Why gametes? Benefits and Consequences:

Male reproductive physiology

from the perspective of the spermatozoon

Ulrik Kvist
M.D. Ph.D.

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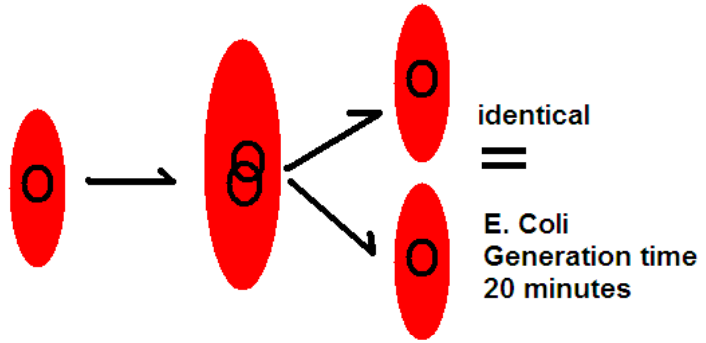
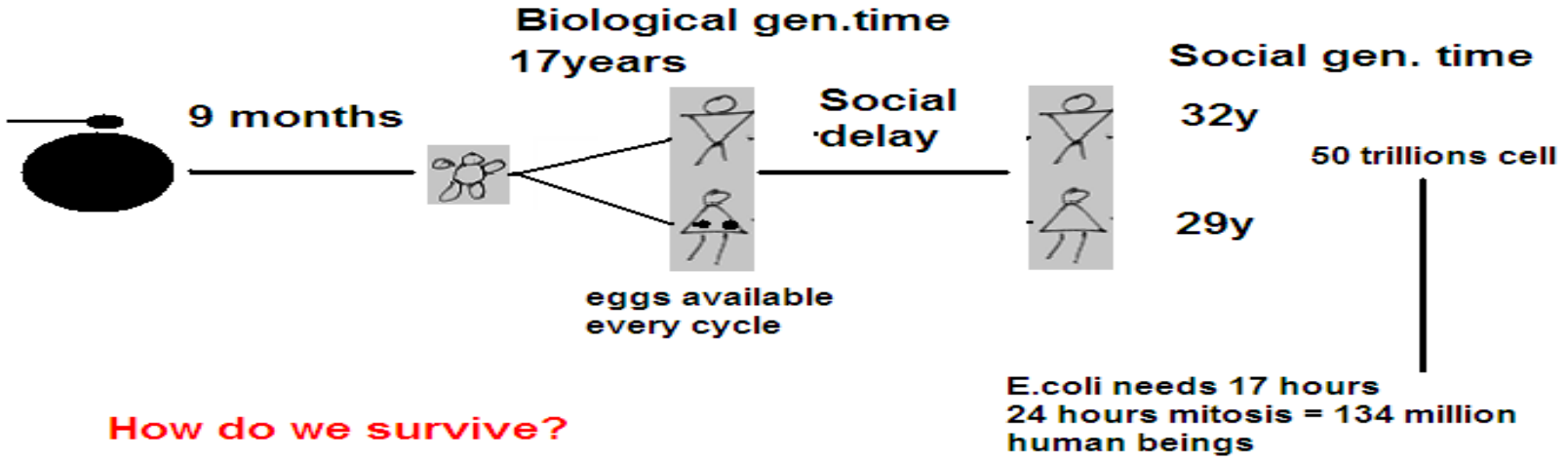
Centre for Andrology and Sexual Medicine

Karolinska Institutet
KAROLINSKA Universitetssjukhuset

Why gametes

- **Mitosis** = the dominating way of reproduction in the world
- all single cell organisms
- **We** are multicellular.





How do we survive – short version

- Outside the body we don´t (Cell culture – needs sterile conditions)
- After death – we are invaded

- **Thus the living intact body survives – how? short version**

- You (as all multi cellular organisms) have your own **unique immune system** directed towards all but yourself (except. Autoimmune disease).

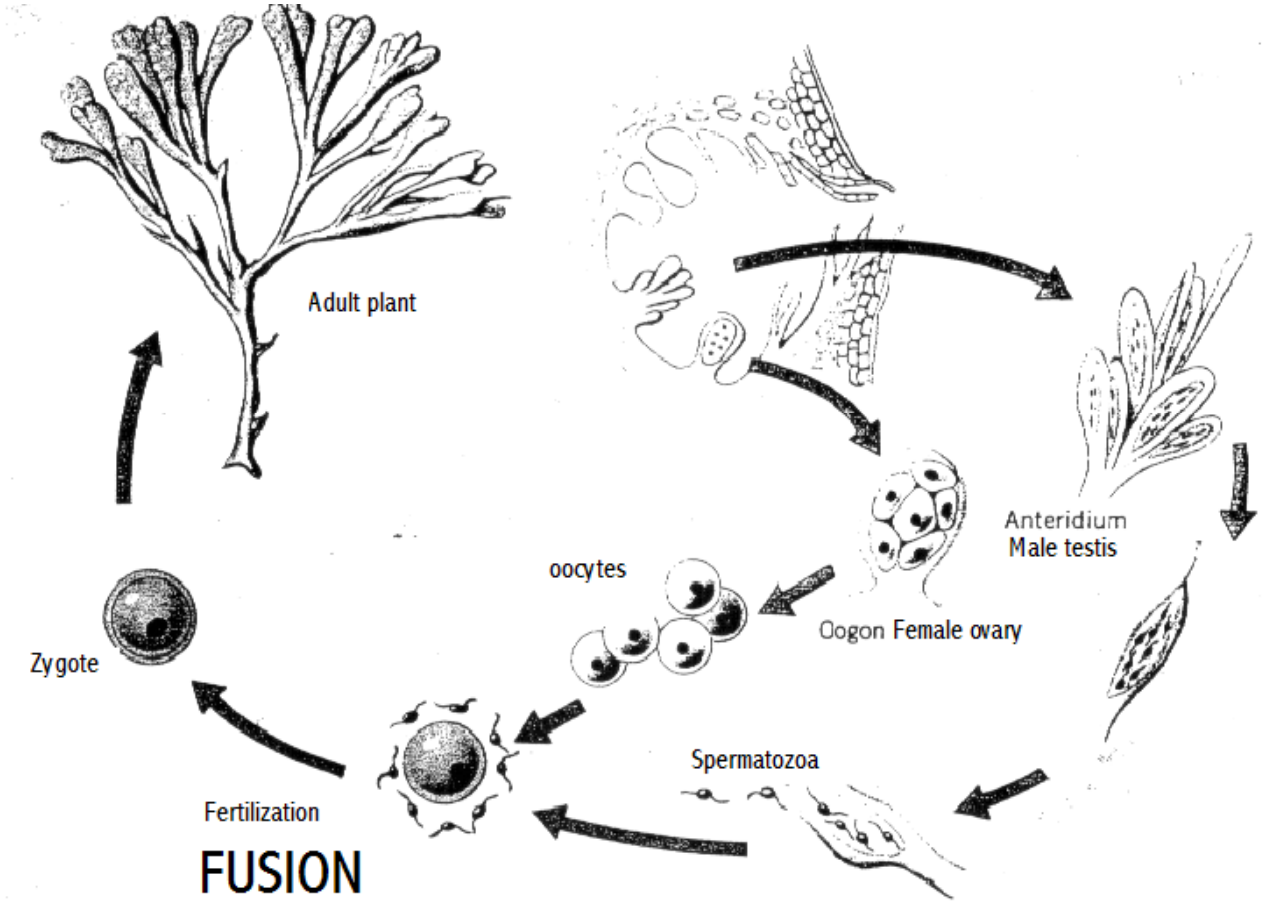
- For this there is a need for you to be unique.
- Mitosis gives identical individuals

- Nature needed an invention to create unique individuals- MEIOISIS

- **MEIOSIS created unique gametes that created unique individuals surviving endless attacks from microrganisms**

Meiosis

Sperm, egg and fusion evolved 600 million years ago

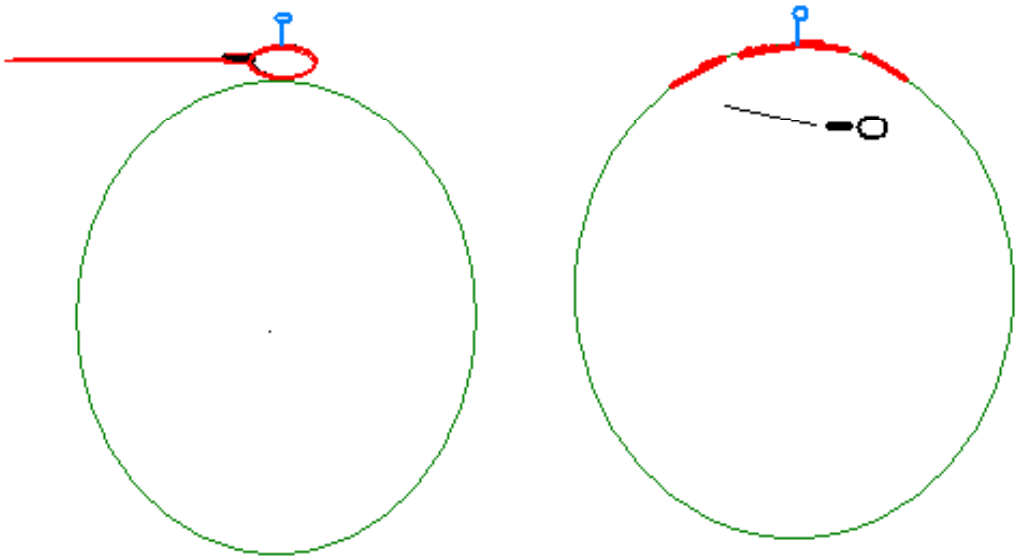


Fertilization by natural course, insemination and IVF means fusion of membranes

FUSION

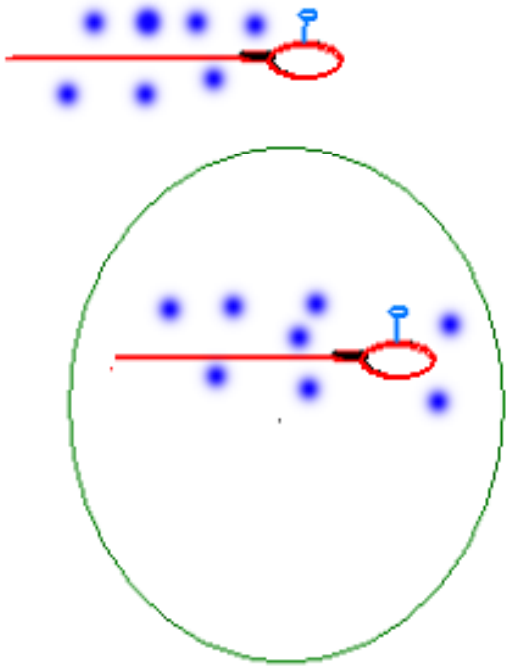
means that the sperm and oocyte mebranes fuse

- the naked sperm enters the ooplasm-.



With ICSI, a new era of man-induced evolution started 1991 - selection and fusion were by-passed by the injecting embryologist

Intra cytoplasmic sperm injection
- means that the sperm membrane + medium enters the ooplasm



Calls for controlled and traceable conditions excluding factors modulating genetics

Controlled conditions?

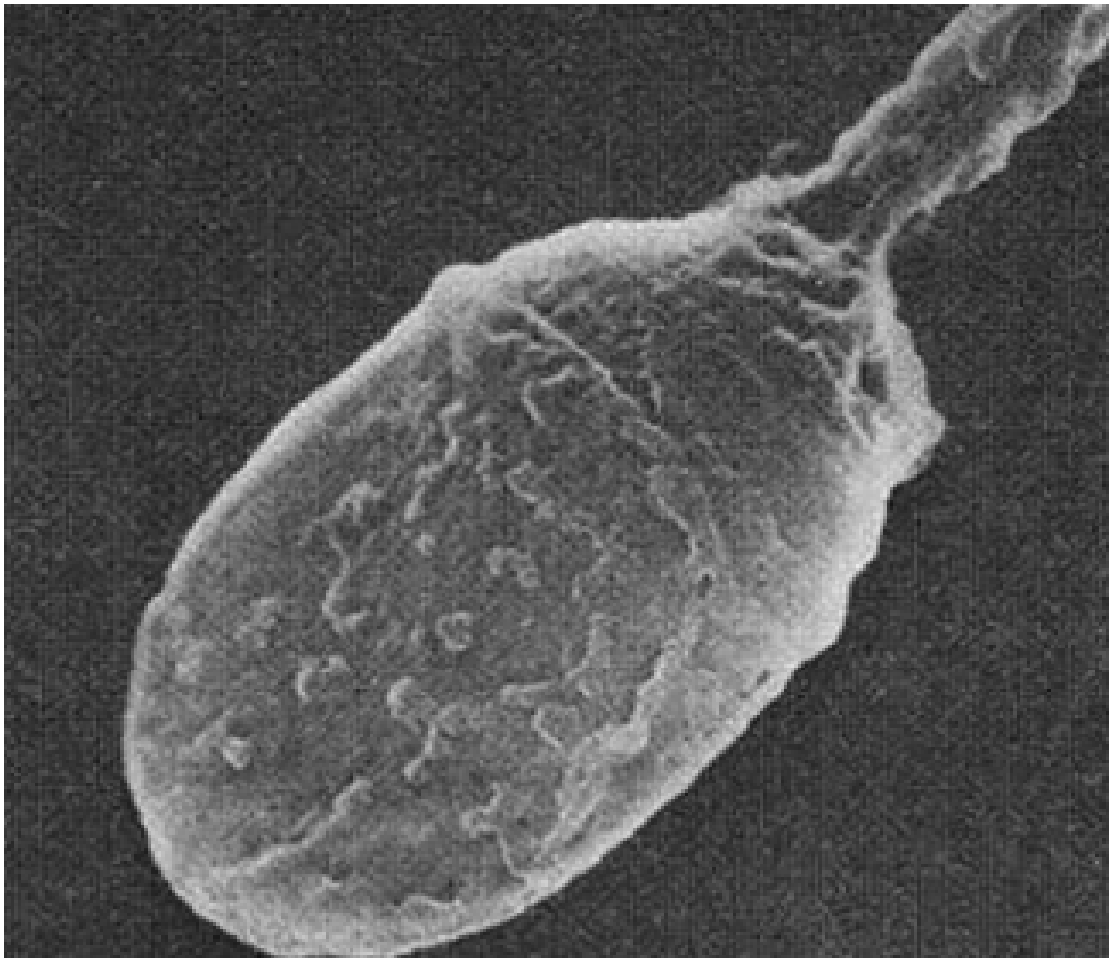
Has the injection into the oocytes of components from chicken and cow been stopped?

- egg-yolk components in cryopreservation media??
- bovine serum albumin in original sperm preparation media??

The spermatozoon

A messenger cell

with messages



Functions of the spermatozoon as a ”messenger cell”

the postman

Functions of the messenger

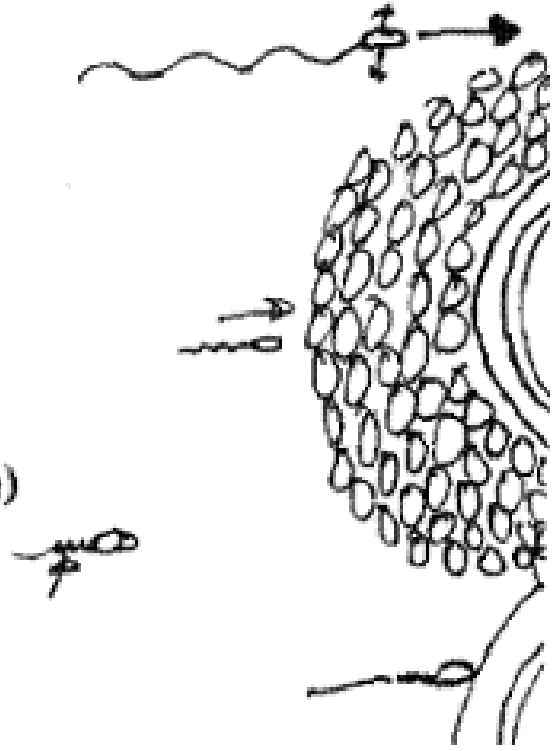
Live
Swim, rapidly, vigoursly, and straight

Penetrate cervical mucus

Pass the uterus (How??), the tubes
Pass the coronacells

Activate to reach the ovum(=Capacitation)

Bind to the zona pelucida



More messenger functions

Undergo acrosome reaction

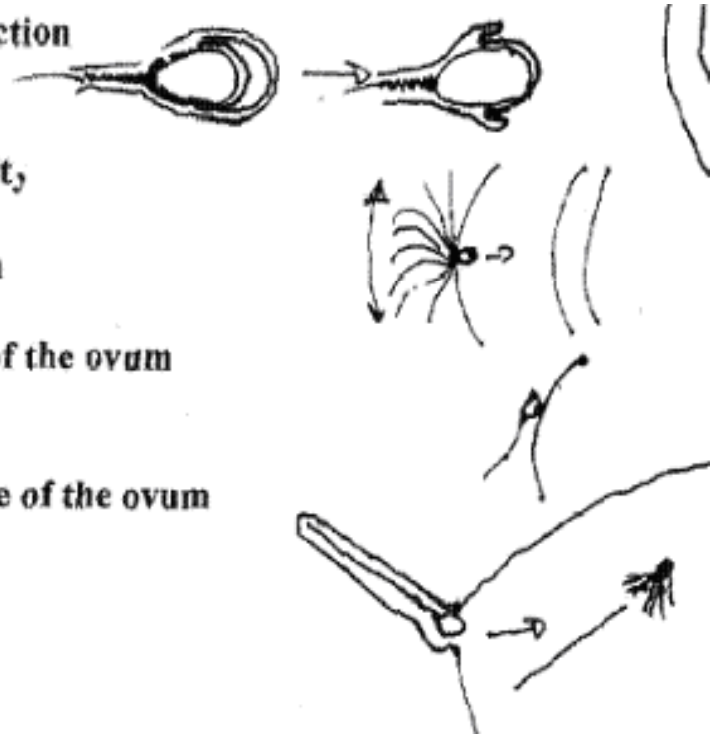
Hyperact vate (?)
= hyperactivated motilit,

penetrate zona pelucida

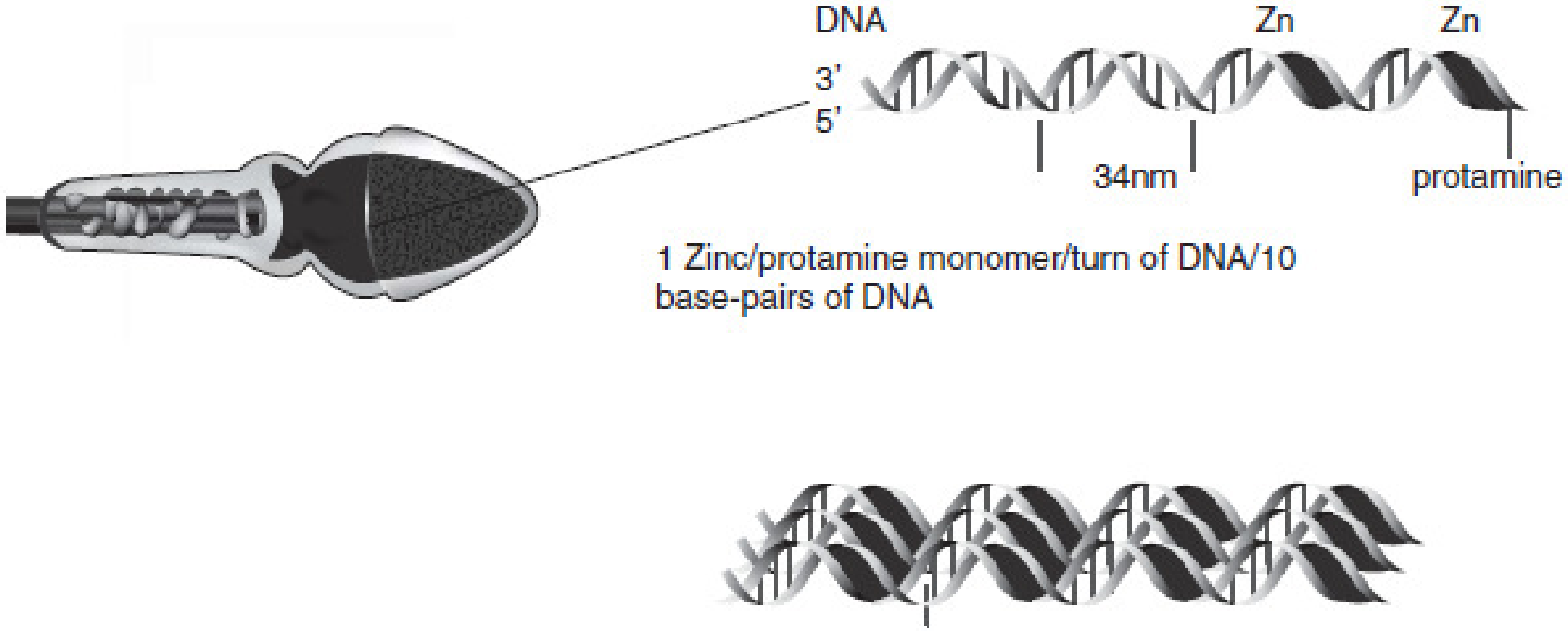
bind to the membrane of the ovum
(equatorial plate)

fuse with the membrane of the ovum

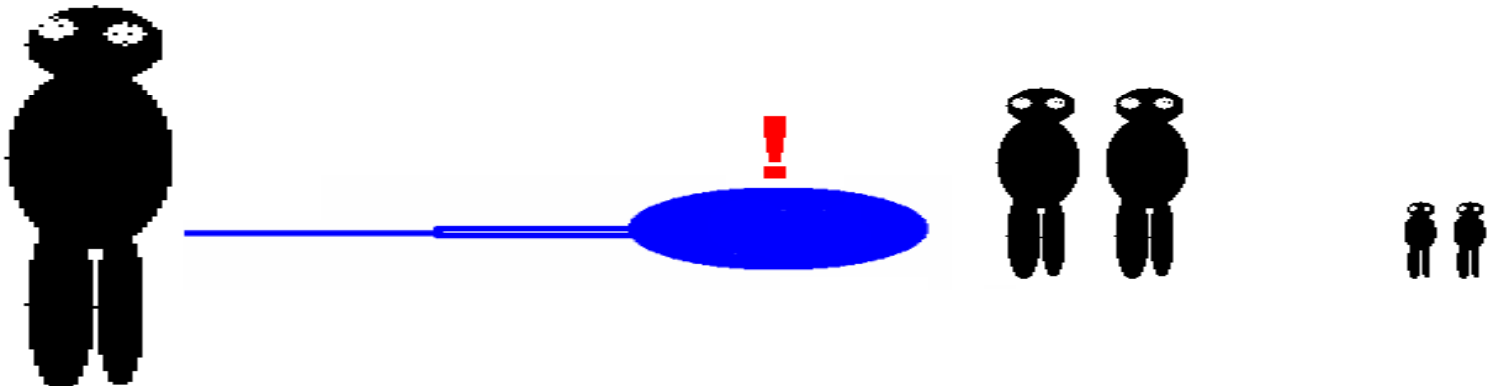
deliver the MESSAGES



Message 1 The haploid intact genome

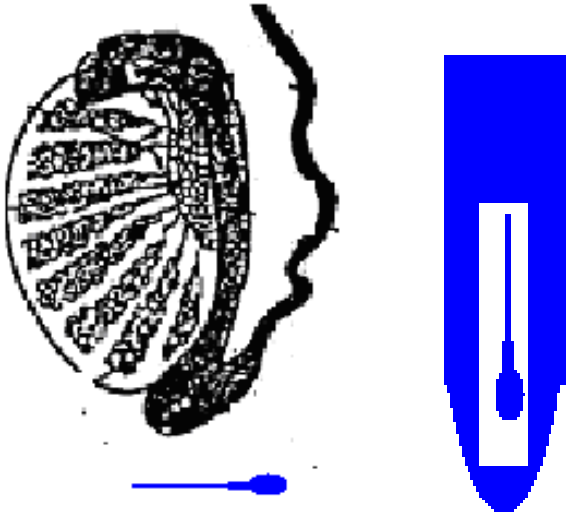


An unaffected Reproductive physiology calls for healthy grand-children



DNA strand breaks

- A hit in a spermatozoon in the epididymis or the test tube now!

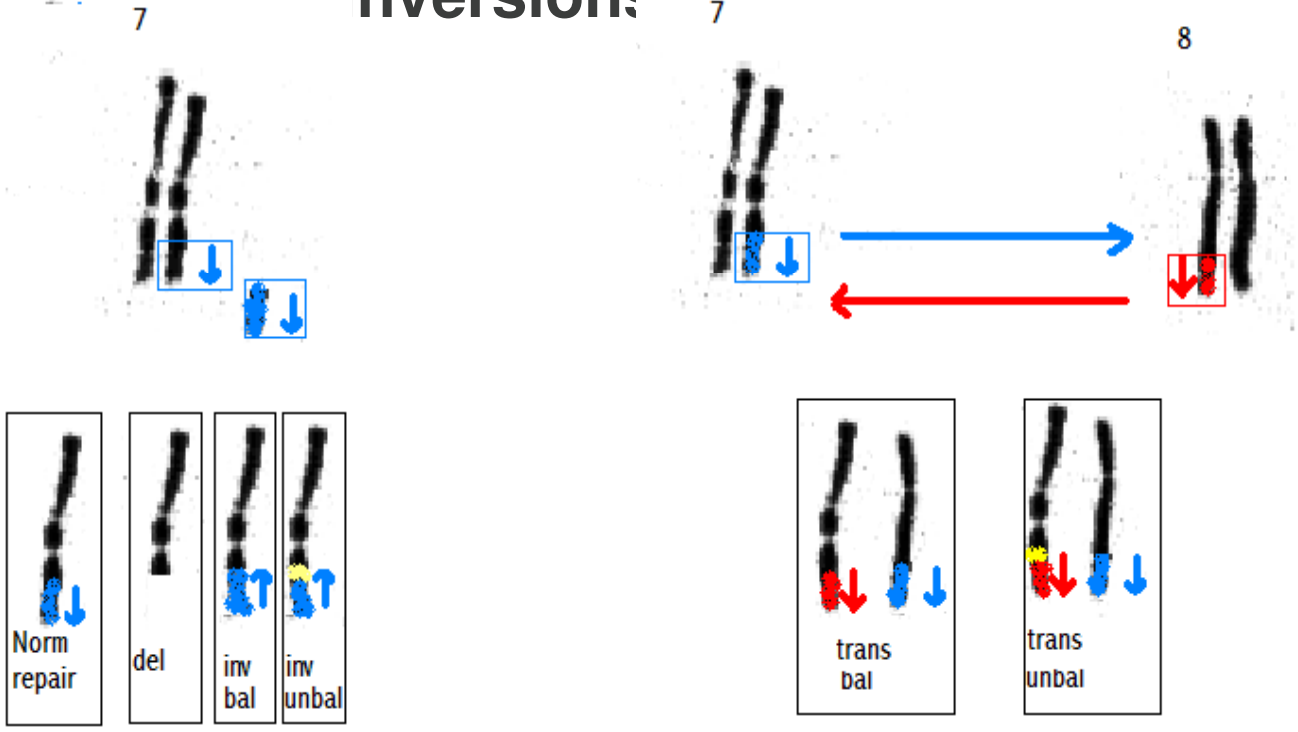


May result in a grand-child with an unbalanced translocation or inversion with

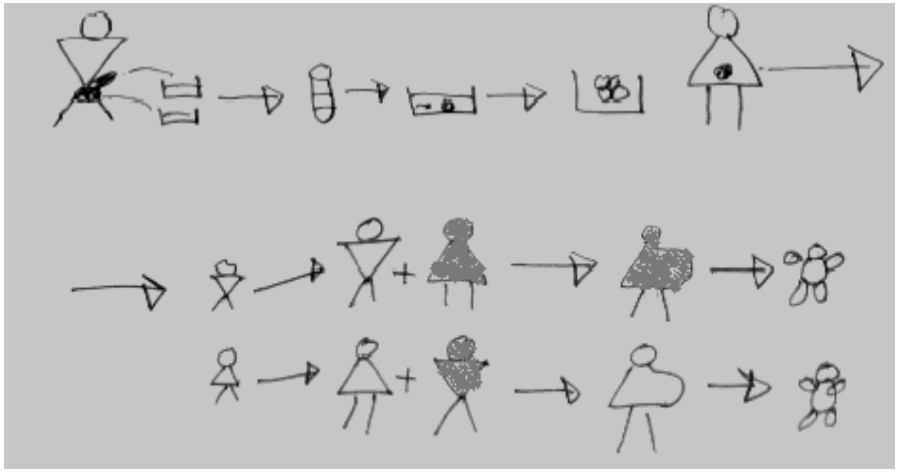
impaired psychomotor development and malformations

The sperm can not repair DNA-strand-breaks.
The oocyte may repair - properly or not
Different women different repair capacity

De novo deletions, inversions, translocations



Consequences of damaged sperm DNA are affected pregnancy>fetus>child>grandchild



Two generation perspective to ensure intact reproductive power

Carrier of balanced translocation (or inversion)

- Creates gametes with

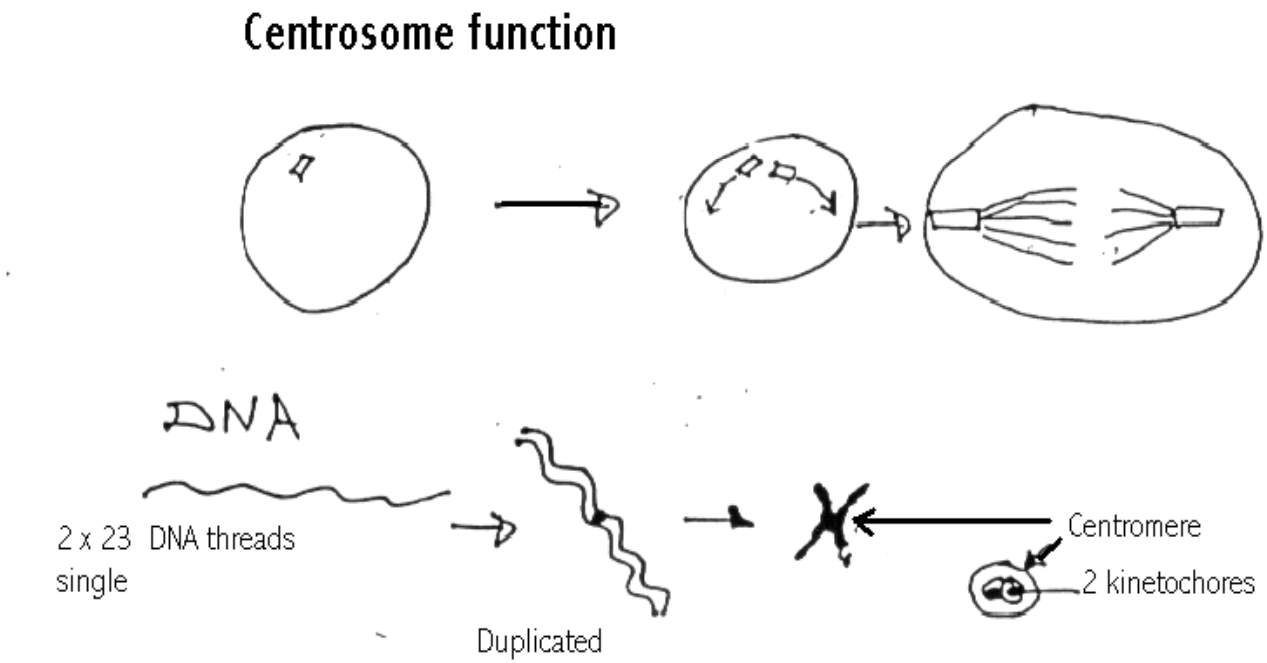
- 1. Too much DNA – affected grandchild
- 2. Too little DNA- affected grandchild
- 3. The balanced translocation grandchild carrier – risk for grand-grand-child.
- 4. Normal DNA

Evaluation of reproduction therefore calls for a

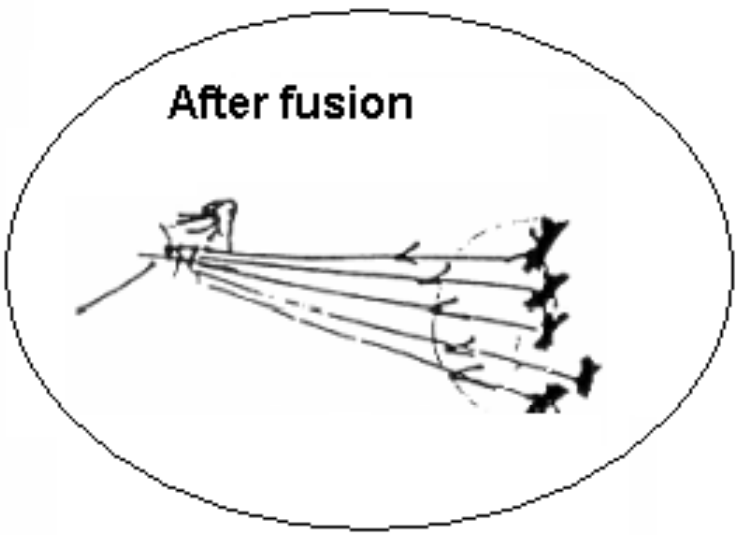
two-generation perspective

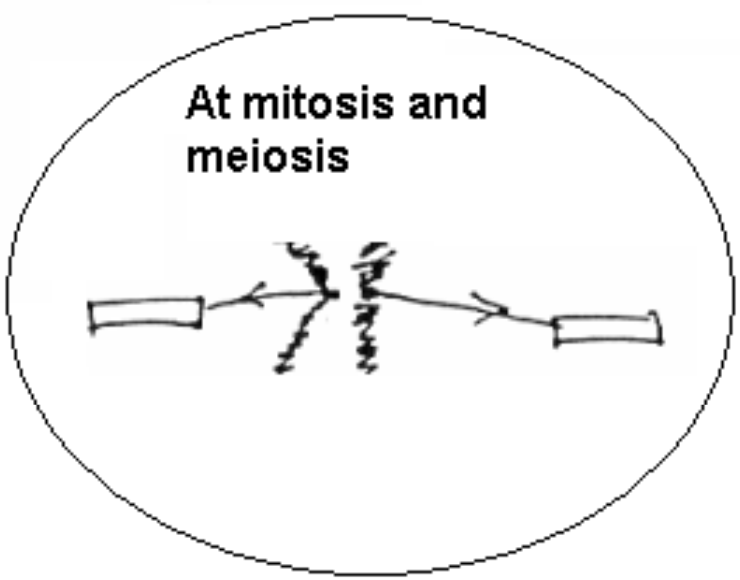
Sperm messages 2: The centrosome 1

for the very first until the very last mitosis and other centrosome based mechanisms.

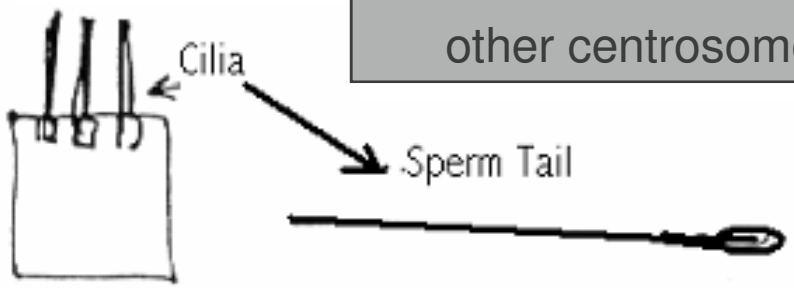


The very first event after fusion, every mitosis and meiosis, and other centrosome based mechanisms



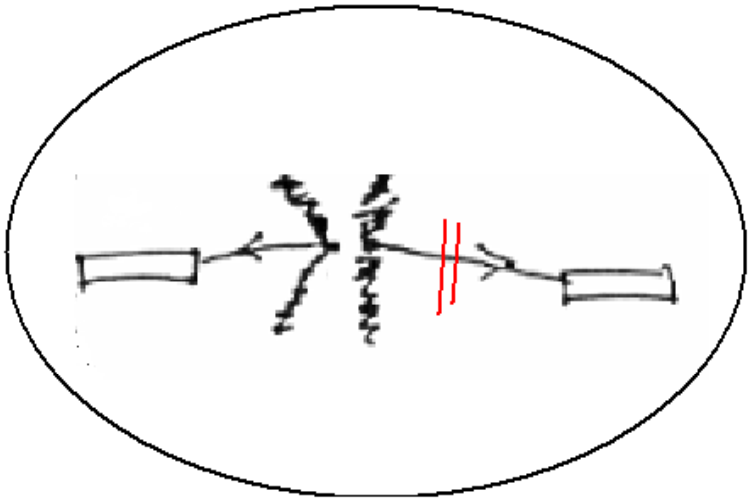


other centrosome functions



Sperm tail length - function

Impaired centrosome spindle function - aneuploidy



- Extra chromosomes 1-23;
- At birth somatic trisomies 8,13,18,21
- Sex chromosome aberrations
- Turner, XO, Klinefelter XXY
- XYY
- XXX

We need basic knowledge of the genetics of the centrosome and Tools to identify men at risk:

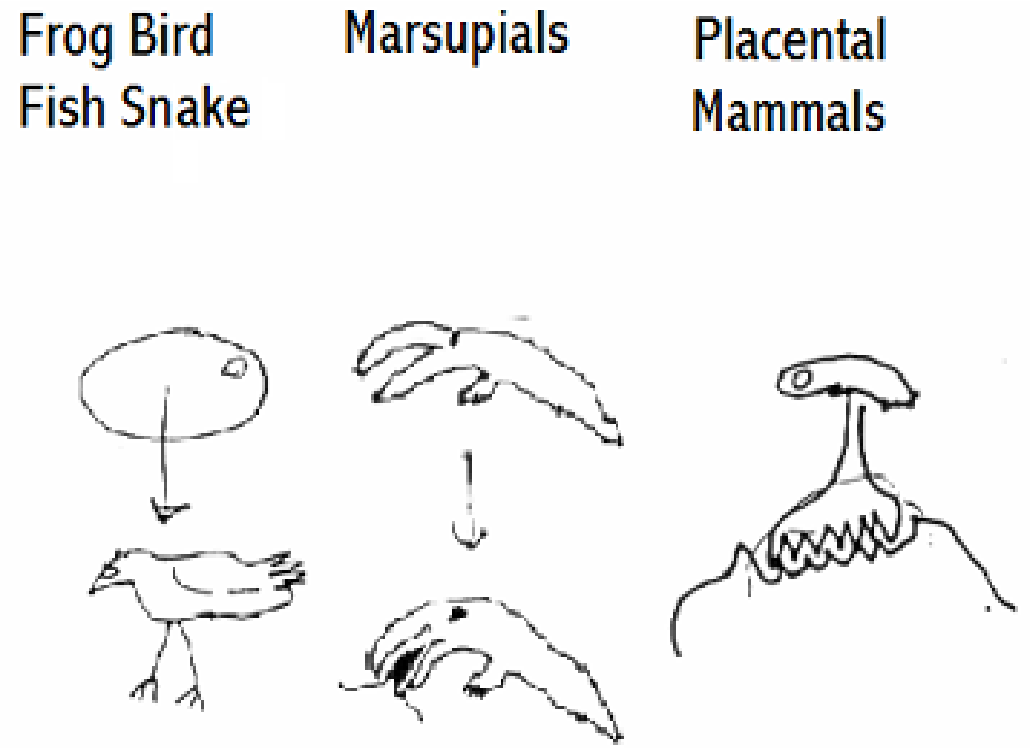
constitutional

by exposure

FISH

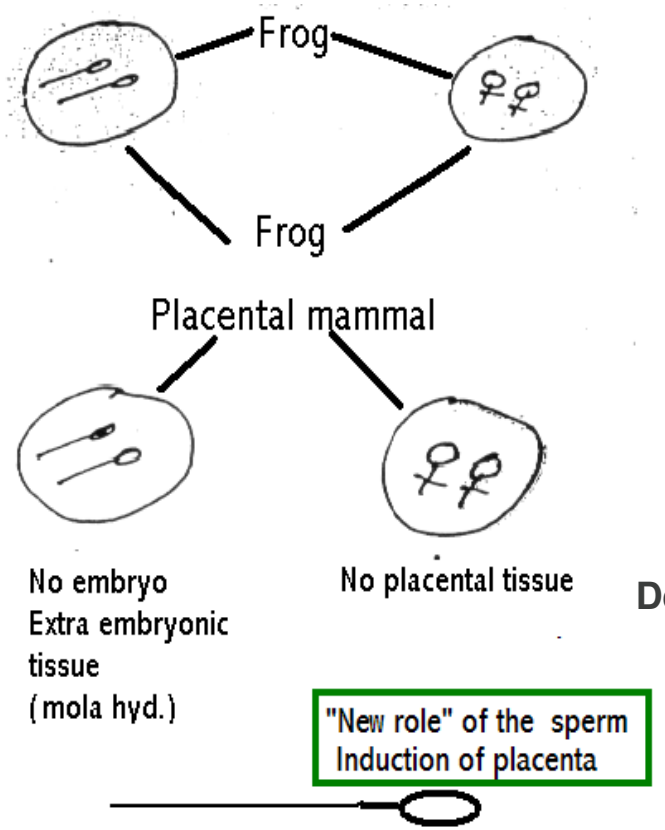
Sperm messages 3: The placenta

Females provide nutrition- the male provides the spoon



Nutrion lats for whole development to an embryo 4 days egg hatches

Sperm message- the placenta



Mola

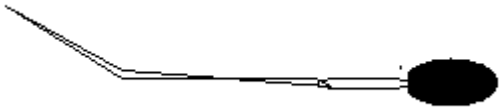
Dermatoid cyst

Sperm message- the placenta

- Pre-eclampsia involves maldevelopment of the placenta

- Questions for future:

- Does an impaired sperm factor contribute to Pre-eclampsia?
- Identify men at risk?
- Prevention- Selection of spermatozoa?



From where comes the germ cells?

1676 van Leeuwenhok saw them in his microscope and his followers thought they may be ”human seeds”.

The Pope and his followers were still ”ovists” and ”knew” that life origins in female eggs if the male ”induce them with the aura”

1750 Linneus who invented the plant sexual system thought they were parasites

1826 Hertwig and Fohl saw the frog spermatozoon fertilizing an oocyte.

1841Kölliker concluded that cell divisions in the testis resulted in spermatozoa.

So how to get a testis?

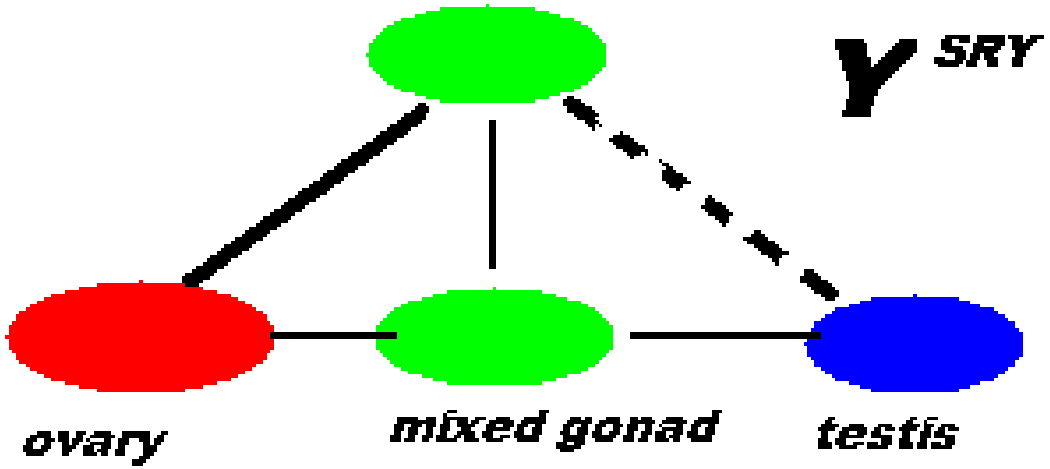
.

The default pathway for embryonic and fetal development creates girls and women.

Male development calls for active deviations from the default pathway at five different steps.

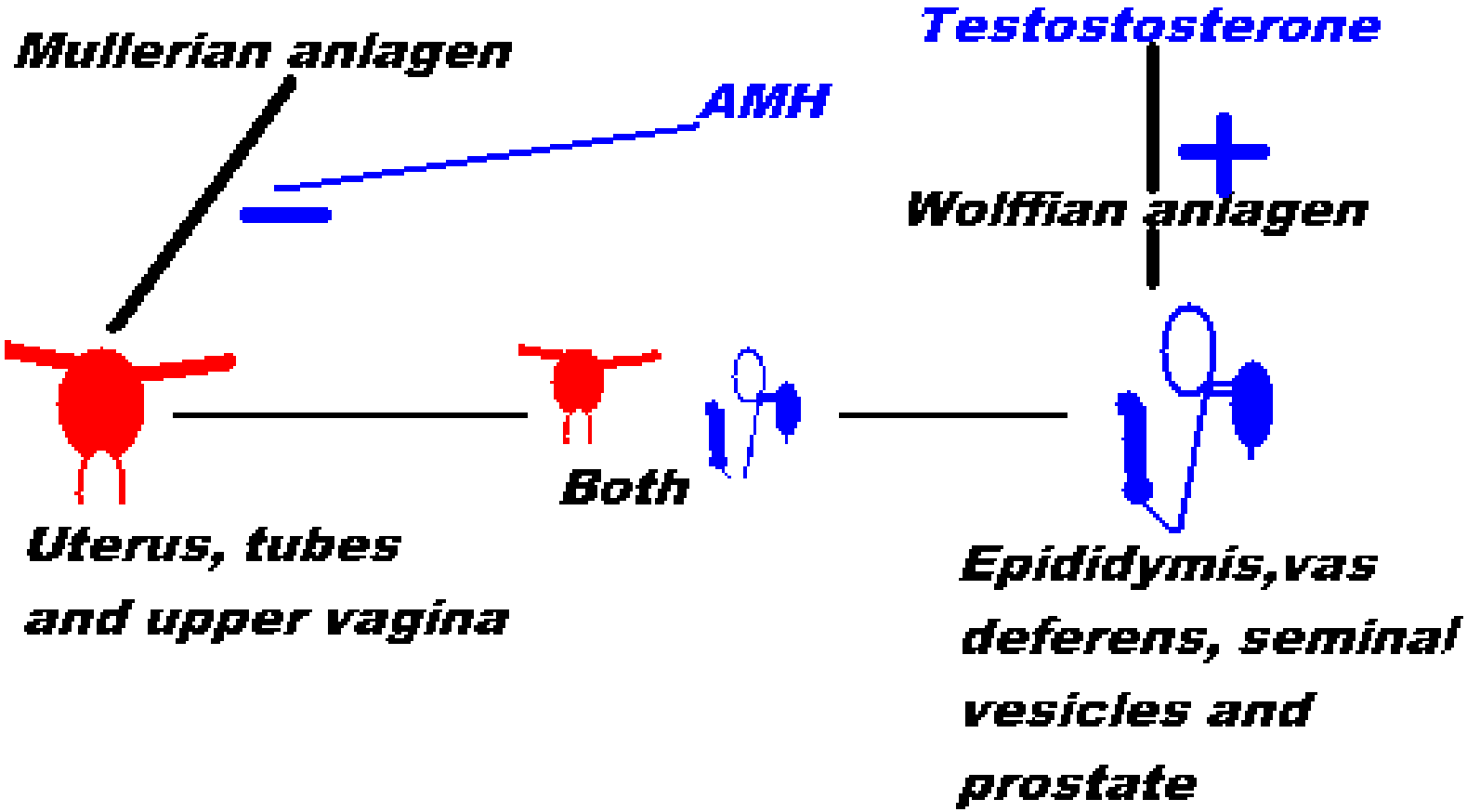
Thus, every man is a unique experiment by nature

Step 1 The gonad

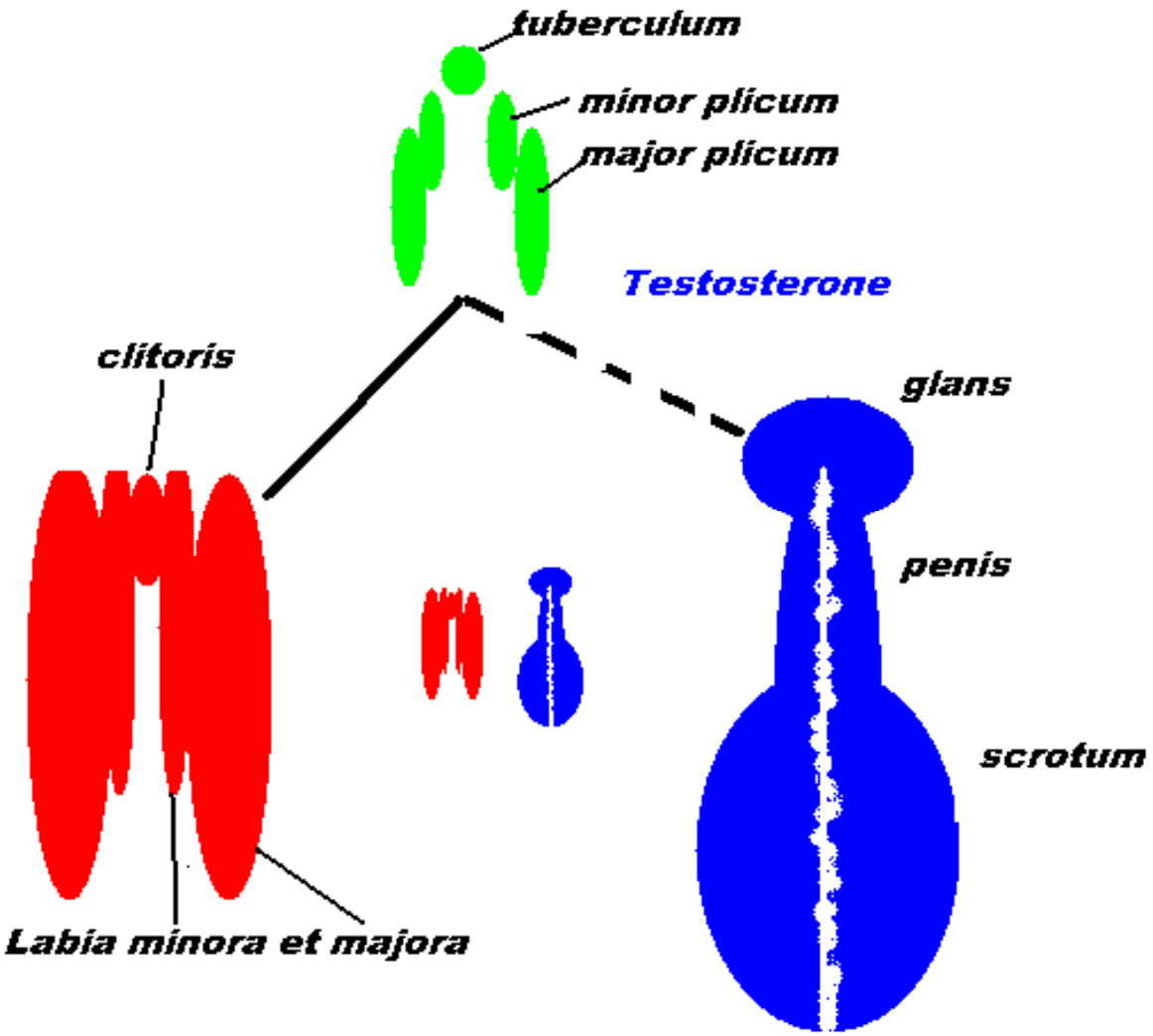


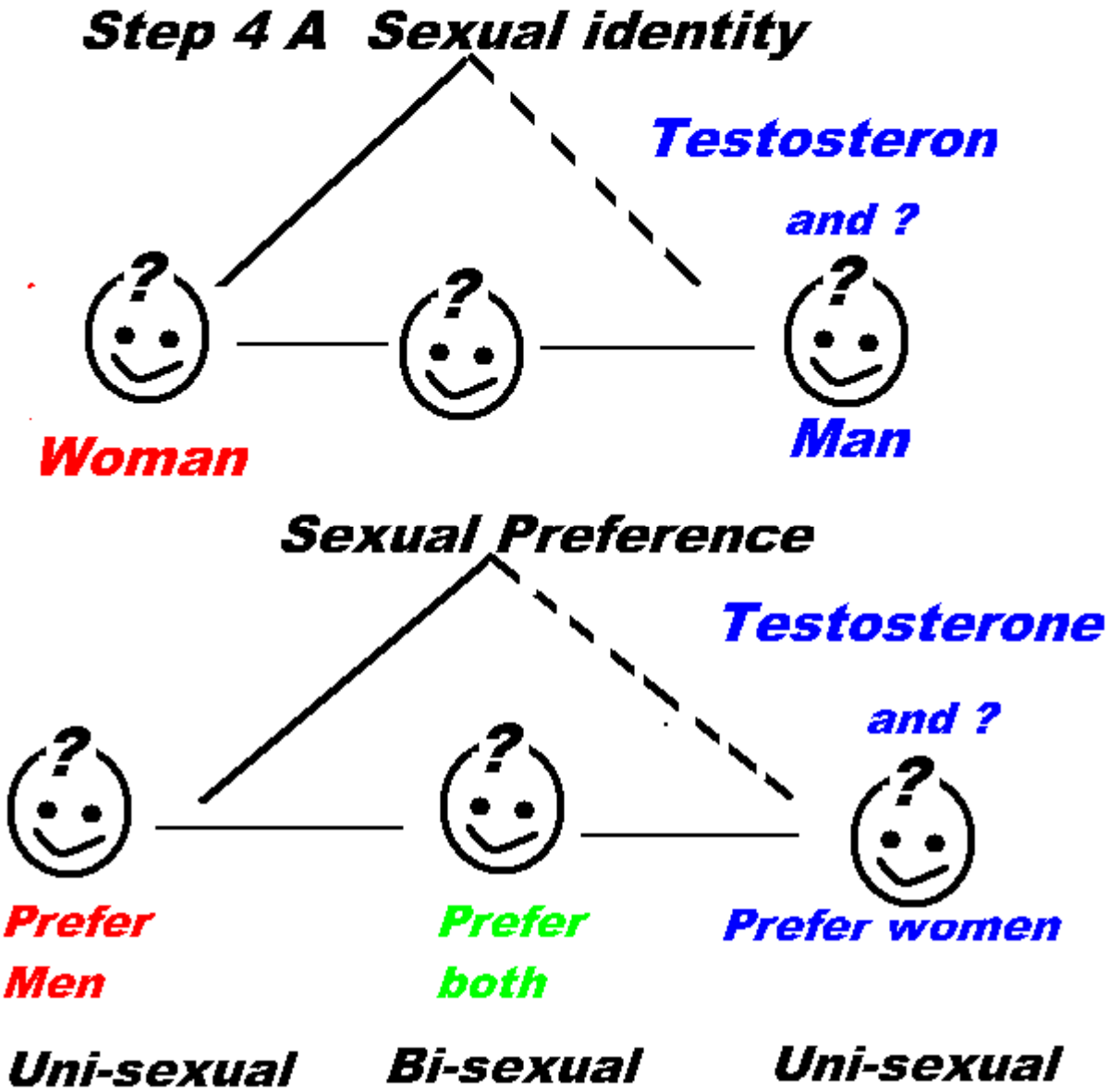
Step 2

The inner genital organs



Step 3 Outer genital organs

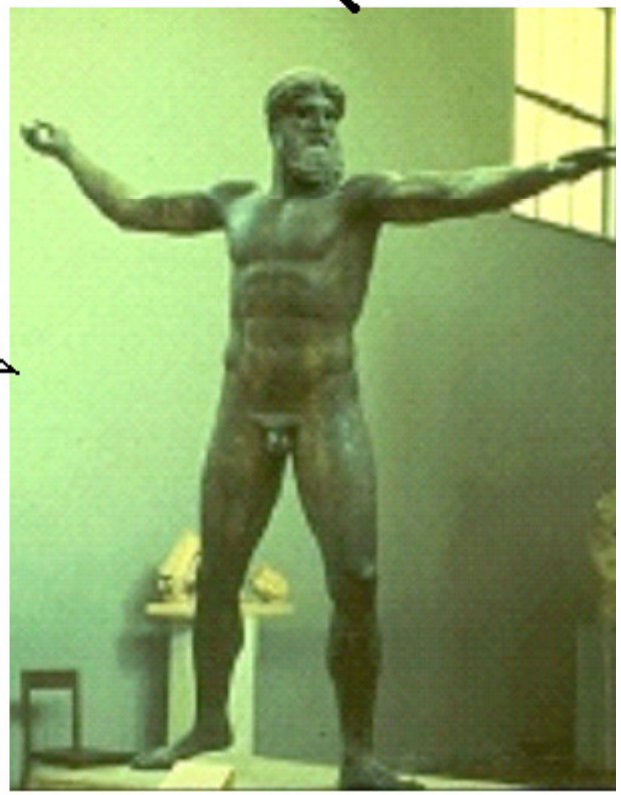


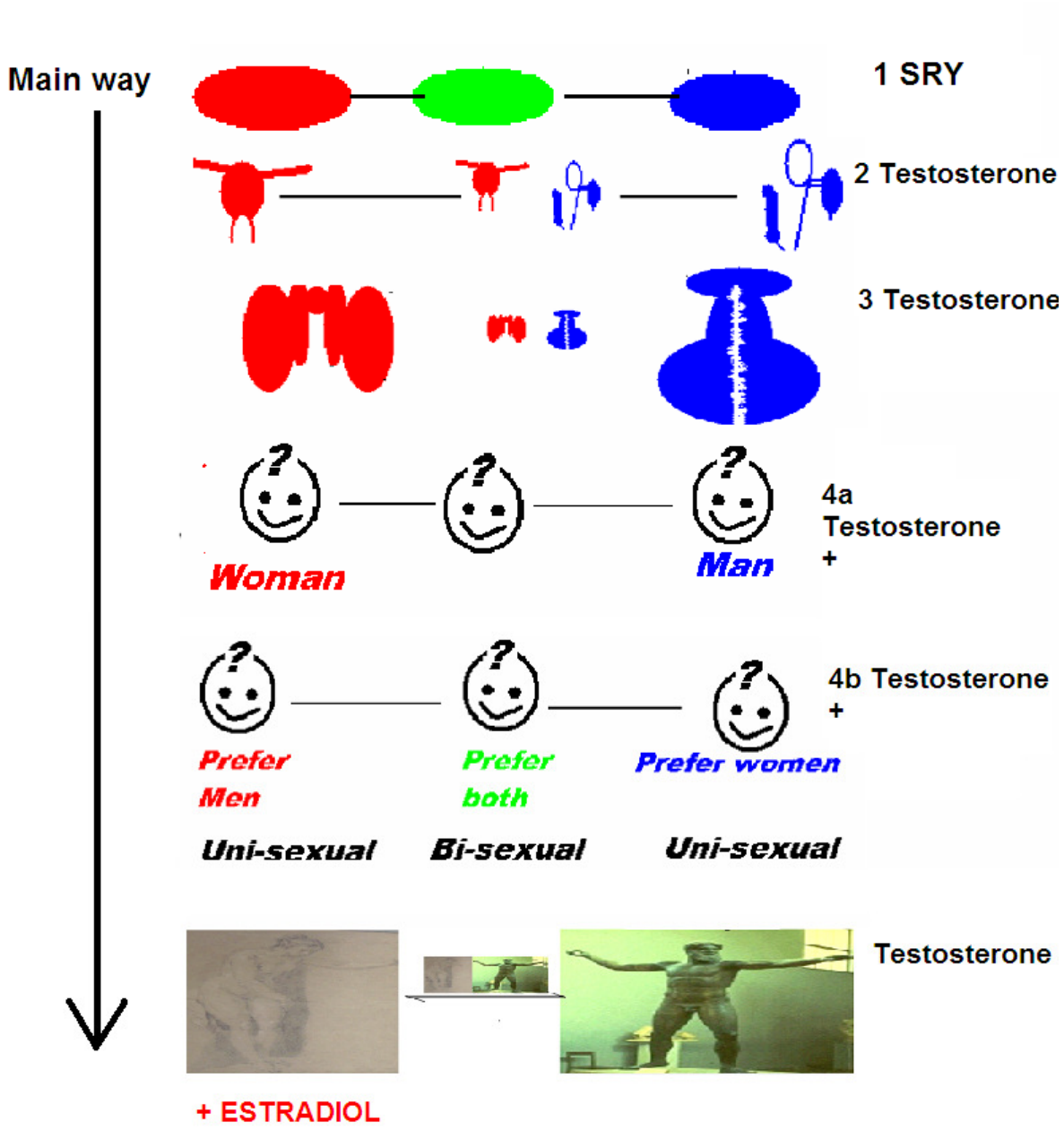

Step 4 A Sexual identity
Testosteron
and ?
Woman
Man
Sexual Preference
Testosterone
and ?
Prefer
Men
Prefer
both
Prefer women
Uni-sexual
Bi-sexual
Uni-sexual

Step 5 Puberty

Estradiol

Testosterone



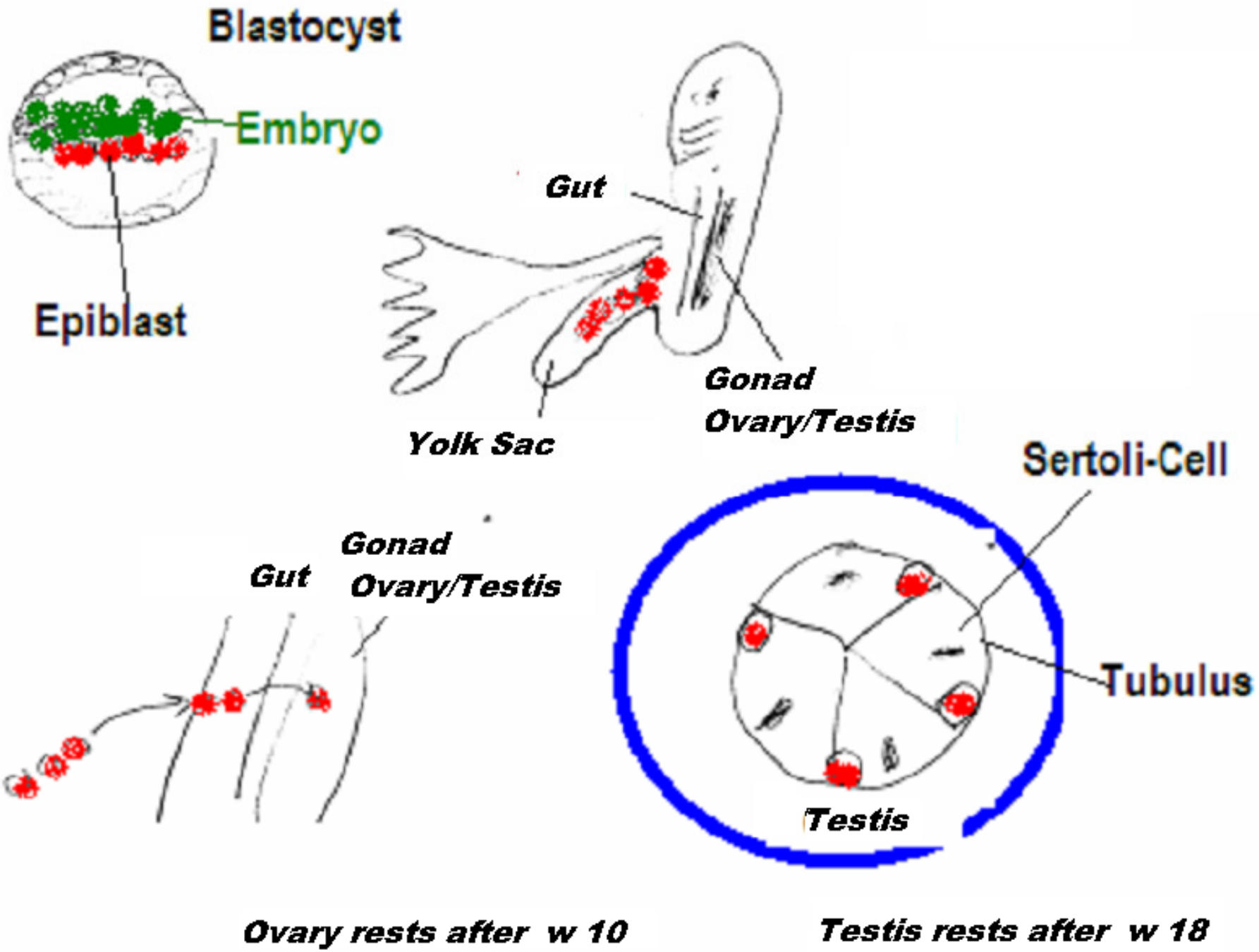


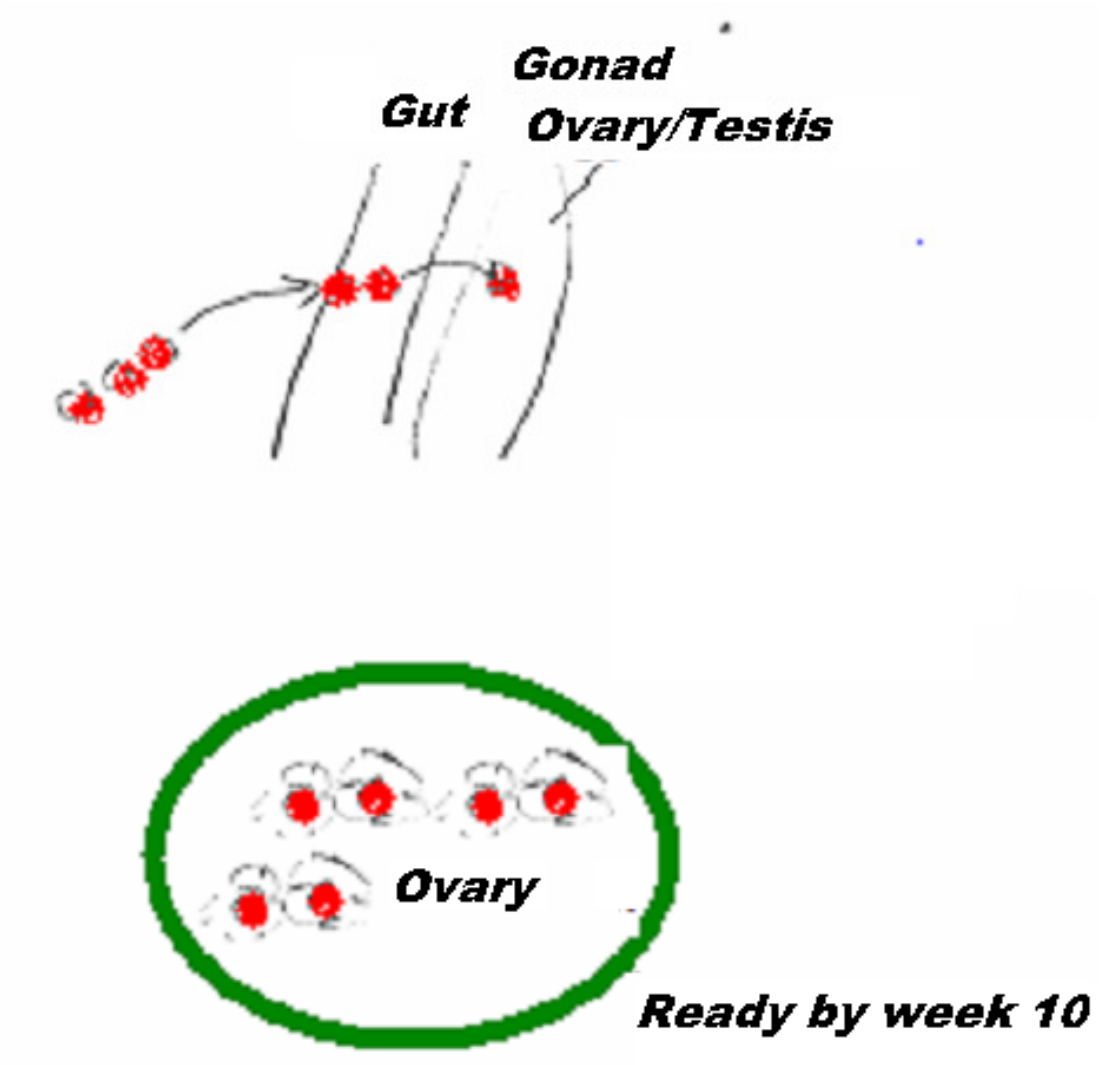
+

The androgen receptor,

the smaller CAG repeats the better effect

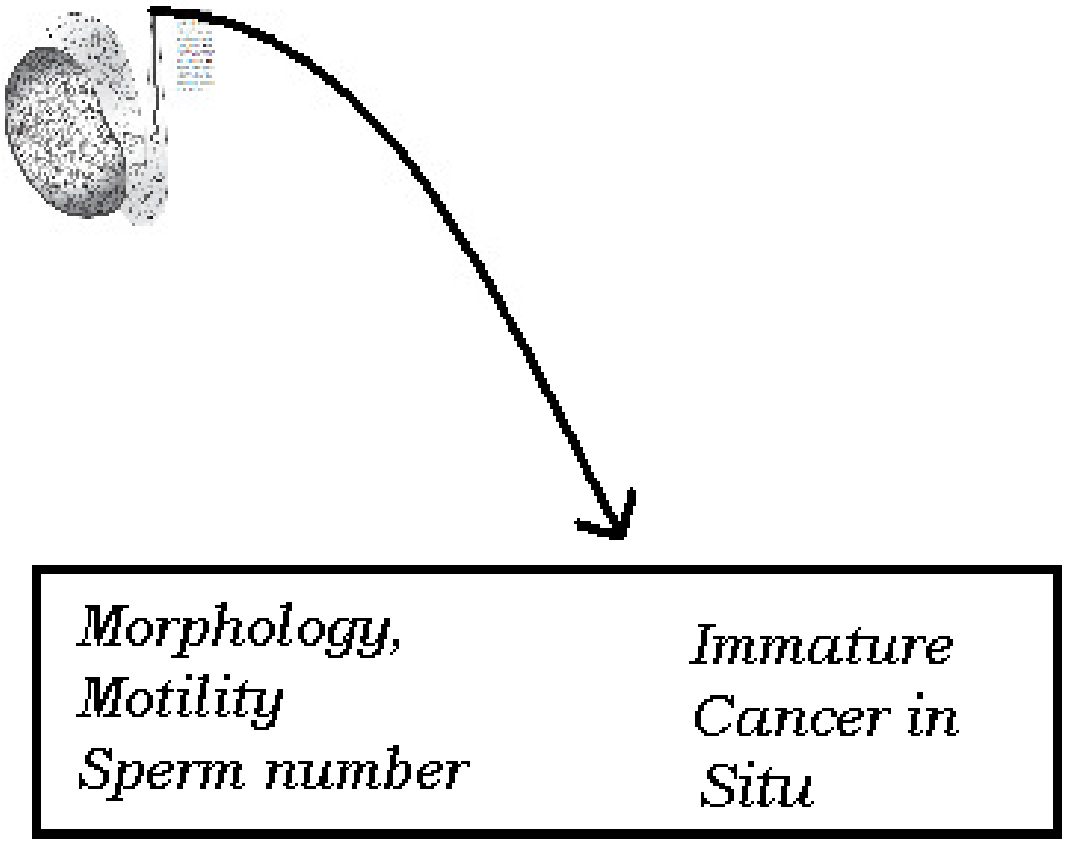
Gonocytes invade the testis


Blastocyst
Embryo
Epiblast
Gut
Gonad
Ovary/Testis
Yolk Sac
Sertoli-Cell
Tubulus
Testis
Gut
Gonad
Ovary/Testis
Ovary rests after w 10
Testis rests after w 18



Take home: Week 10-18 only critical for testis, ovary rests

Semen analysis tells about the testis and sperm production



250 lobuli with 1000 tubules

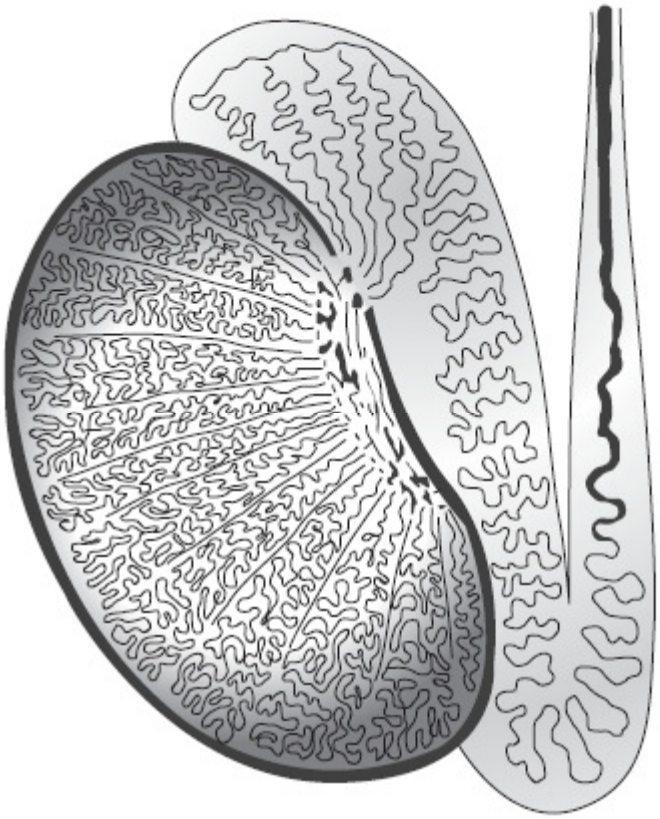
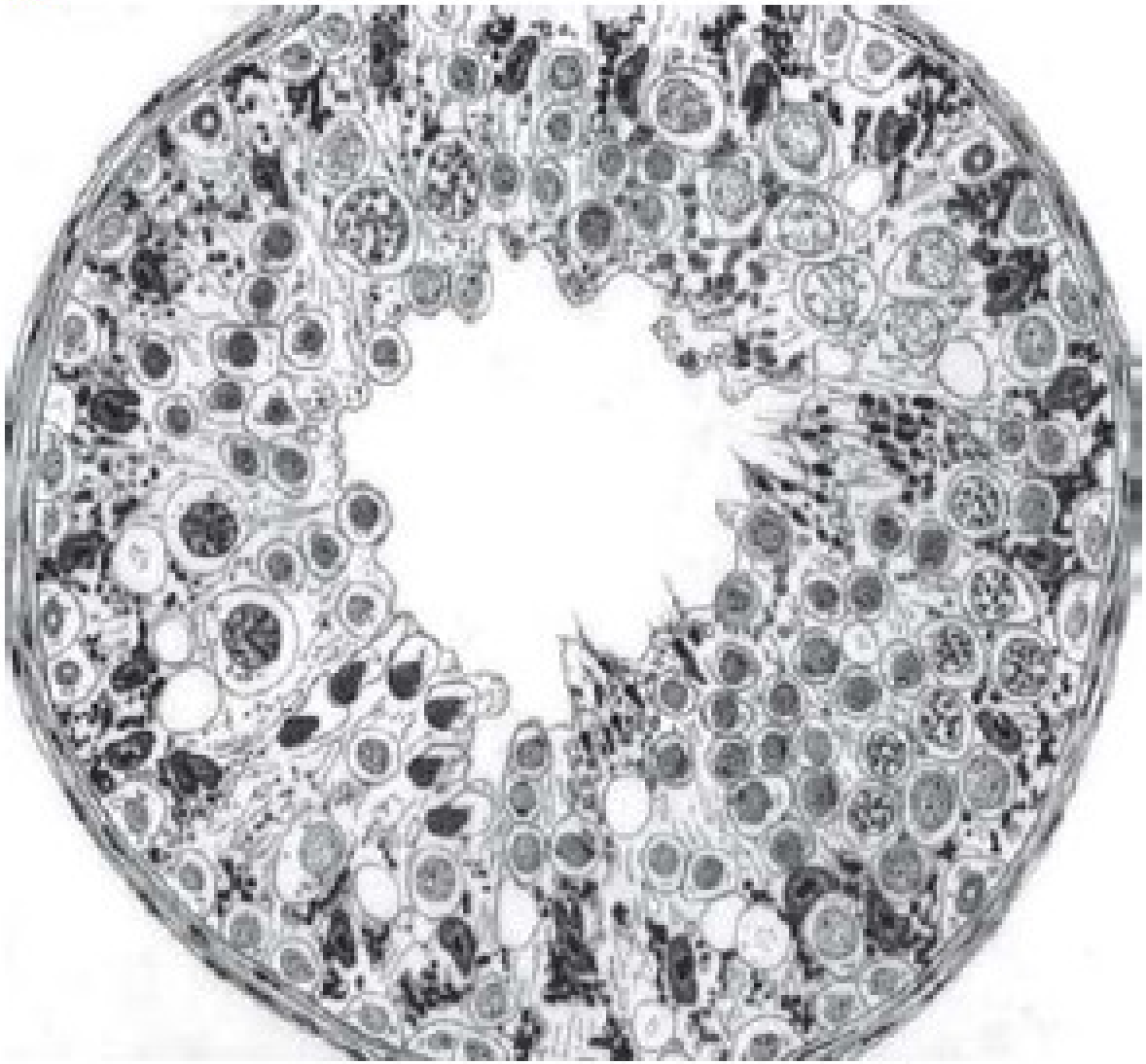


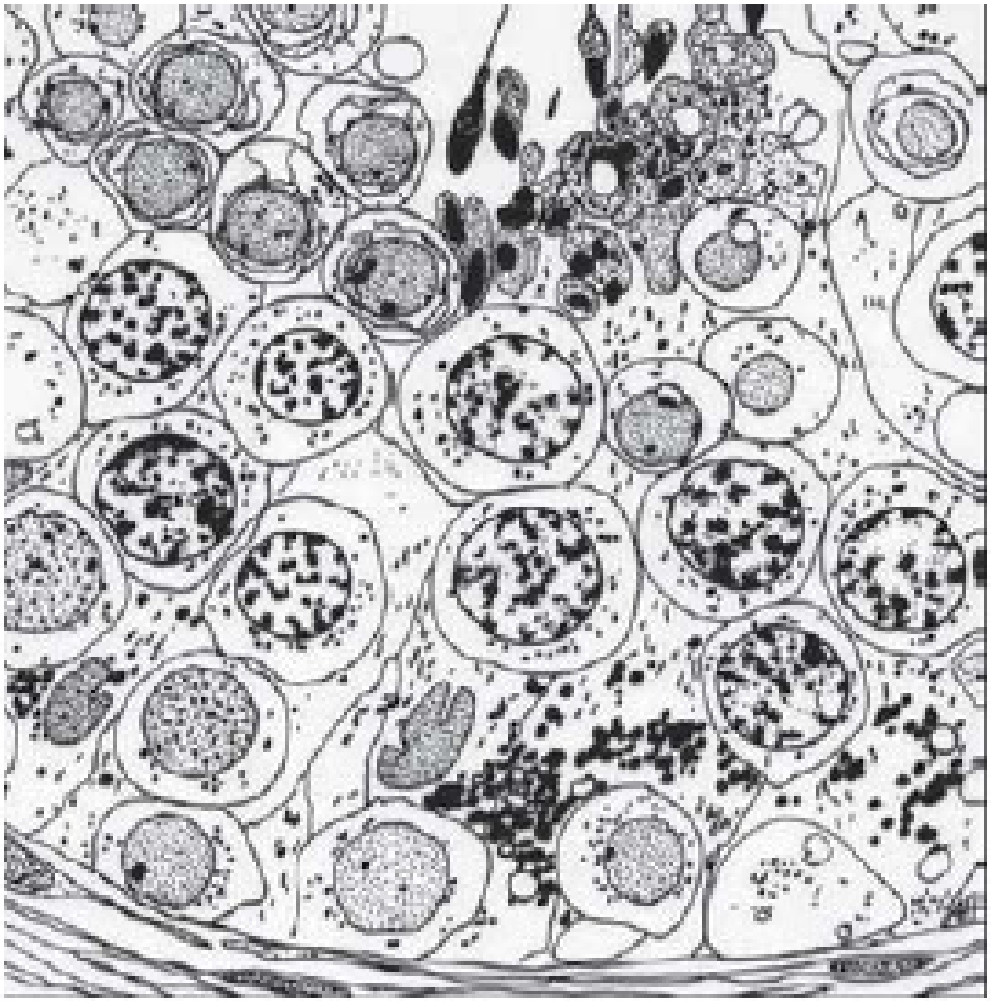
Figure 2.6 A semi-schematic drawing by AF Holstein [4] showing the arrangement of the seminiferous tubules in the human testis, the efferent ductules (6 of 15–10 shown) connecting the rete testis to the epididymal duct, and the continuation of the epididymal duct into the vas deferens.

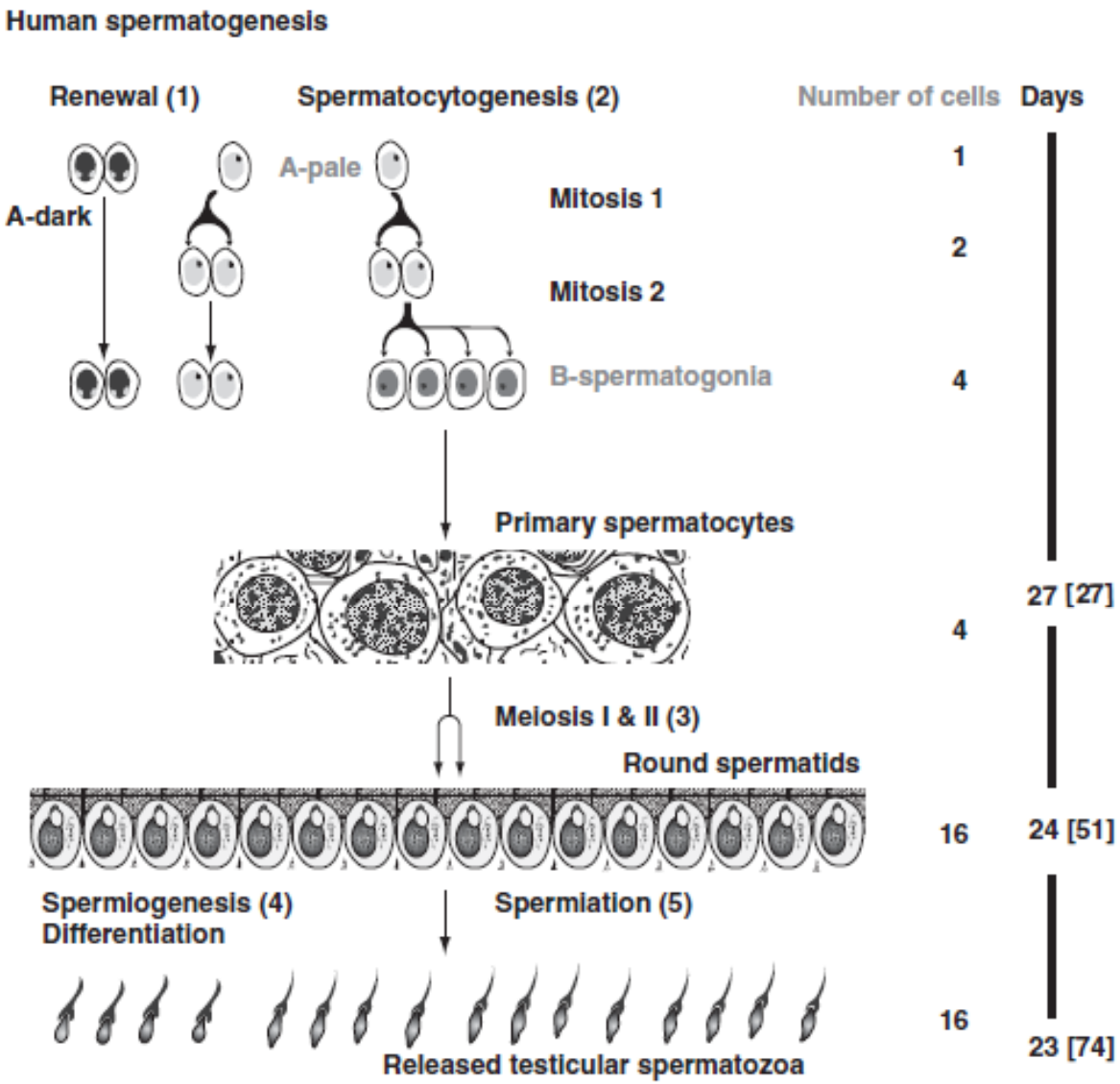
NB All drawings by AF Holstein

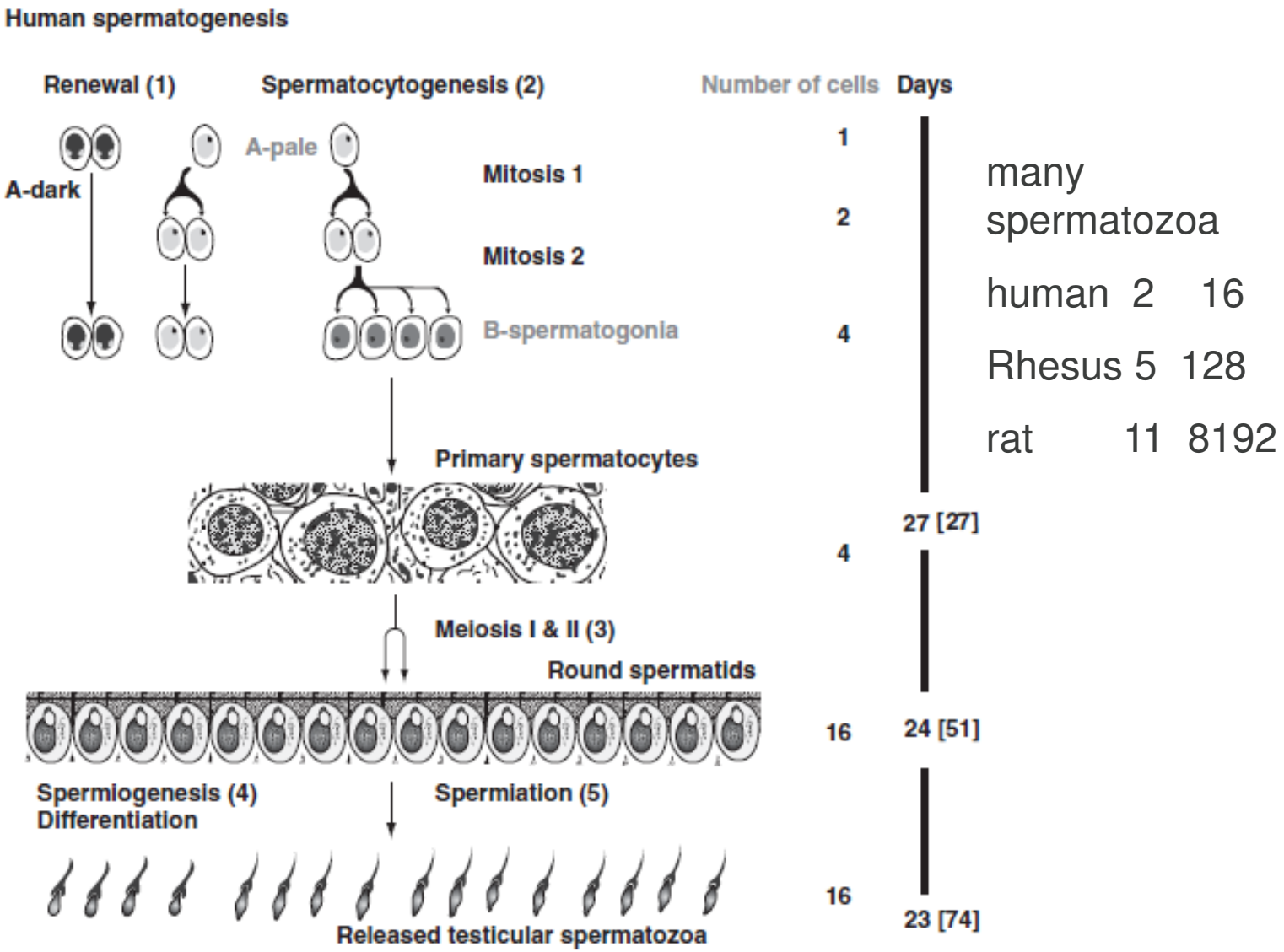
A



B




Human spermatogenesis
Renewal (1)
Spermatocytogenesis (2)
Number of cells
Days
A-dark
A-pale
Mitosis 1
Mitosis 2
B-spermatogonia
1
2
4
Primary spermatocytes
4
27 [27]
Meiosis I & II (3)
Round spermatids
16
24 [51]
Spermiogenesis (4)
Differentiation
Spermiation (5)
16
23 [74]
Released testicular spermatozoa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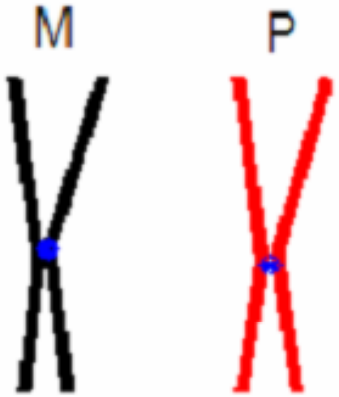
Released testicular spermatozoa
many spermatozoa
human 2 16
Rhesus 5 128
rat 11 8192

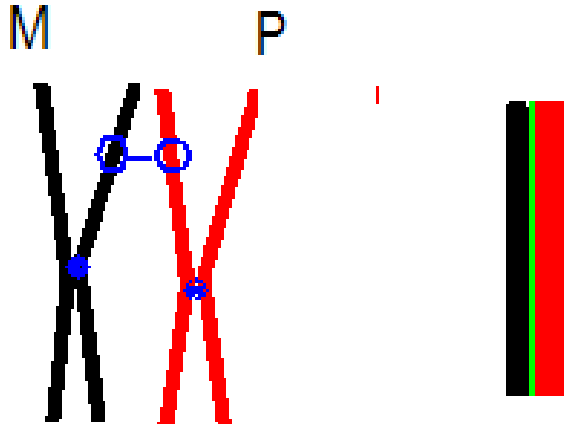
46 chromosomes
23 pairs
one paternal (P)
one maternal (M)



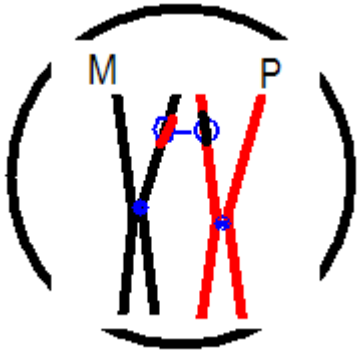
At Meiosis I
in the primary
spermatocyte

Chromosomes are
paired

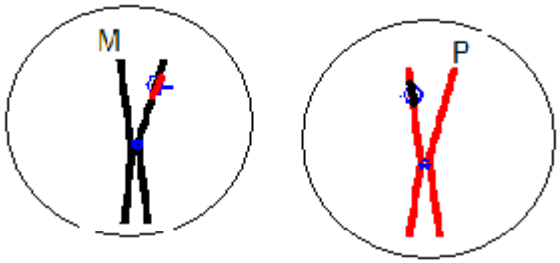
Recombination !



Chiasmata=crossing over = recombination

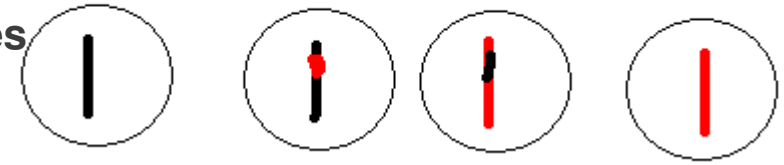


Meiosis I



Meiosis II

Four different outcomes



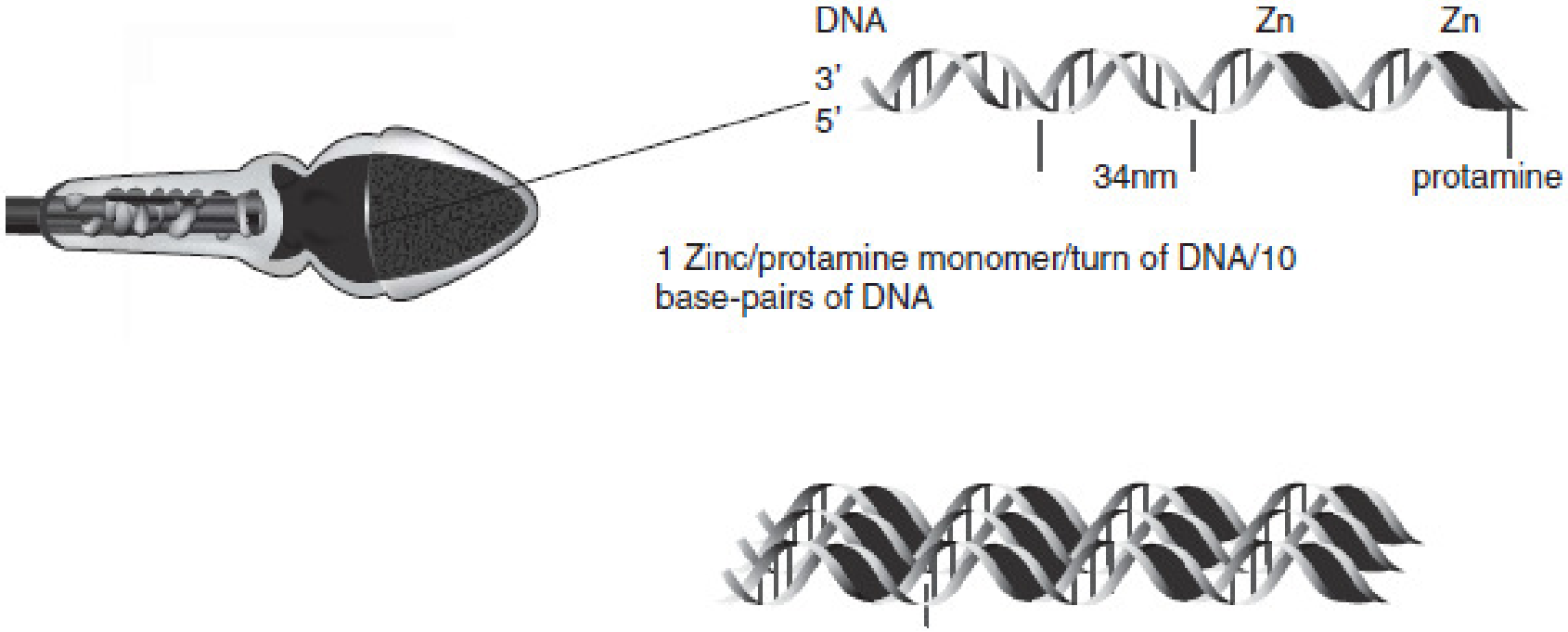
$4^{23} = 70*10^{12}$

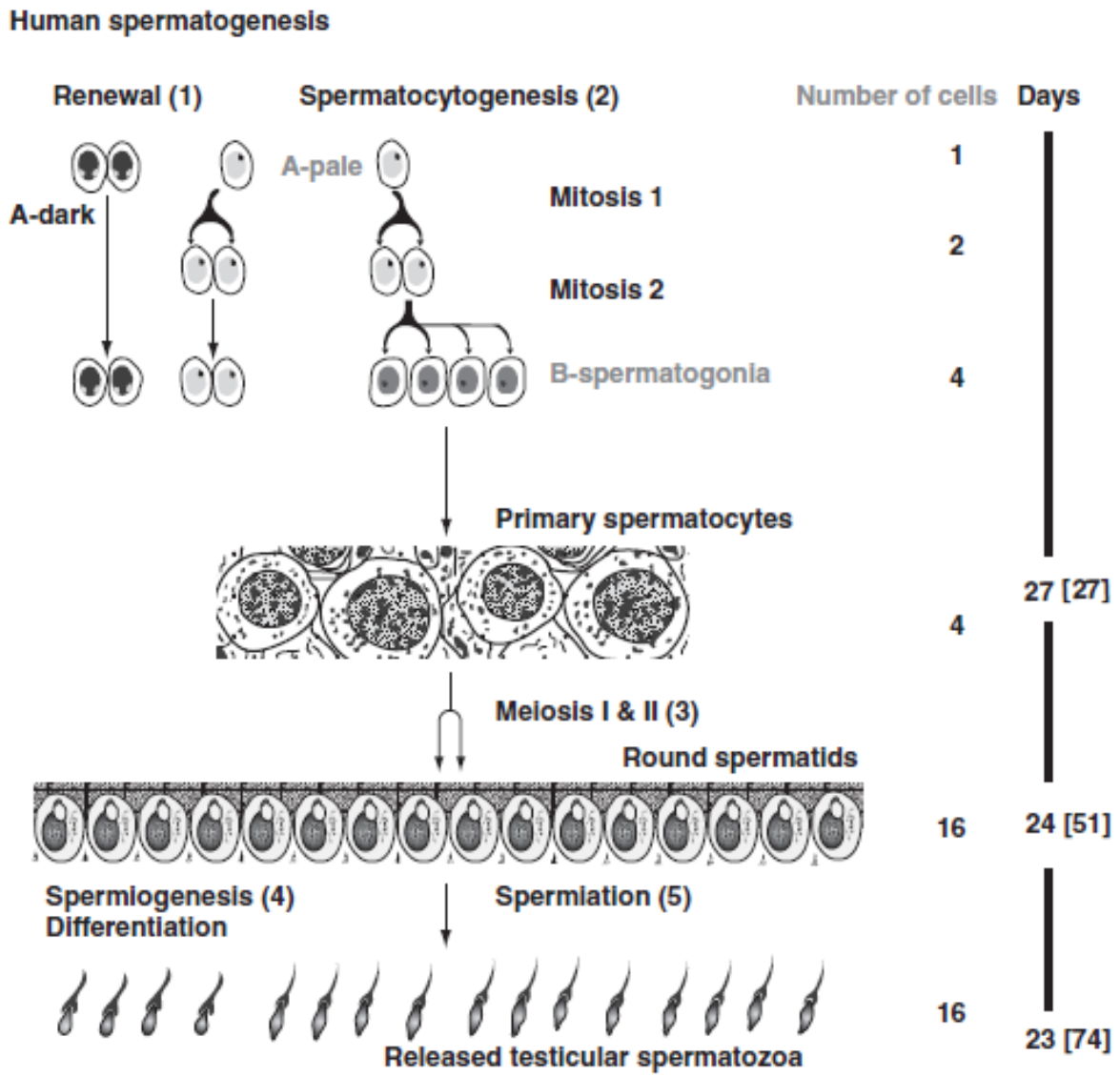
Every spermatozoon and oocyte is unique: 1 combination out of 70 trillions
Every human being is unique: 1 combination out of 4900 trillions trillions

Exception homozygotic twins and other cloning activities

Histones are exchanged for protamines

Chromsomal fiber composed of three strings the DNa helix and the protamine-polymer

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24 [51]
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Differentiation
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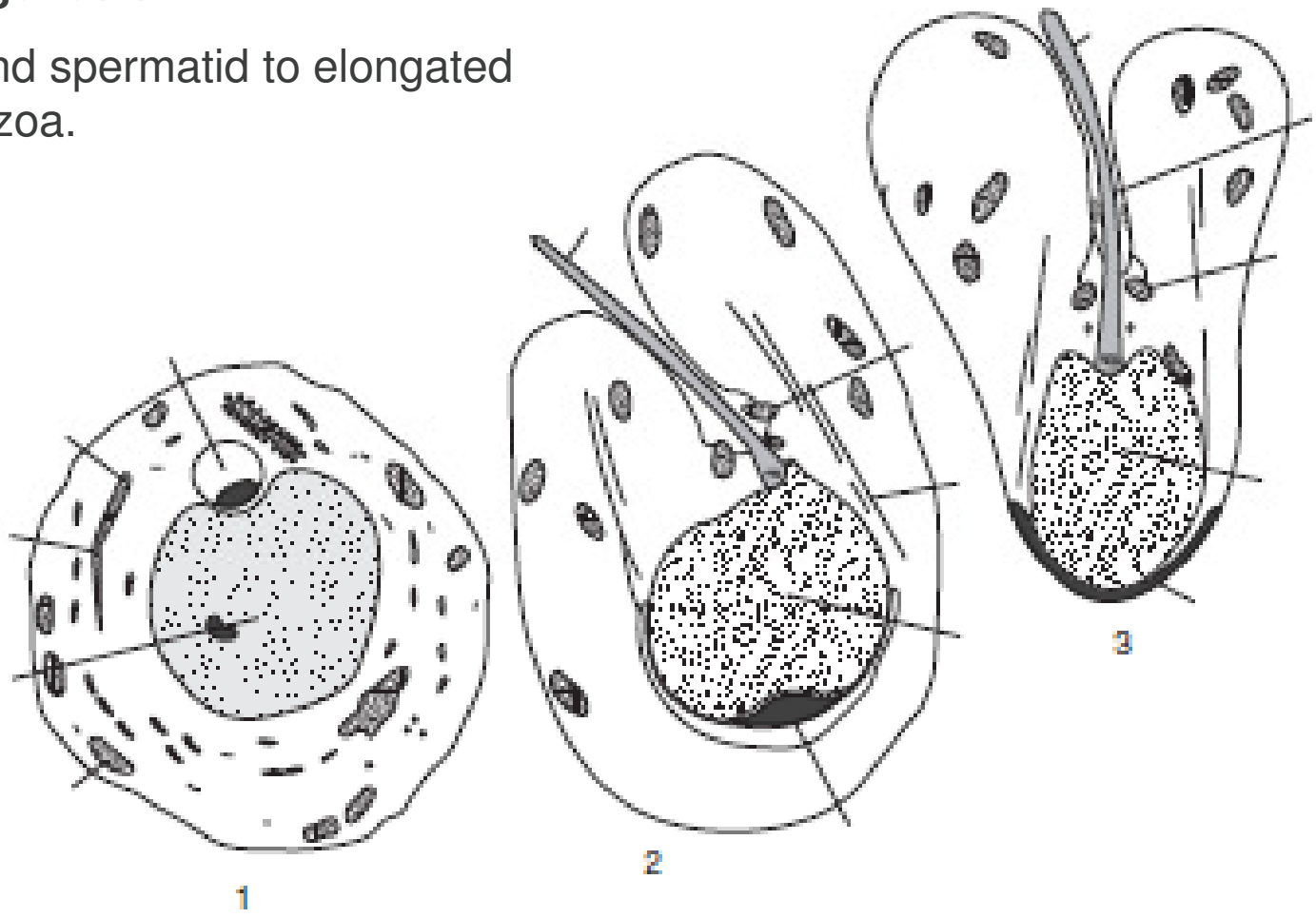
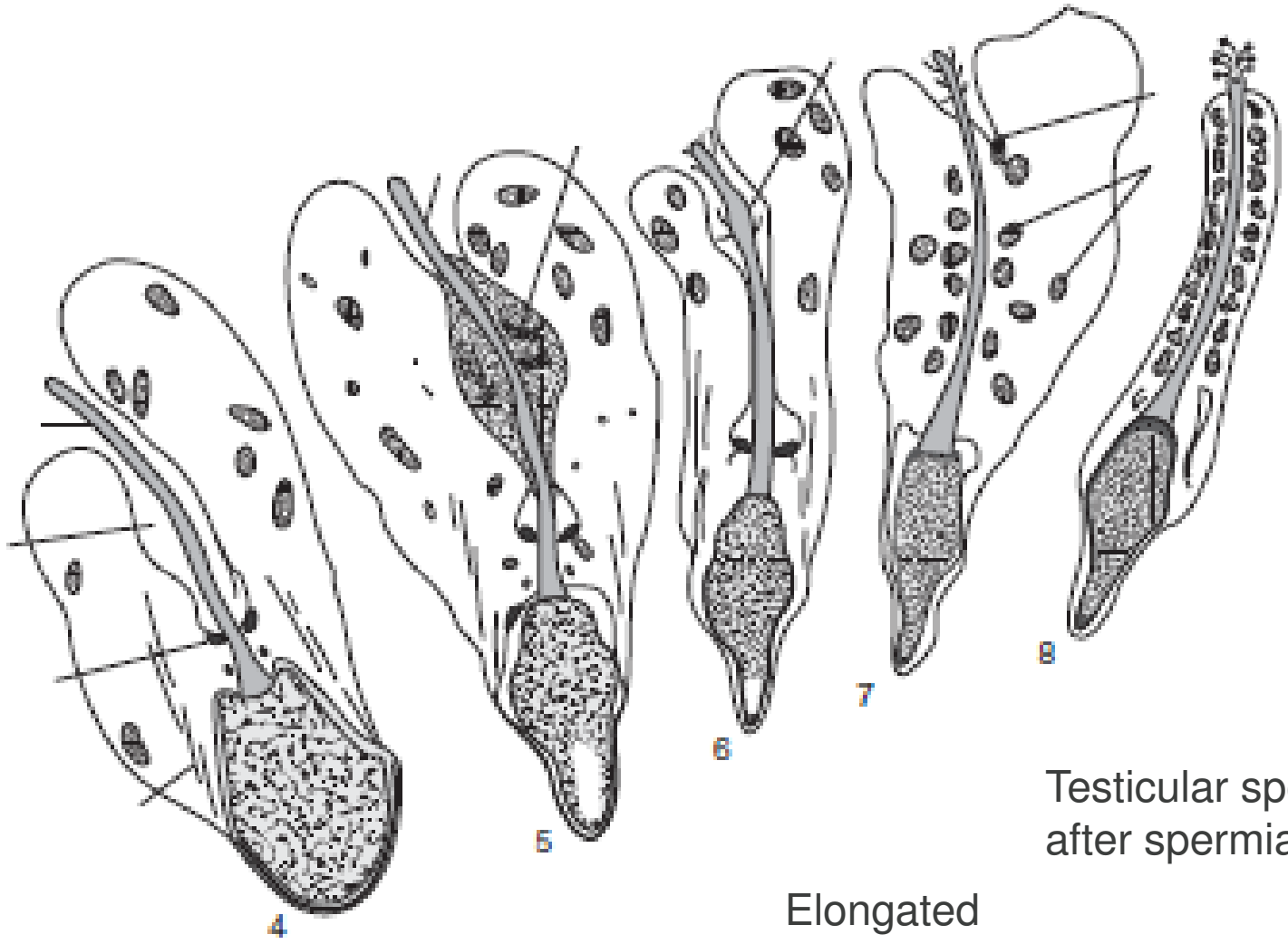
Released testicular spermatozoa

74 days

Spermiogenesis

From round spermatid to elongated spermatozoa.

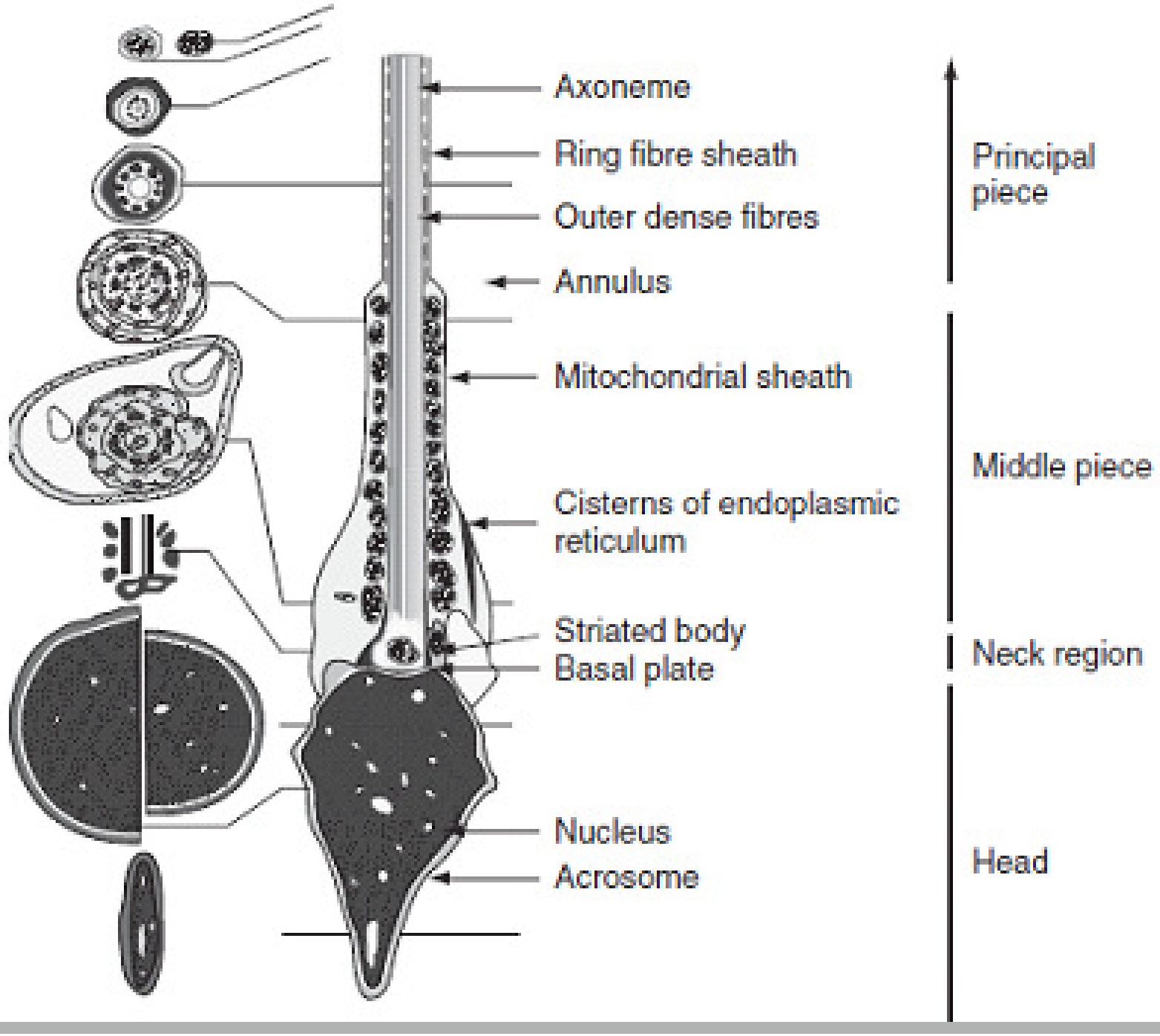




Elongating

Elongated

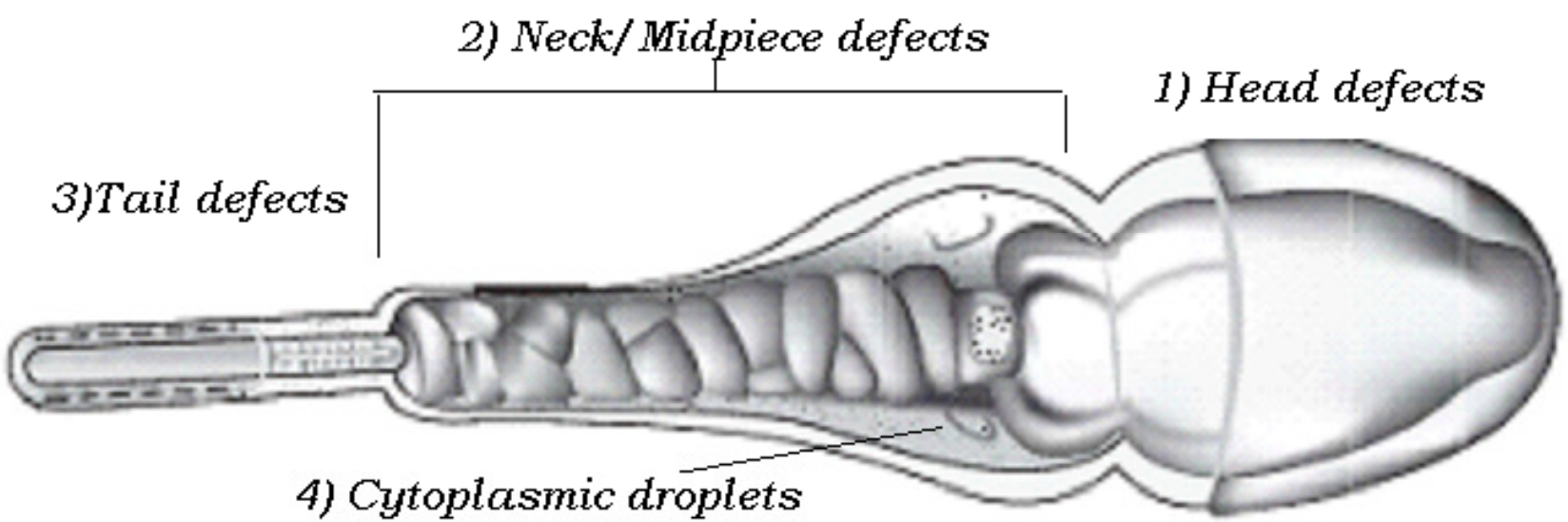
Testicular spermatozoon
after spermiation


Axoneme
Ring fibre sheath
Outer dense fibres
Annulus
Mitochondrial sheath
Cisterns of endoplasmic reticulum
Striated body
Basal plate
Nucleus
Acrosome
Principal piece
Middle piece
Neck region
Head

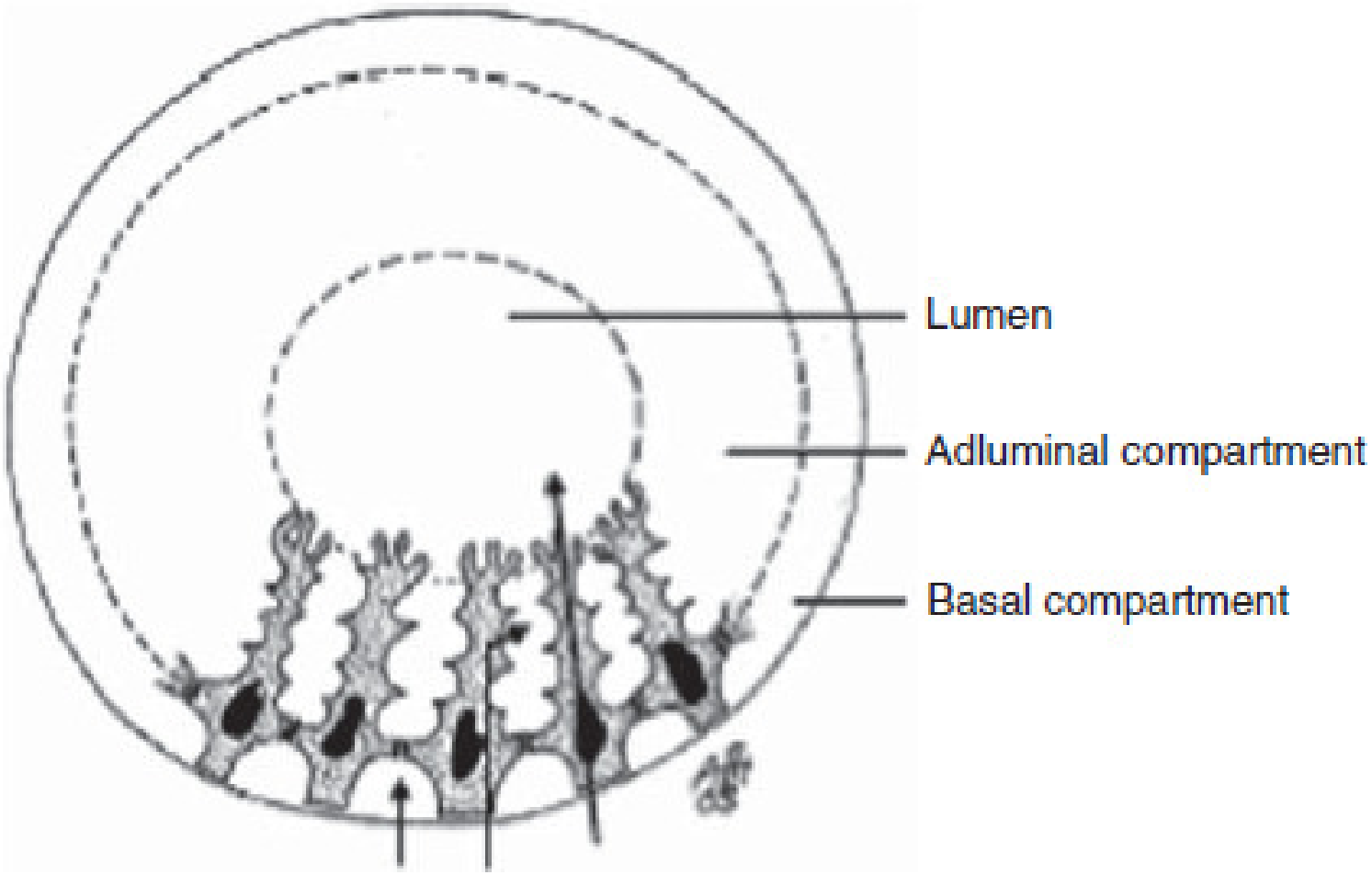
Sperm morphology
Normal or
Not normal

$$TZI = \text{Teratozoo-index} = \frac{\%\ (1+2+3+4)}{\%\ \text{Not Normal}}$$

1.00-4.00
> 2,00 not good



Blood testis barrier



Spermatogenesis conclusions

- (1) Renewal of stem cells. (20 mitosis every year; at 35 year 20 x 20 = 400
- mitosis) Consequences for DNA and mtDNA

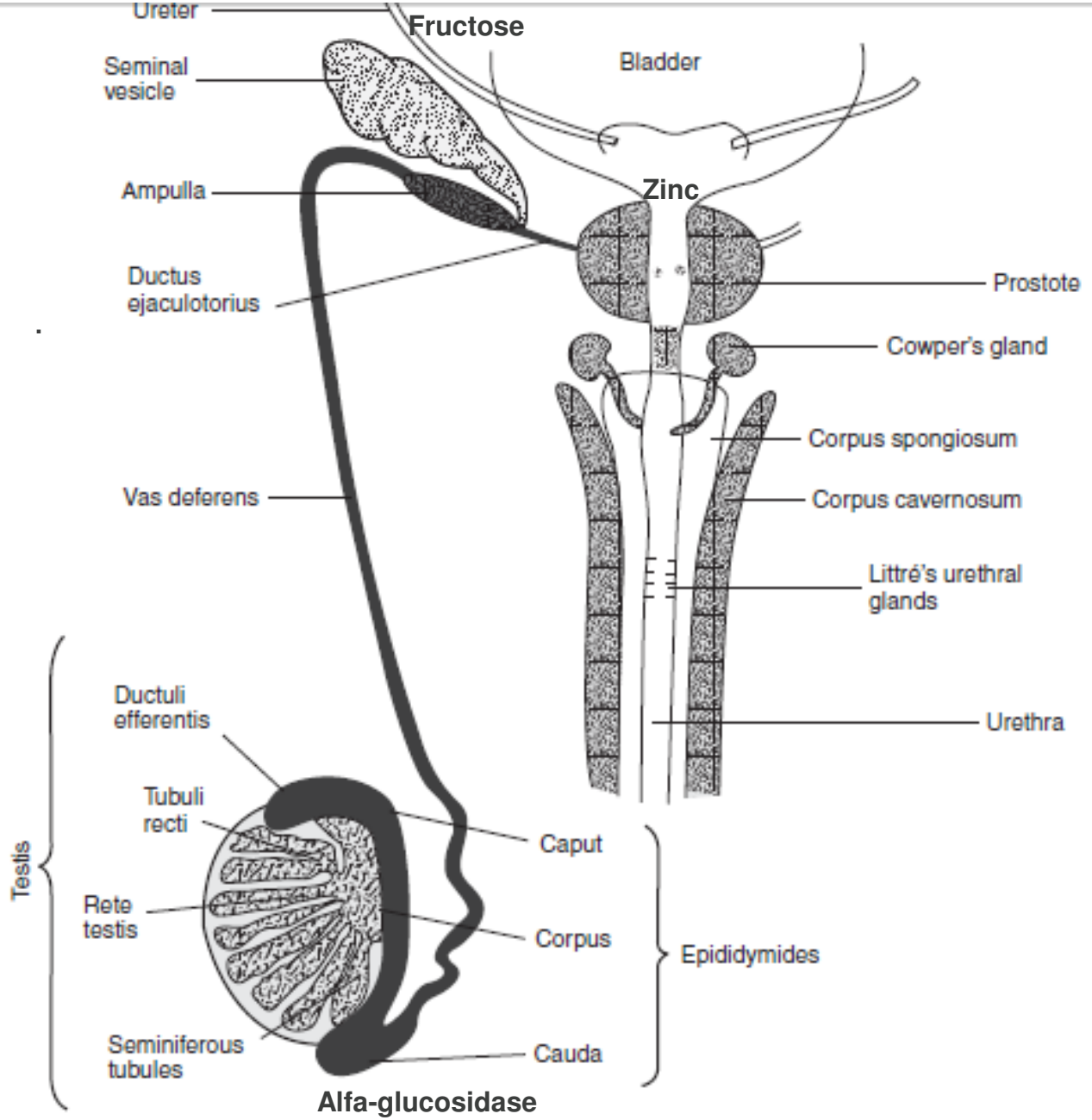
- (2) Spermatocytogenesis, by which two extra mitotic divisions in human, makes possible the production of 16 spermatozoa from one spermatogonium.

- (3) Meiosis by which unique and haploid cells are formed.

- (4) Spermiogenesis i.e the metamorphosis into a spermatozoon The sperm chromatin becomes a semi-crystalline temporary structure comprised of one zinc, for every protamine molecule for every turn of the DNA-helix.

- (5) Spermiation by which the Sertoli Cell liberate the single spermatozoon from the cluster of 16.

The way out!



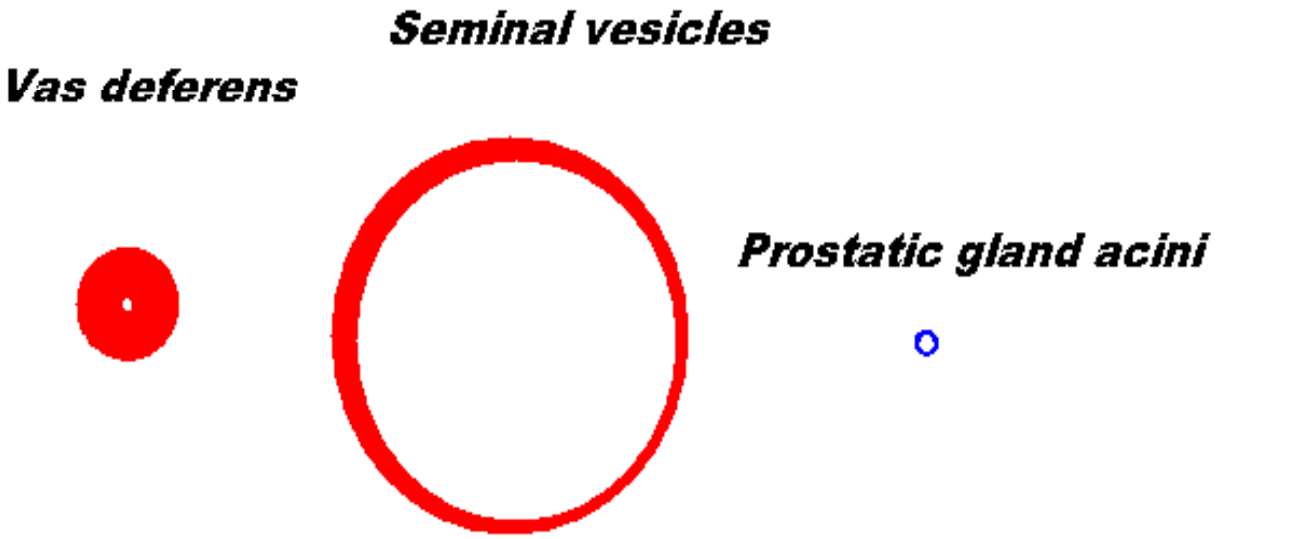
Testosterone converted to DHT

By luminal fluid to caput (Type I converting enzyme)

By blood to the rest (Type 2 (blocked by eg finasteride)

Emission before ejaculation

- **Emission = The emptying of spermatozoa and fluids into the urethra**
- Symphatic system; **noradrenalin** as transmittor, acting on **alfa-1 receptors** of smooth muscles in
- Epididymal cauda Vas deferens Seminal vesicles Prostatic glands (20-25)
- (+ inner sfinkter of bladder)
- **substances interacting with alfa-1 affects transport**

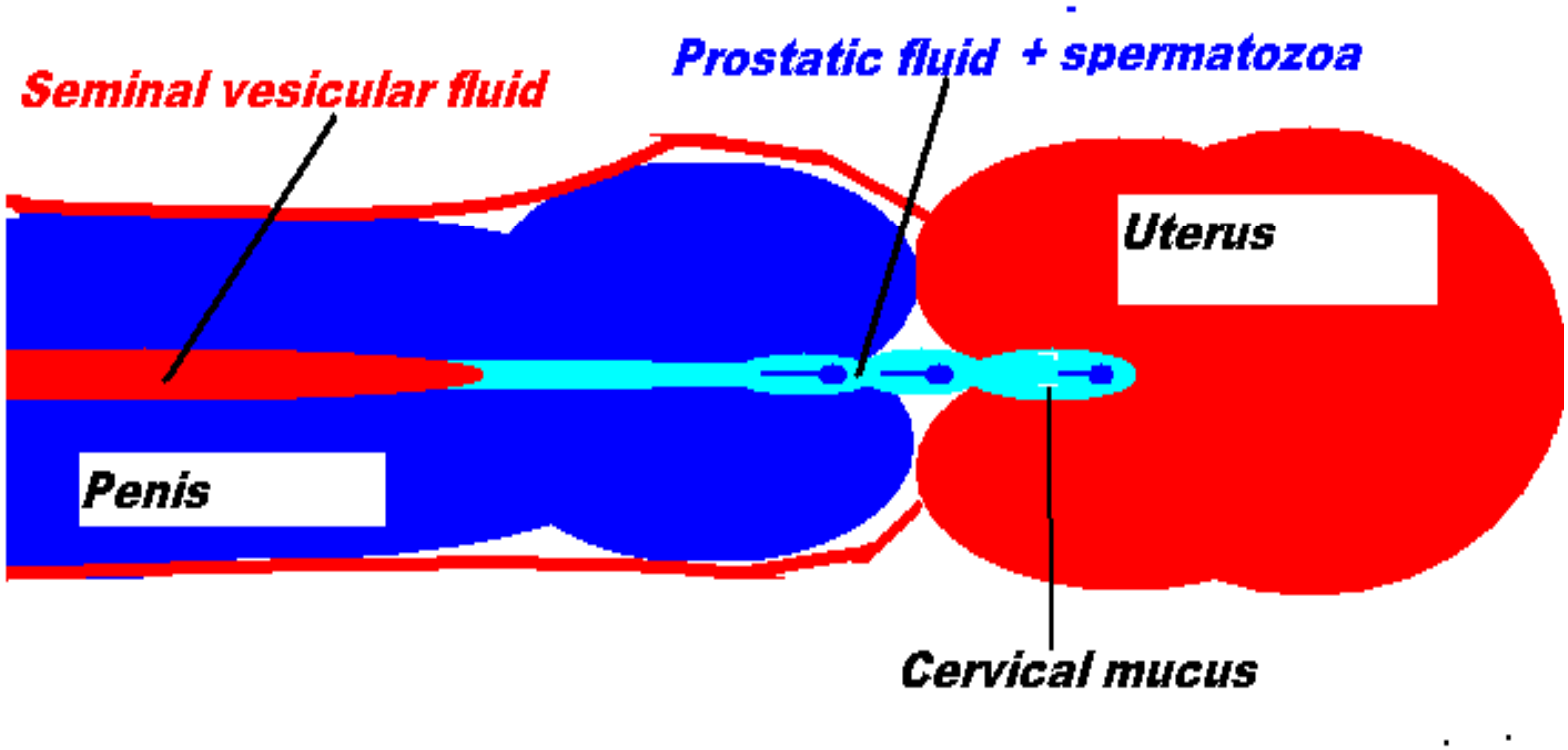


The geometry of the lumina and smooth muscle walls determine the order of emptying.

The Sequence of Ejaculation

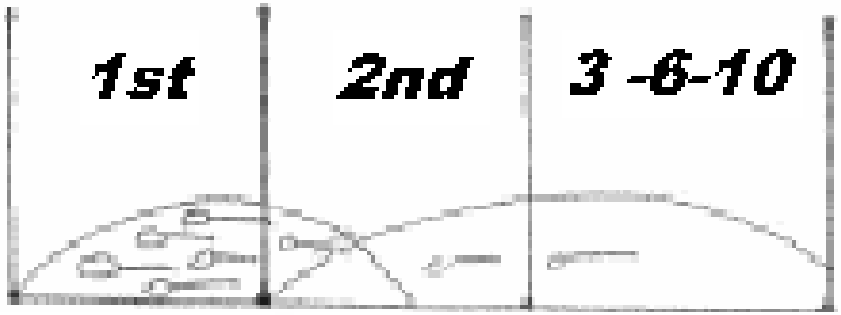
– Man offers the woman spermatozoa in prostatic fluid

Physiology of ejaculation



Ejaculatory sequence

1949



Prostatic fluid

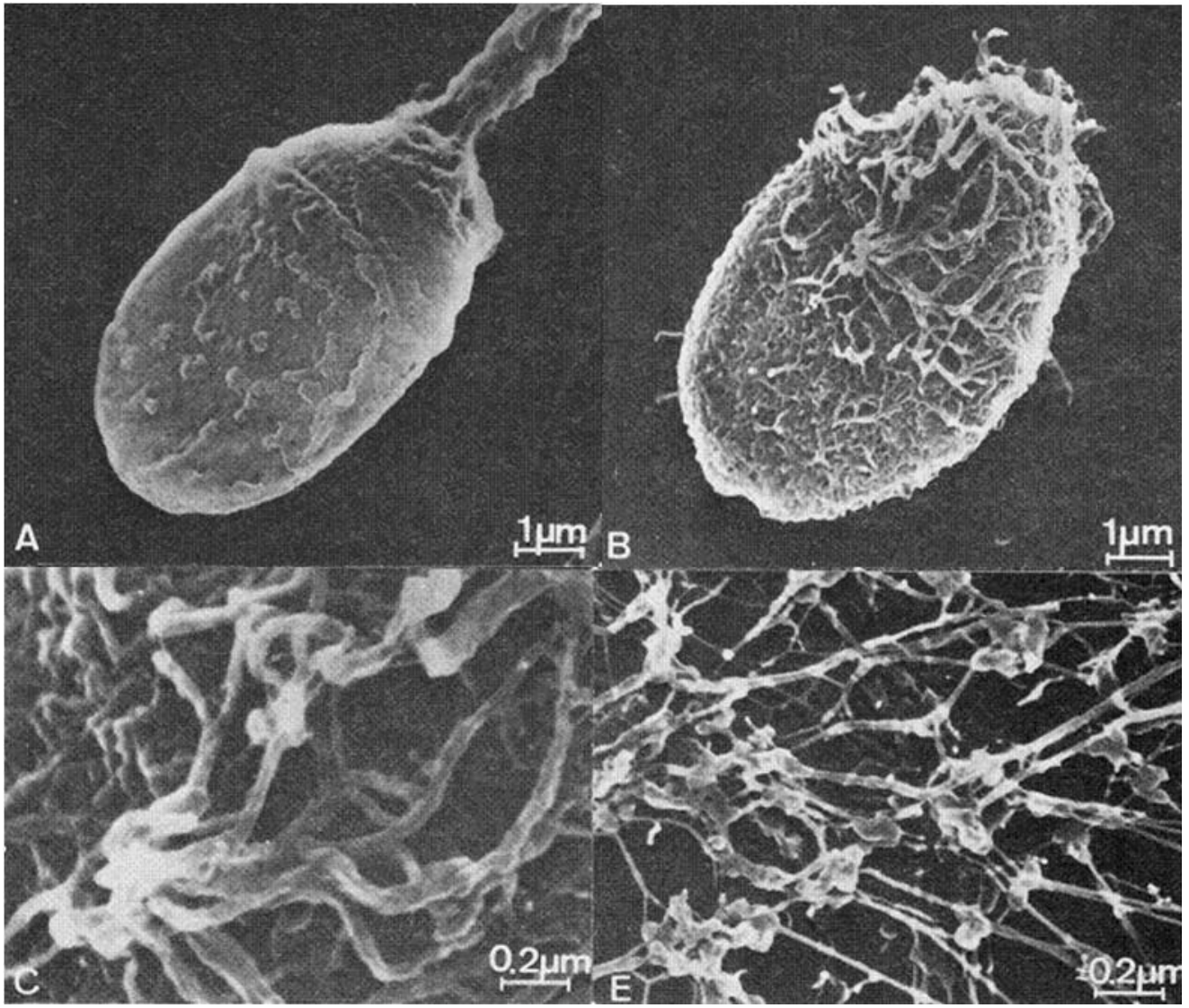
Seminal vesicular fluid

No gel
Vitality +
Motility +

Chromatin stability +
Chromatin zinc +

Gel
Motility-
Vitality-

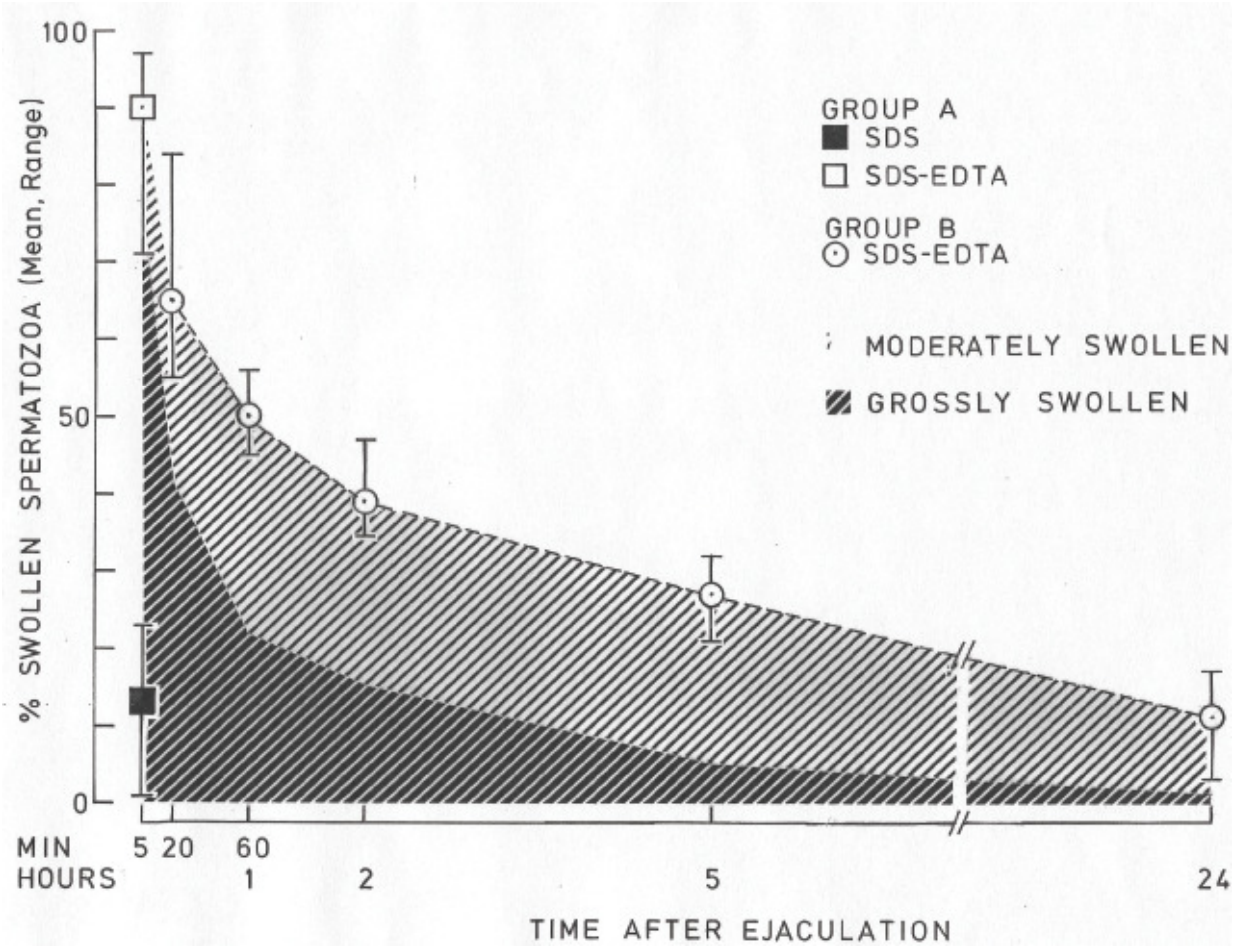
Chromatin zinc -
Chromatin stability -- ++ [55]



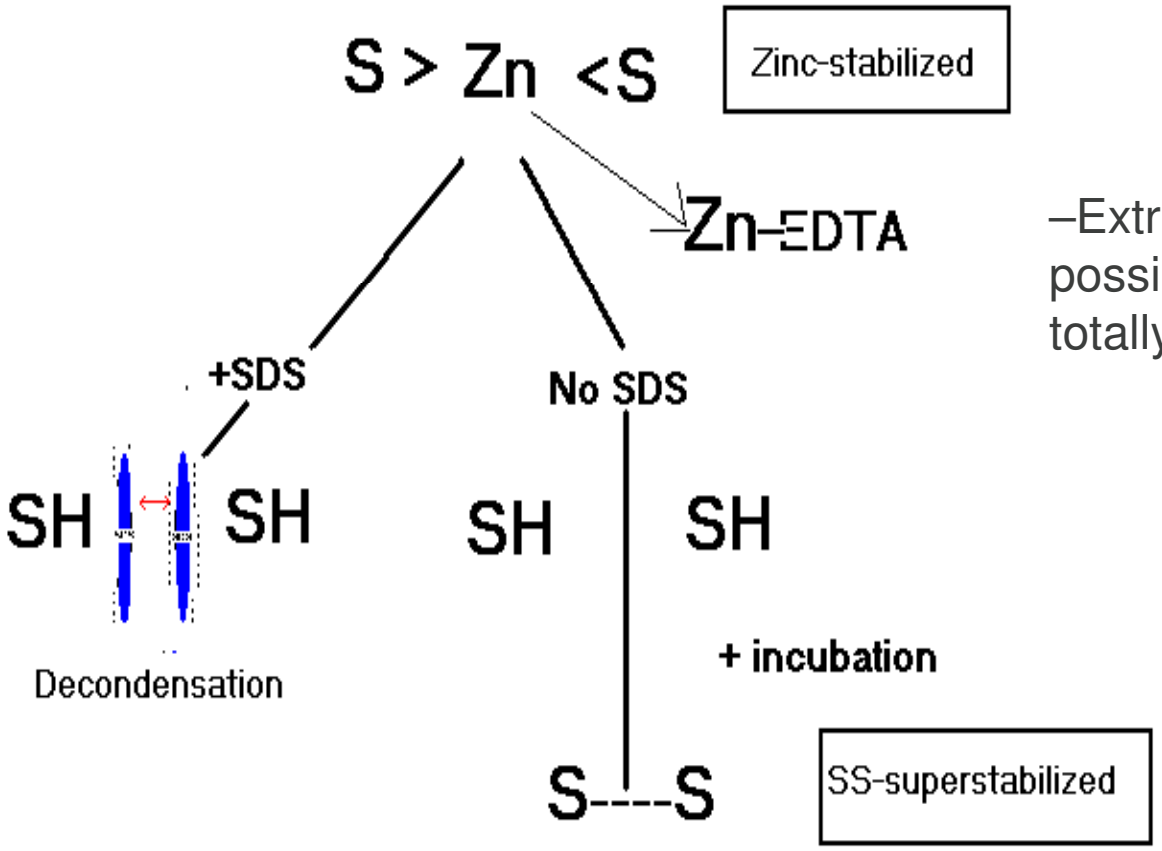
SDS

SDS + EDTA

At Ejaculation and after

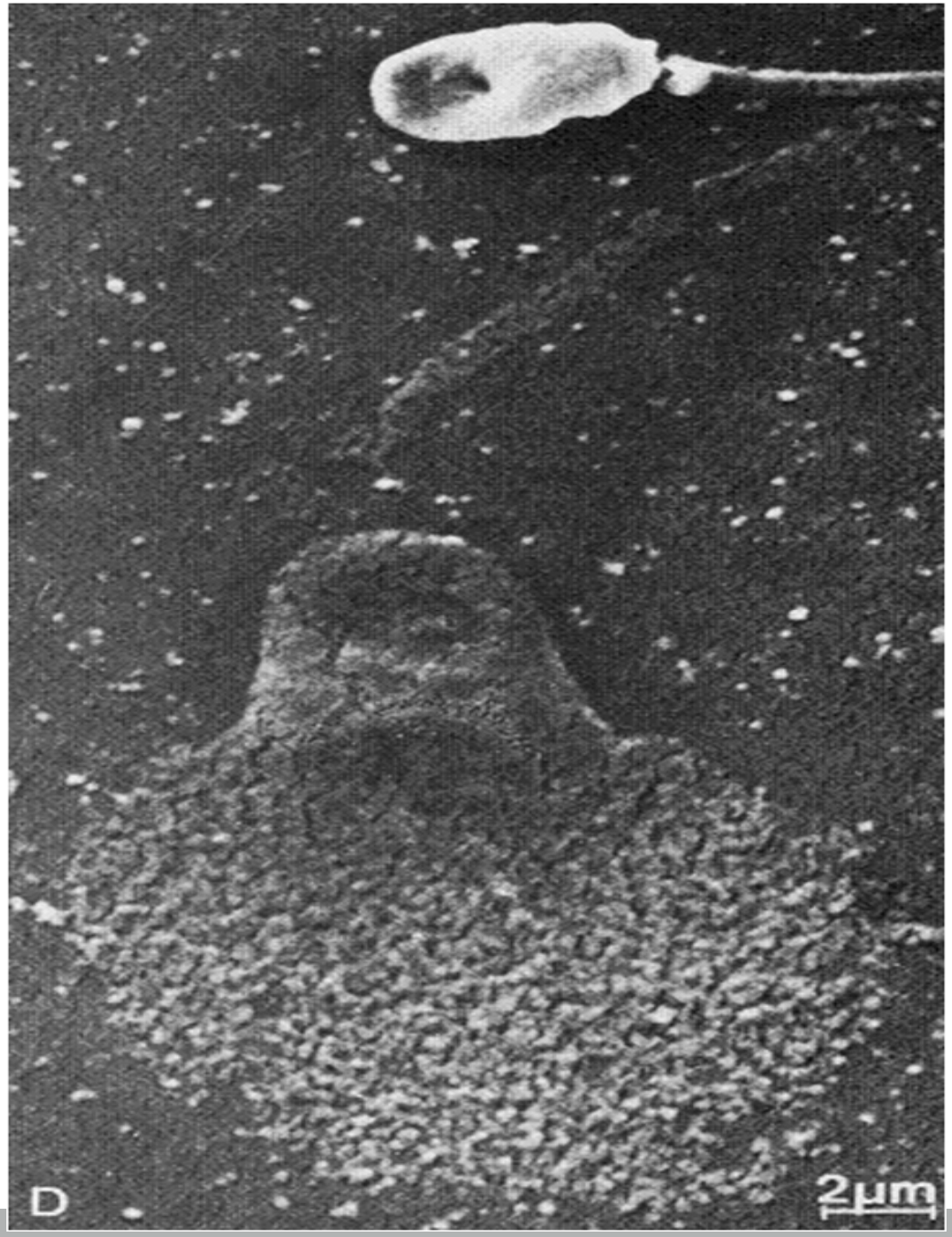


Zn2+ (Zn) *stabilizes* the structure and *prevents* formation of disulfide bridges by a single mechanism: formation of salt bridges with protamine thiols (S).

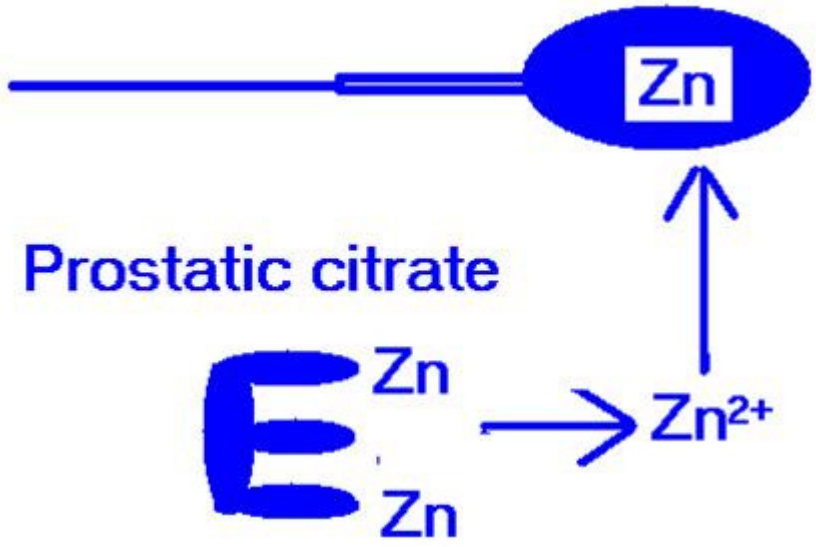


–Extraction of zinc makes possible two biologically totally different outcomes:

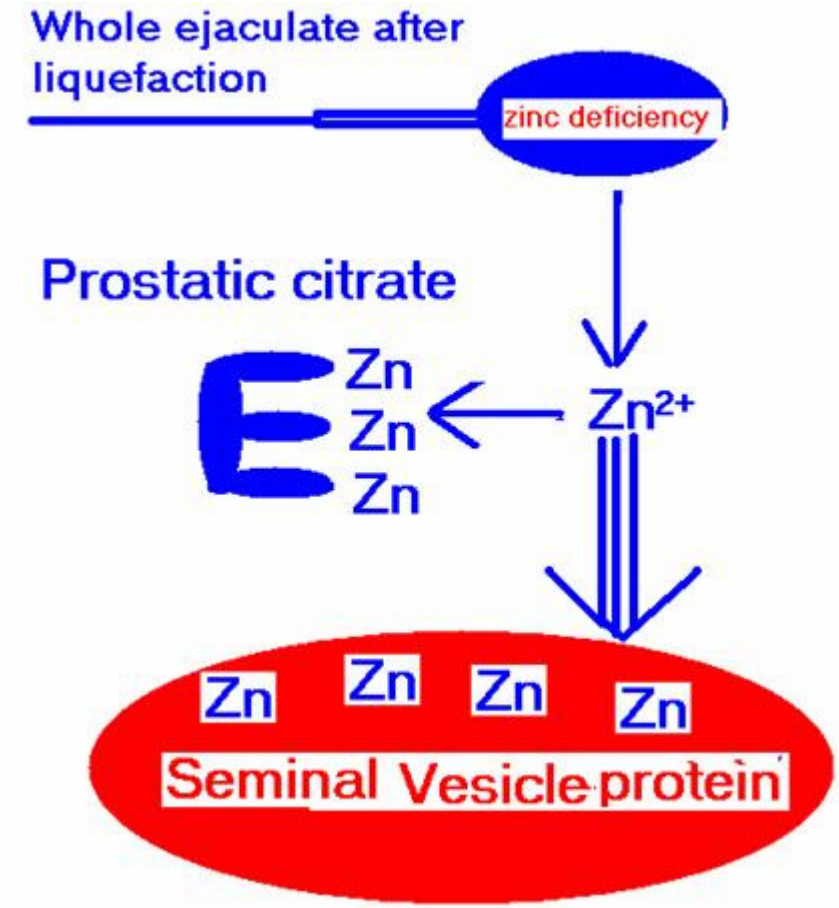

m
s
g


D
2µm

In the prostatic fluid expelled onto the cervical mucus

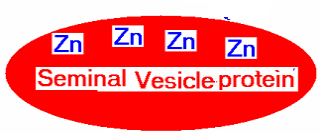


In the liquefied semen collected in one single container (=WHO sample)

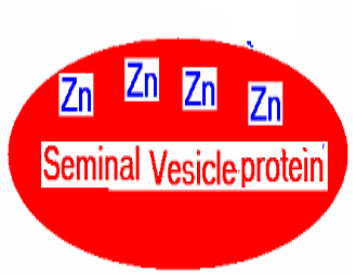


Liquefied ejaculate can act zinc-chelating, % HMW-Zn

- 20 fertile men mean 13% (Arver 1982)
- 13 fertile donors < 10% (Kjellberg, 1993)
- 115 infertile men 2-67% (Kjellberg 1993)



Liquefied whole ejaculate can act as a zinc-chelating medium, especially in men with low zinc concentration, indicating abundancy of seminal vesicular fluid

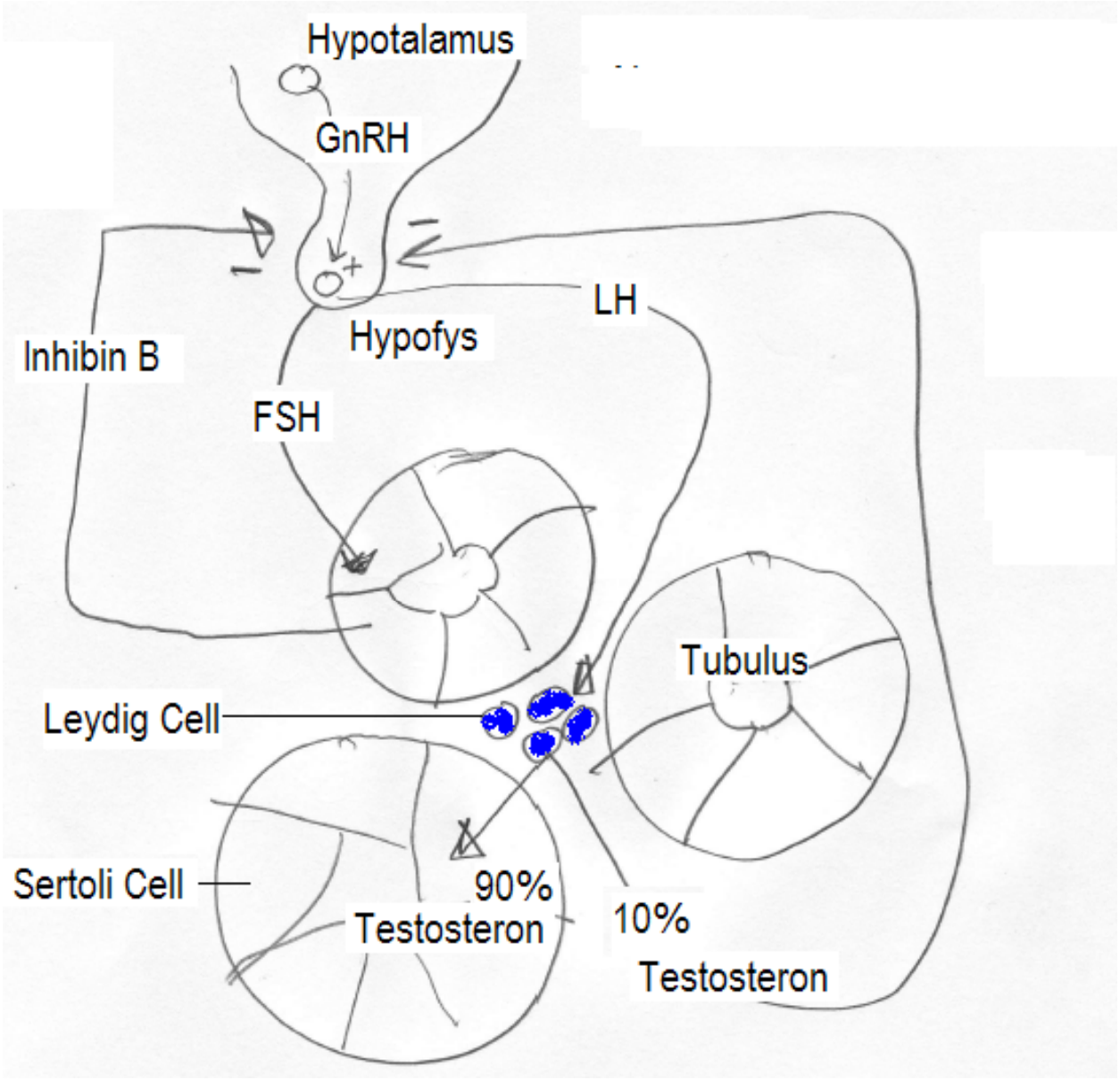


- Spermatozoa in the zinc rich prostatic fluid represent the physiological situation.

- Extraction of chromatin zinc can be accomplished by the seminal vesicular fluid.

- Collection of the ejaculate in one single container causes abnormal contact between spermatozoa and seminal vesicular fluid affecting the sperm chromatin stability.
- There are men in infertile couples with low content of sperm chromatin zinc due to loss of zinc during ejaculation and liquefaction.

- Tests for sperm DNA integrity may give false negative results due to decreased access for the assay to the DNA in superstabilized chromatin.


Hypotalamus
GnRH
LH
Hypofys
Inhibin B
FSH
Tubulus
Leydig Cell
Sertoli Cell
90%
Testosteron
10%
Testosteron

Take home message

- **In the embryo**
- **hCG** from the **placenta-**anlagen of the embryo stimulate the **Leydig Cells** to produce**Testosterone.**

- Disturbed function of the early ”placenta” jeopardize male development.- not female (testosteron dependent).

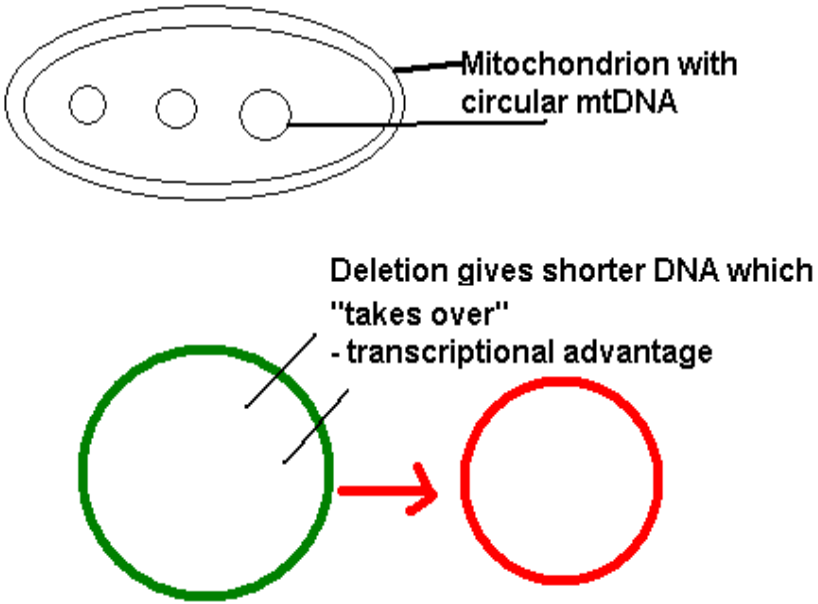
- **In the adult: GnRH** from the hypothalamus gives **LH** from the piturity which stimulates the Leydig Cells to produce **Testosterone.**

What about mitochondria?

Bacteria producing ATP!

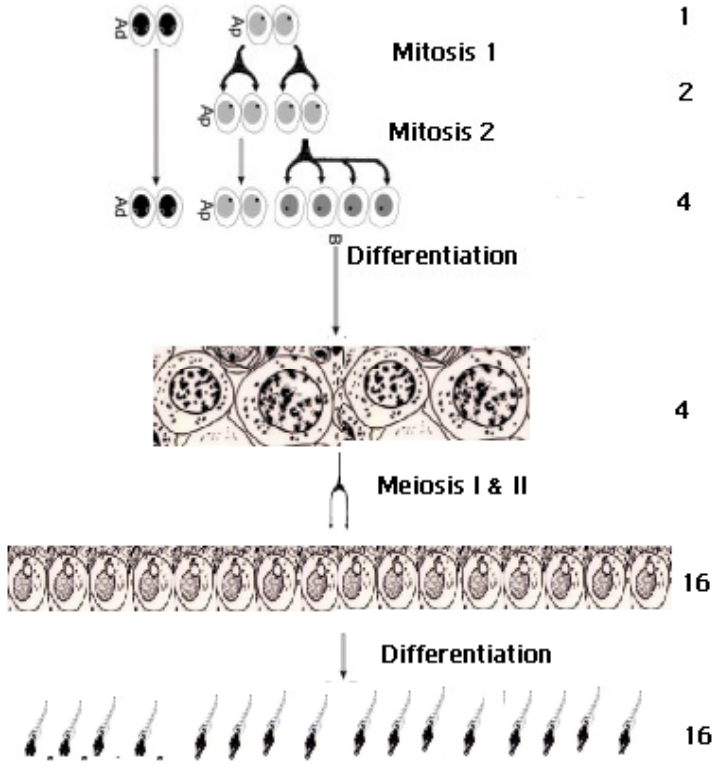
Male gonadal mitochondria detoriate because of cell divisions!

Deleted mitochondrial DNA propagates and take over



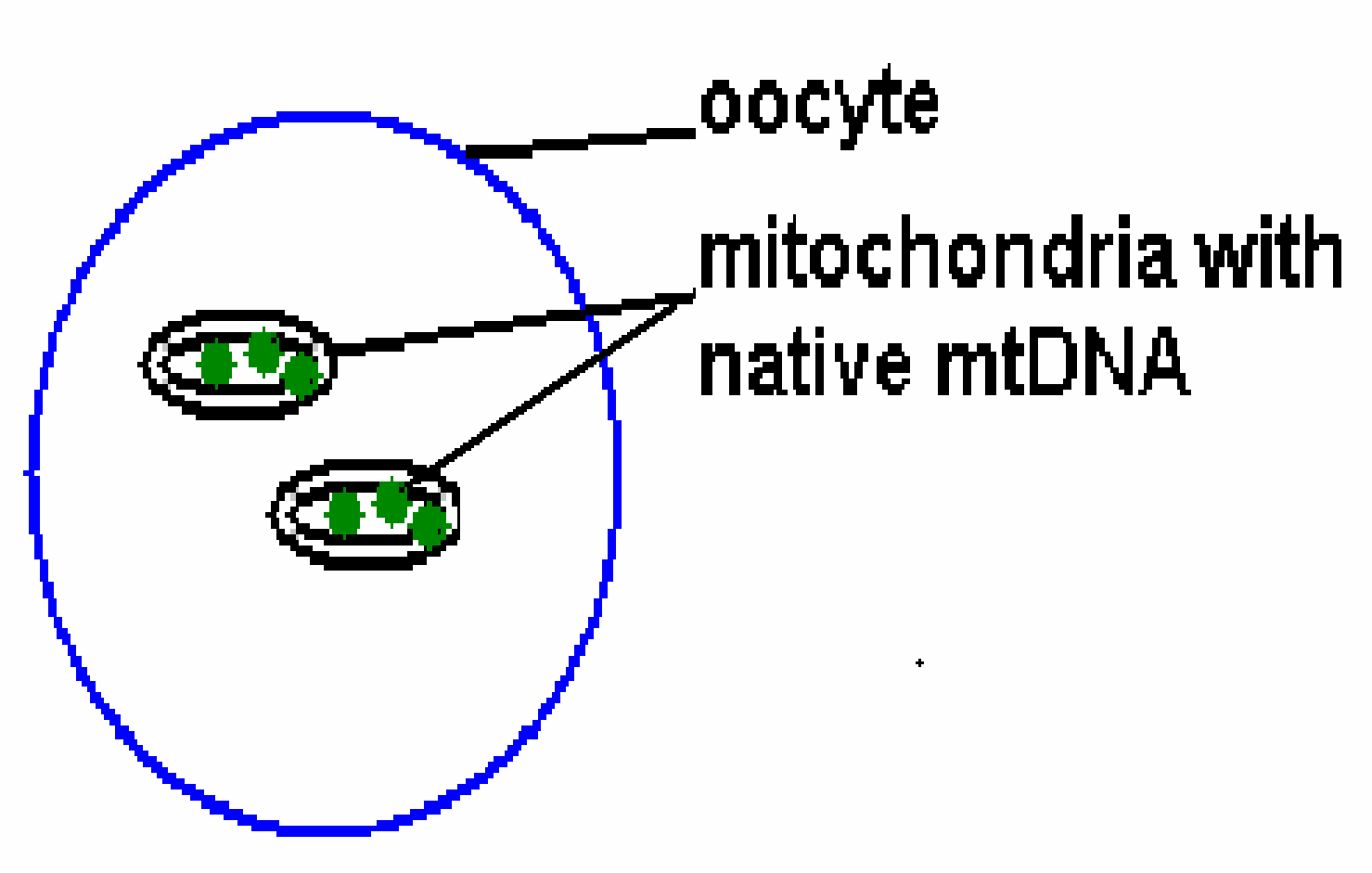
Deleted male germ mtDNA may propagate at > 400 cell divisions while the oocyte rests

- Mitosis of gonocytes fetal week 10-18 in man
- Plus 400 mitosis events of spermatogonia at 35 years of age
- Plus mitosis and meiosis at spermatogenesis

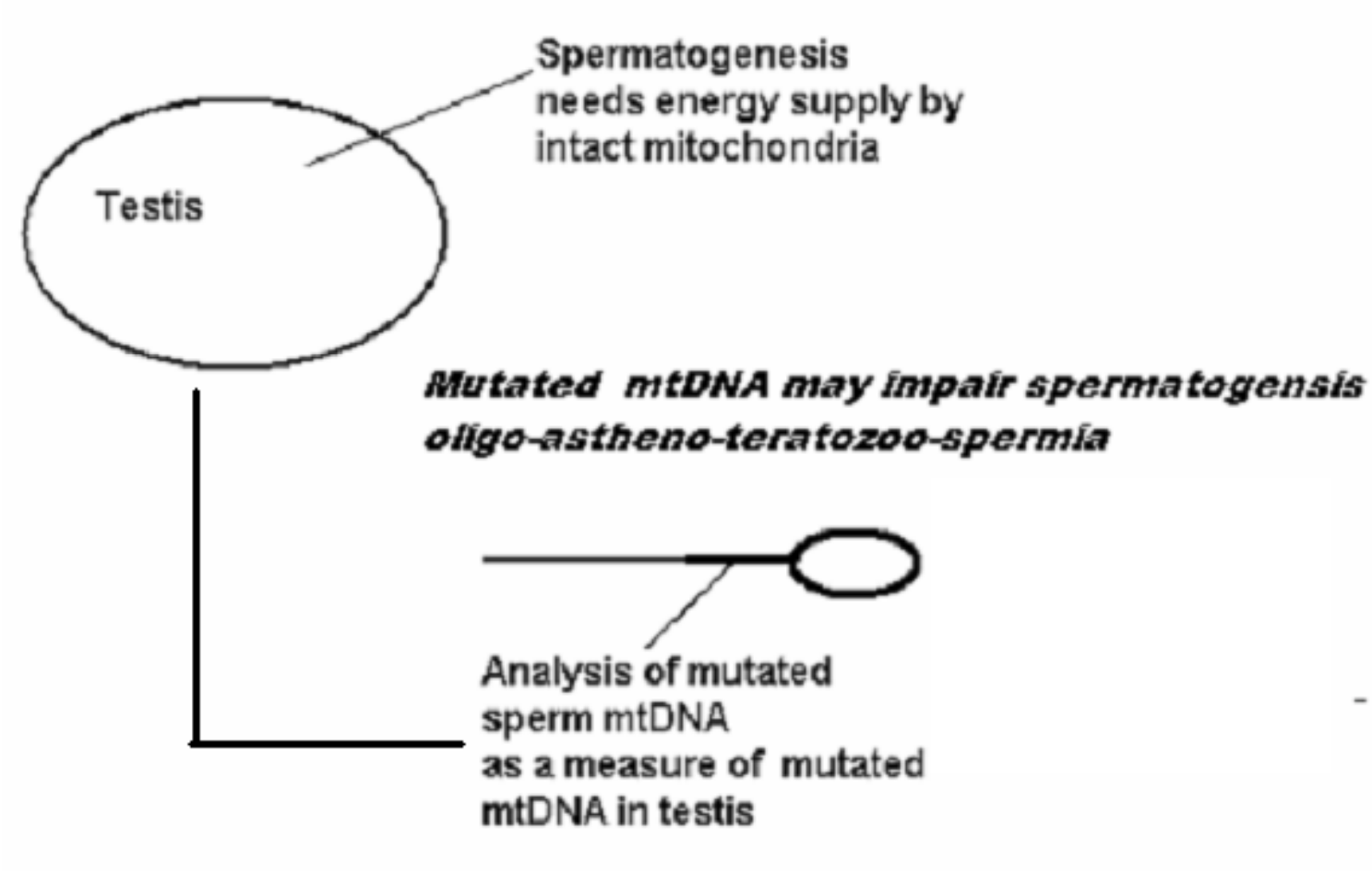


Efter Ehmcke & Schlatt, Reproduction 2006,132:673-680
Holstein, Schultze & Davidoff, Reproductive Biology and Endocrinology 2003, 1:107

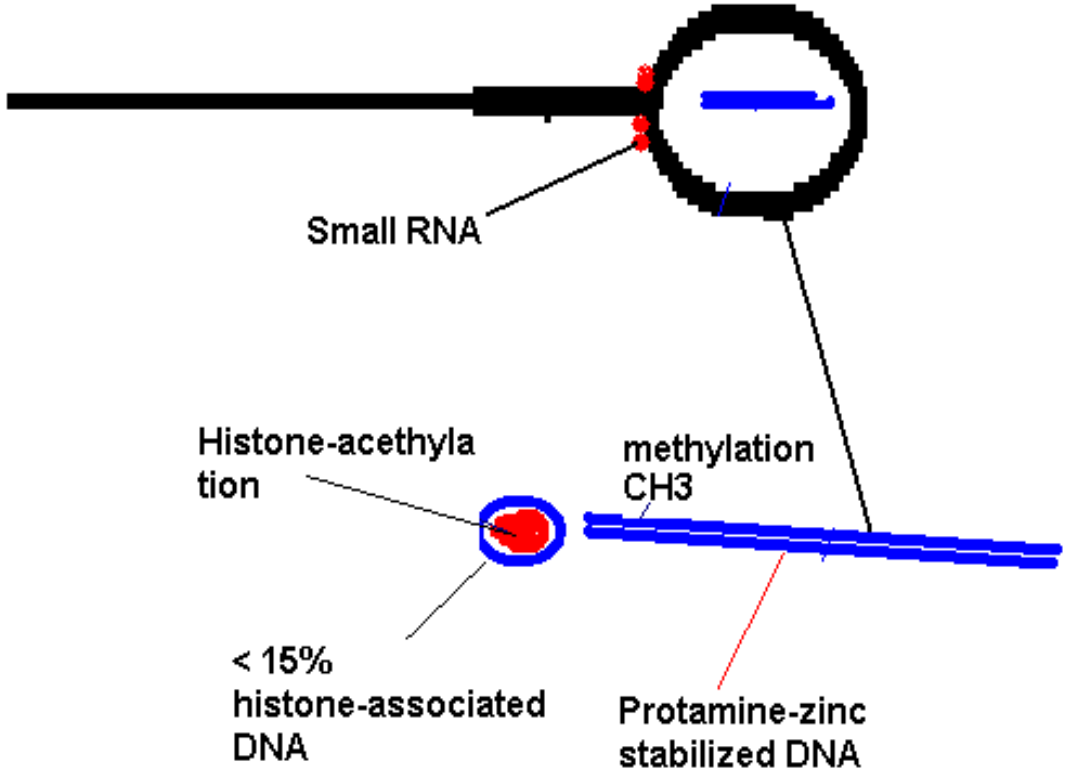
Mitochondrial DNA -The oocyte provides native mitochondrium for the next generation



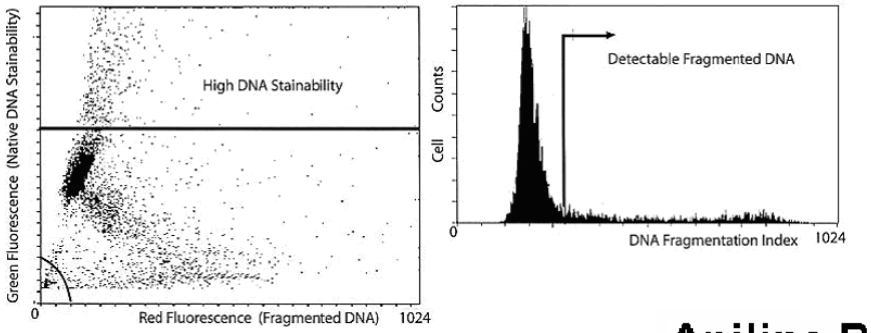
Analysis of sperm mtDNA as marker of deleted mtDNA in the testis

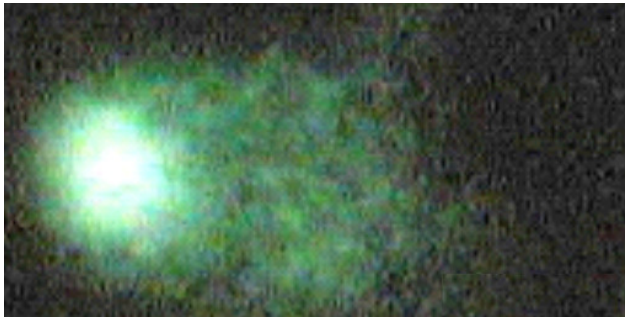


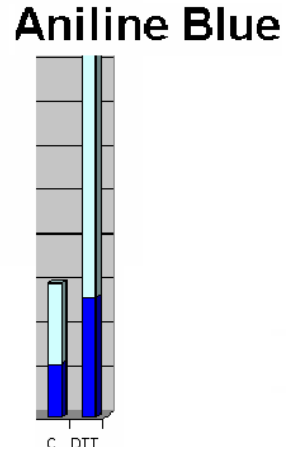
Sperm RNA and Epigenetic modulations.

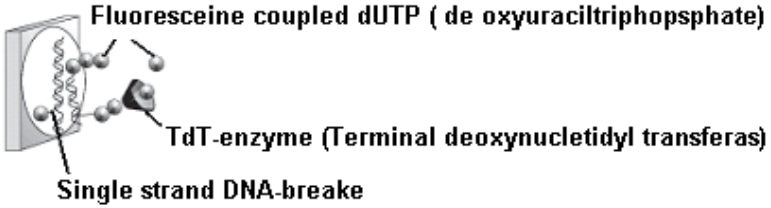


Superstabilization results in low accessability









False negatives

Calls for more methodological work and standardisation

Learning Objectives 1

- That the spermatozoon is a messenger cell carrying messages
- That the intact messages are the pre-requisite for a healthy child and healthy grand-children.
- That the question whether reproductive function of a man is affected or not, needs the observations of two generations to be answered.
- That the messages as far as is known are
- (1) factors for oocyte activation,
- (2) an intact haploid genome,
- (3) a centrosome (needed for mitotic divisions in the new individual) and
- (4) factors necessary for the initiation of placental development.
- That meiosis is the tool by which genetically unique gametes are produced resulting in genetically unique individuals.
- That the evolution of meiosis and unique gametes was a prerequisite for an individual immune defence, which in turn was a prerequisite for the evolution and survival of multi-cellular organisms exposed to endless attacks by micro-organisms.

Learning Objectives 2

- That the production of spermatozoa comprises five phases:
- (1) Renewal of stem cells.
- (2) Spermatocytogenesis, by which two extra mitotic divisions in human, makes possible the production of 16 spermatozoa from one spermatogonium.
- (3) Meiosis by which unique and haploid cells are formed.
- (4) Spermiogenesis i.e the metamorphosis into a spermatozoon
- (5) Spermiation by which the Sertoli Cell liberate the single spermatozoon from the cluster of 16.
- That the sperm chromatin is a semi-crystalline temporary structure comprised of one zinc, for every protamine molecule for every turn of the DNA-helice.
- That sperm mitochondrian DNA (mtDNA) undergoes mutations.

Learning Objectives 3

- That the emission of spermatozoa and fluids are effectuated by symphathic nor-adrenergic neurons acting on alfa-1 receptors.

- That there is a normal sequence of ejaculation meaning that spermatozoa are expelled with zinc-rich prostatic fluid in the first expelled fractions of the ejaculate, onto the cervical mucus.

- That admíxture of seminal vesicular fluid depresses sperm motility, vitality and affects the packaging of the sperm chromatin.

- That a WHO liquefied semen sample in which all fractions are collected in one vessel and allowed to mix, only exists in the lab.

- References: Kvist U. Basic Physiology. In Practical guide to basic laboratory andrology.Cambridge University Press 2009-10
- ISBN 0521735904

Ulrik.Kvist@ki.se

Thank you for listening!

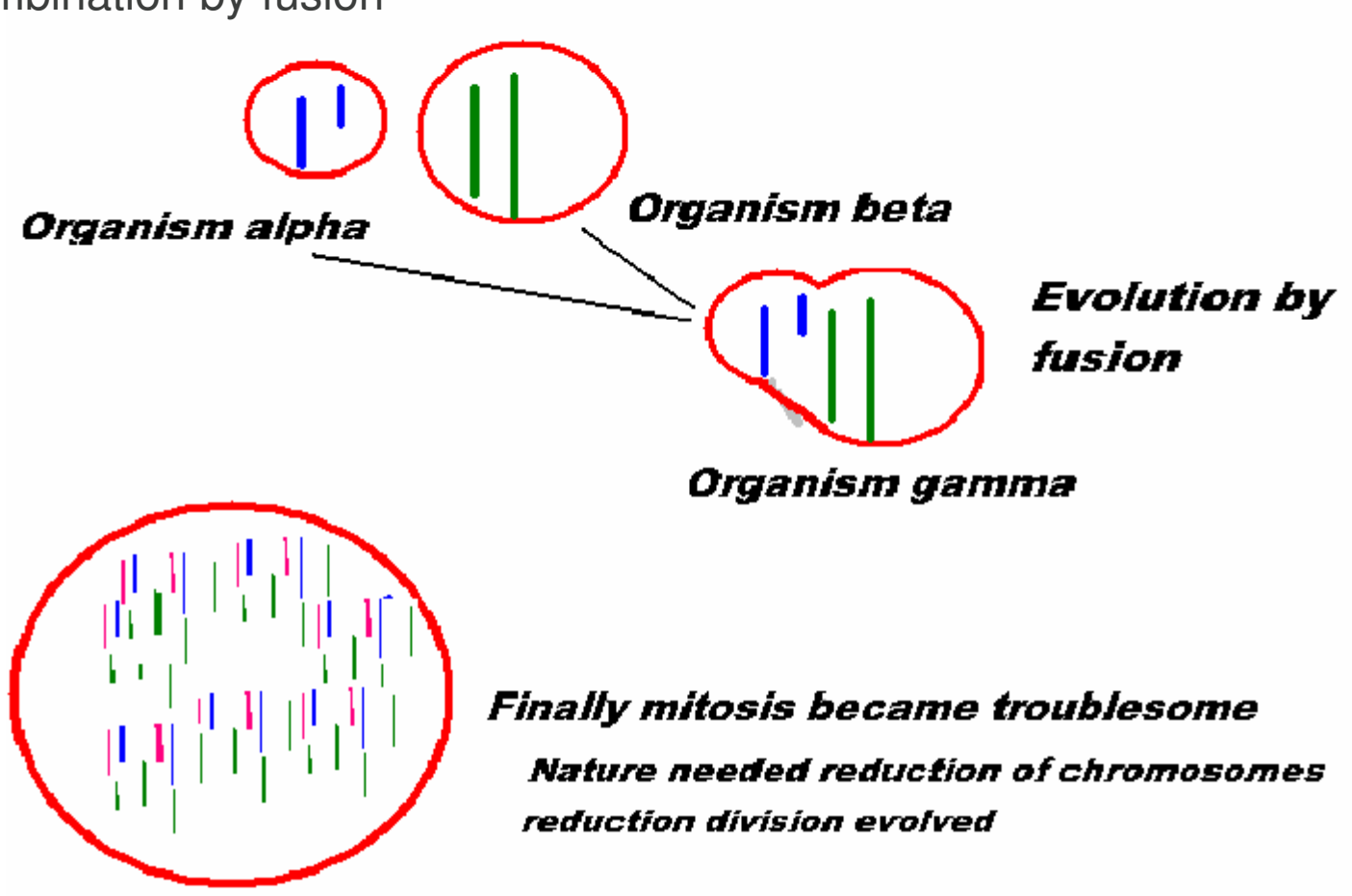
Why gametes?

- The spermatozoon, as well as the final oocyte that is formed after fertilization and meiosis II are

- haploid cells = ½ of the DNA
- with unique DNA 100 000 spermatozoa per minute – all different
- 100 million a day…

The need for reduction of chromosomes?

Recombination by fusion



The need or urge to create new possibilities resulted in unique gametes

– recombination = crossing-over= chiasmata at the beginning of meiosis.