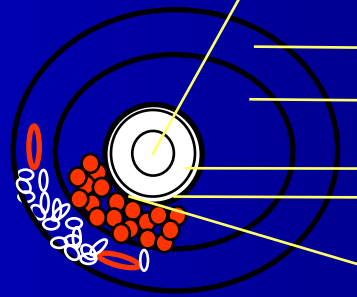
flattened mesenchymal (granulosa) cells

Growth

3 stages of development

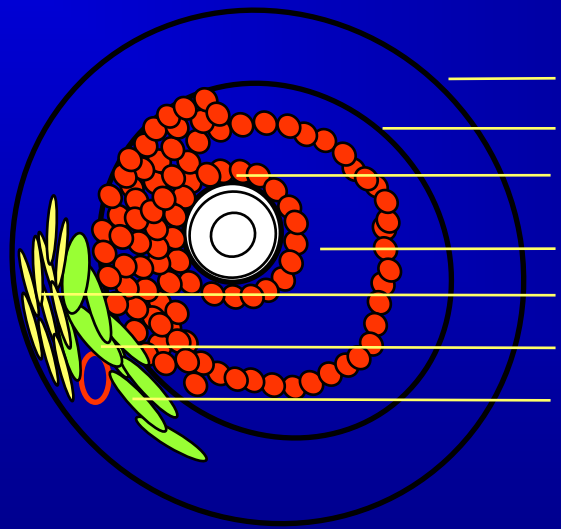
Primary or preantral follicles





germinal vesicle
theca
granulosa cells
oocyte I
zona pellucida
gap junctions

Secondary or antral follicles



stroma
membrane
cumulus oophorus
follicular antrum
theca externa (fribrous)
theca interna (vascular)
blood vessel

Preovulatory follicles

Preantral phase: 85 days
Antral phase: 8-12 days
Preovulatory phase: 37 hours
Luteal phase: 12-15 days
(corpus luteum = postovulatory follicle)

Follicular grow and mature

Fetal and neonatal life:

A few primordial follicles may resume development sporadically and incompletely

Puberty:

Regular recruitment of primordial follicles into a pool of growing follicles
A few primordial follicles recommence growth every day, so that a continuous trickle of developing follicles is formed.

Primordial to preantral transition:

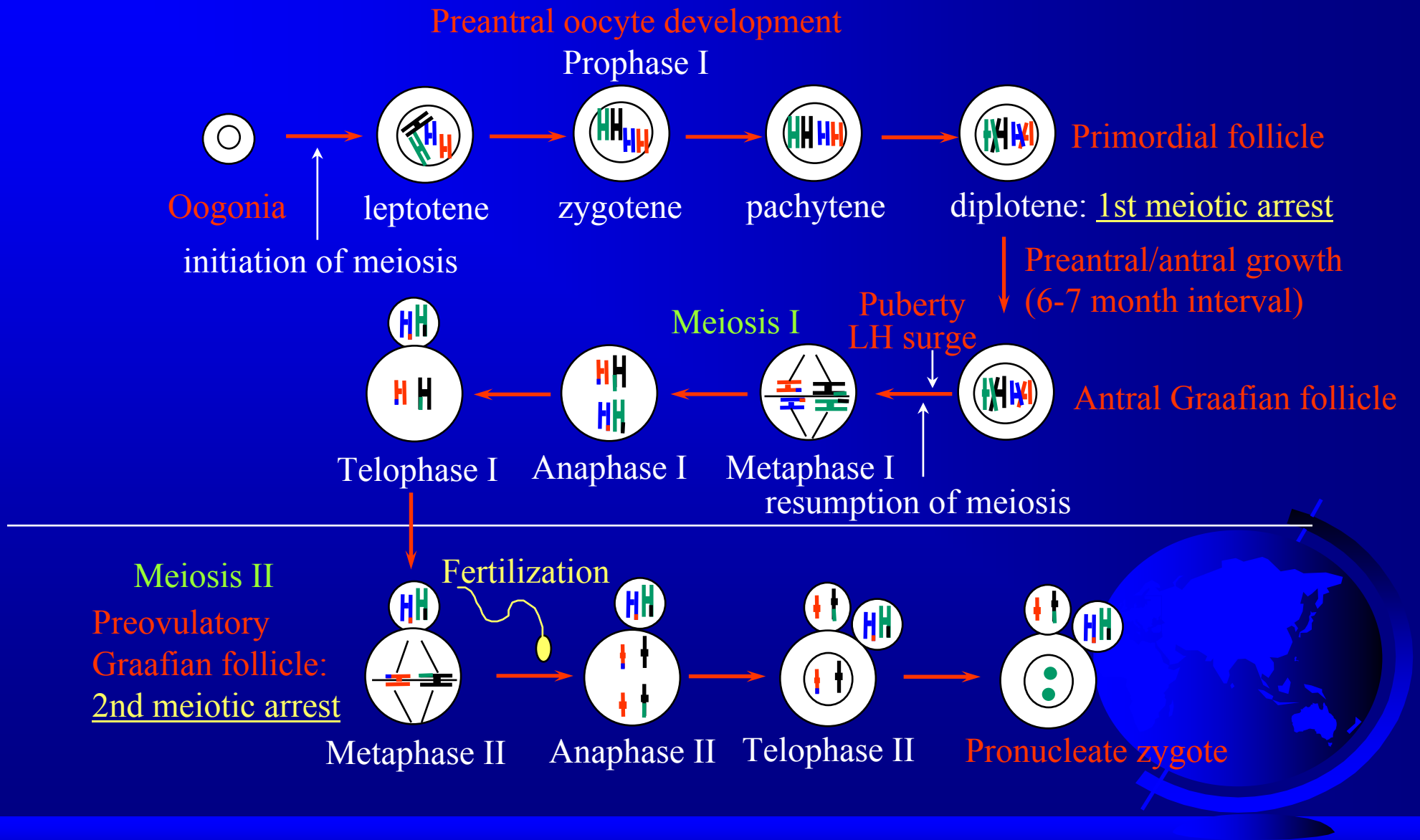
Increase in the diameter of the primordial follicle from 20 to 500 μm
Increase in the diameter of the oocyte I from 60 to 120 μm (its final size)
RNA synthesis and protein turnover in oocyte (essential for oocyte maturation, early embryo)
Bidirectional communication between oocyte and granulosa cells

Preantral to antral transition:

Proliferation of granulosa cells
Appearance of a viscous fluid between granulosa cells = follicular fluid
and formation of the follicular antrum
RNA synthesis and protein turnover in oocyte

granulosa cells secretions
(mucopolysaccharides)
serum transudate

Stages of meiosis during oocyte/follicle development



Role of hormones in oogenesis

How a few primordial follicles start to develop as preantral follicles?

Not well understood

Seems to occur independently of any extraovarian controls

Involvement of paracrine action of cytokines, EGF, within the ovary

The growth of the follicle is regulated by gonadotrophins in its later stages (antral follicles)

Follicular atresia is prevented by the presence of gonadotrophins:

- FSH = Follicle-Stimulating Hormone
- LH = Luteinizing Hormone

FSH and LH bind to their follicular receptors, expressed in the early preantral phase, to induce the production and release of steroids stimulating further antral growth:

- FSH receptor on granulosa cells: aromatization of androgens provided from thecal cells into oestrogens
- LH receptor on thecal cells: androgens and oestrogens

Oestrogens can bind granulosa cells to stimulate their proliferation and together with FSH stimulate the appearance of LH receptors on the outer layers of granulosa cells critical for entry into the preovulatory phase of follicular growth

Oogenesis and apoptosis

Germ cell apoptosis during fetal ovarian development

Granulosa cell apoptosis and postnatal follicular atresia

- Basic atresia of immature follicles (initiated in the oocytes)
- Cyclic atresia of maturing and fully mature follicles (initiated in granulosa cells) (anti-apoptotic action of FSH)

~ 500'000 non-atretic follicles per ovary at birth (stock of follicles)

↓ Follicular atresia

~ 400 will develop to the preovulatory stage and release an oocyte for possible fertilization

Oocyte apoptosis during aging and cancer therapies

- cumulus-enclosed oocytes harvested from aged women by superovulation
- oocytes exposed to chemo- and/or radiotherapy

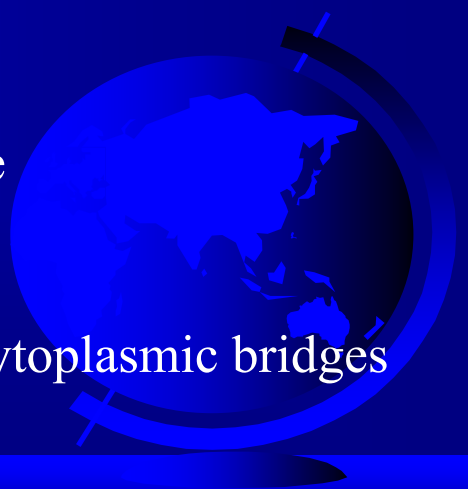
Comparison between spermatogenesis and oogenesis

Similarities

- Phases of gametogenesis:
  - Mitotic proliferative phase: mitosis of spermatogonia or oogonia
  - Growth phase: increase in cytoplasmic volume of spermatogonia or oogonia to become spermatocytes I or oocytes I respectively.
  - Meiotic phase: mechanism of meiosis

Differences

- Duration and timing of gametogenesis
- Duration of the proliferative phase
- Importance of the growth phase and timing of the differentiation phase
- Timing and results of meiosis
- Syncytia between spermatogenic cell types: cells connected through cytoplasmic bridges



In male: male gonad undergo SRY-directed changes in organization

- Proliferation of the sex cord cells deep into the medullary region establishing contact with ingrowing medullary cords of mesonephric tissue
- Formation of the definitive testis cords which incorporate PGC and secrete an outer basement membrane = seminiferous cords (seminiferous tubules of adults)
- In seminiferous cords: PGC become Spermatogonia and mesodermal cord cells give rise to Sertoli cells
- The loose mesenchyme vascularizes and develops as stromal tissue within which cells condense in cluster to form the interstitial glands, Leydig cells

In female: female gonad continues to appear indifferent and does not express SRY

- Sex cords are ill-defined
- Small cell clusters surround the PGC, named Oogonia, to initiate the formation of the Primordial follicles
- Mesenchymal cells give rise to the Granulosa cells of the follicle and oogonia become Oocytes

Differentiation of the 2 sexes

Depends on the endocrine activity of the fetal testis