UMC St Radboud

Testis biopsies for studying human spermatogenesis

Wetenschapsmiddag KLEM

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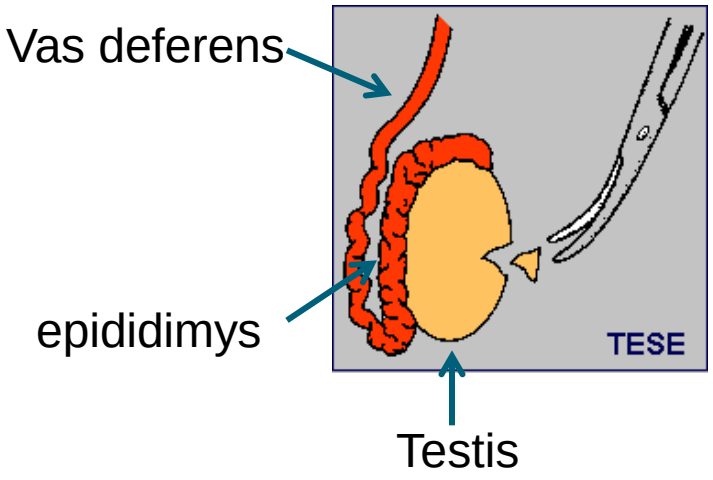
Testis biopsies for studying human spermatogenesis

- Non obstructive azoospermia patients:

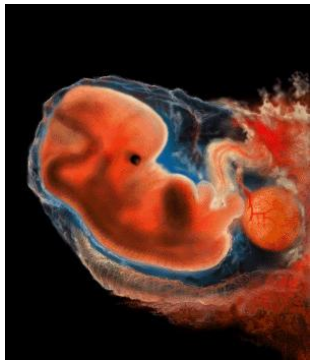
  - no spermatozoa in ejaculate because of a.o. disturbed spermatogenesis

- Last chance at own child → TESE biopsy



How safe is this technique?

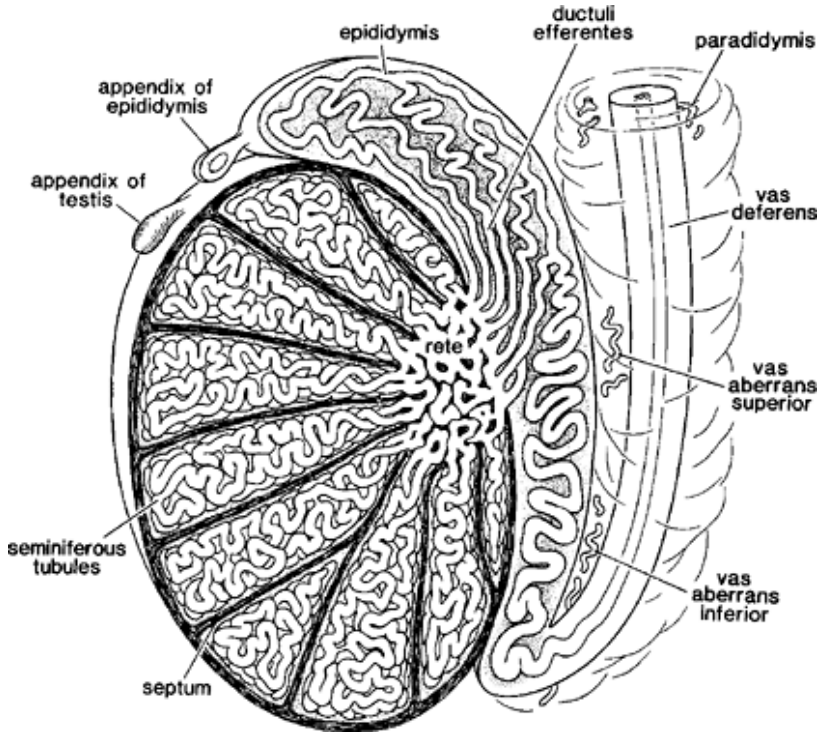
Testicular spermatozoon Normal?

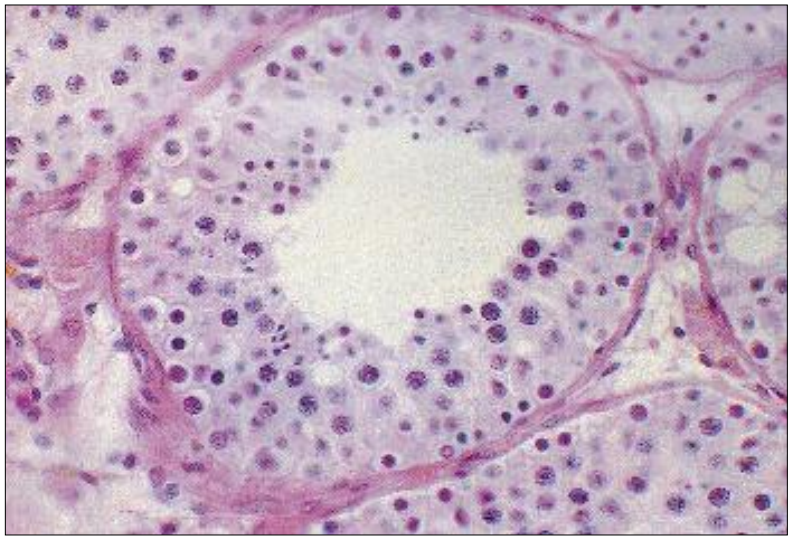


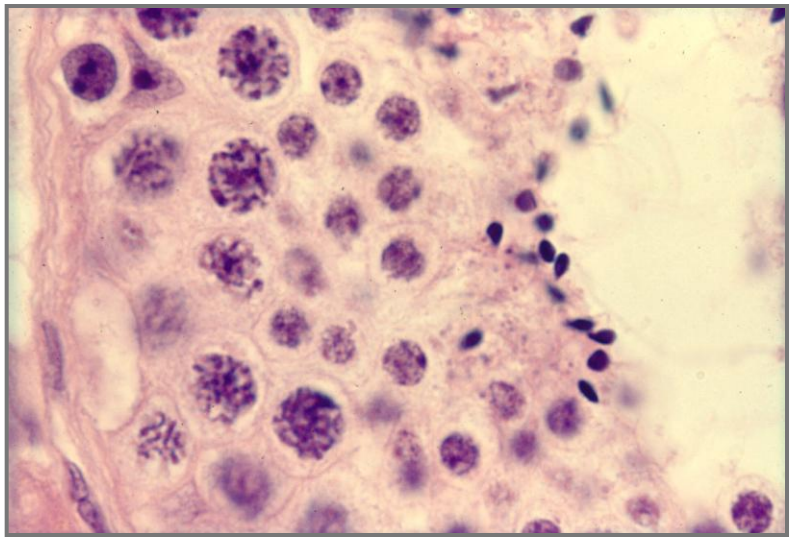
Healthy?

- To answer this question we have to understand spermatogenesis

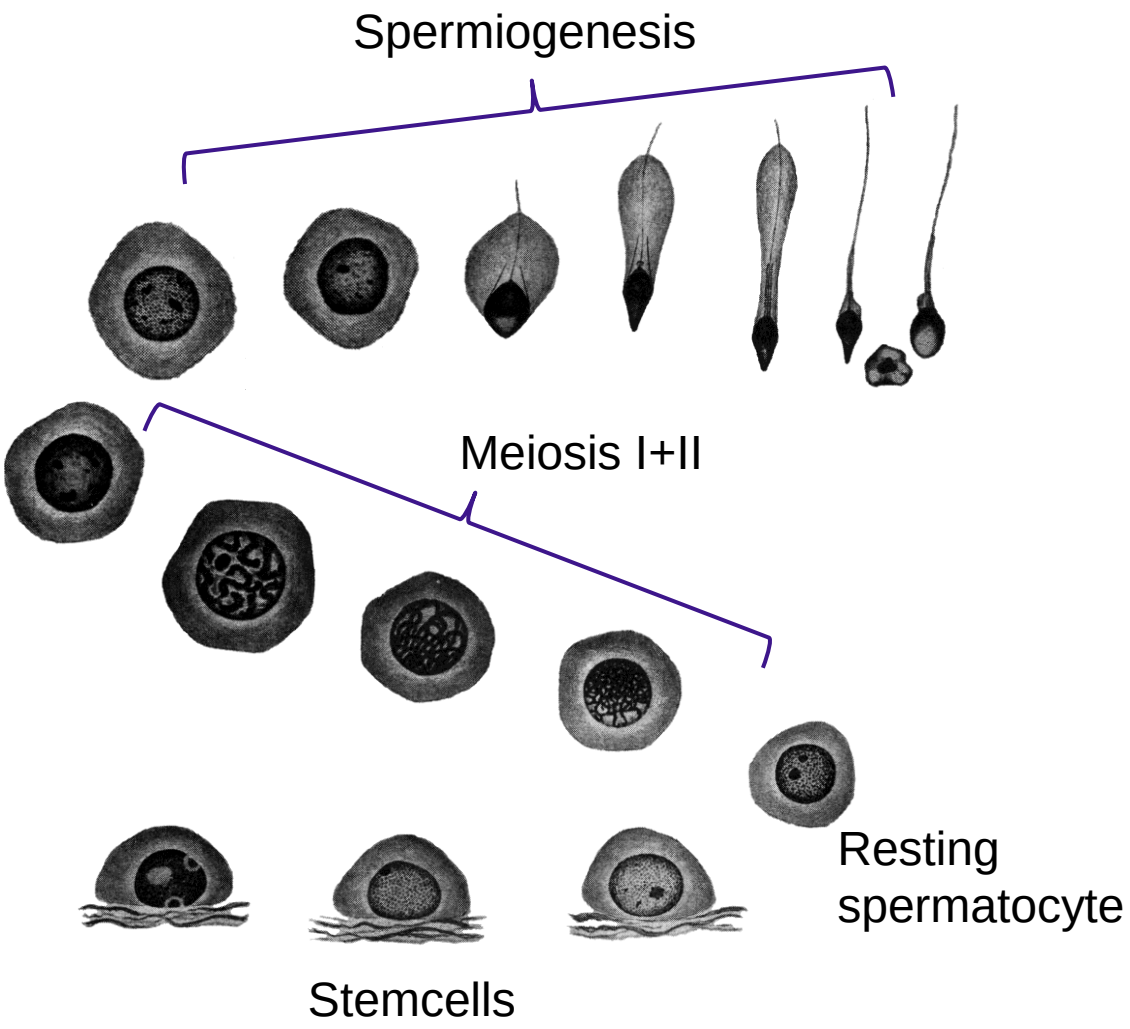
Normal human testis





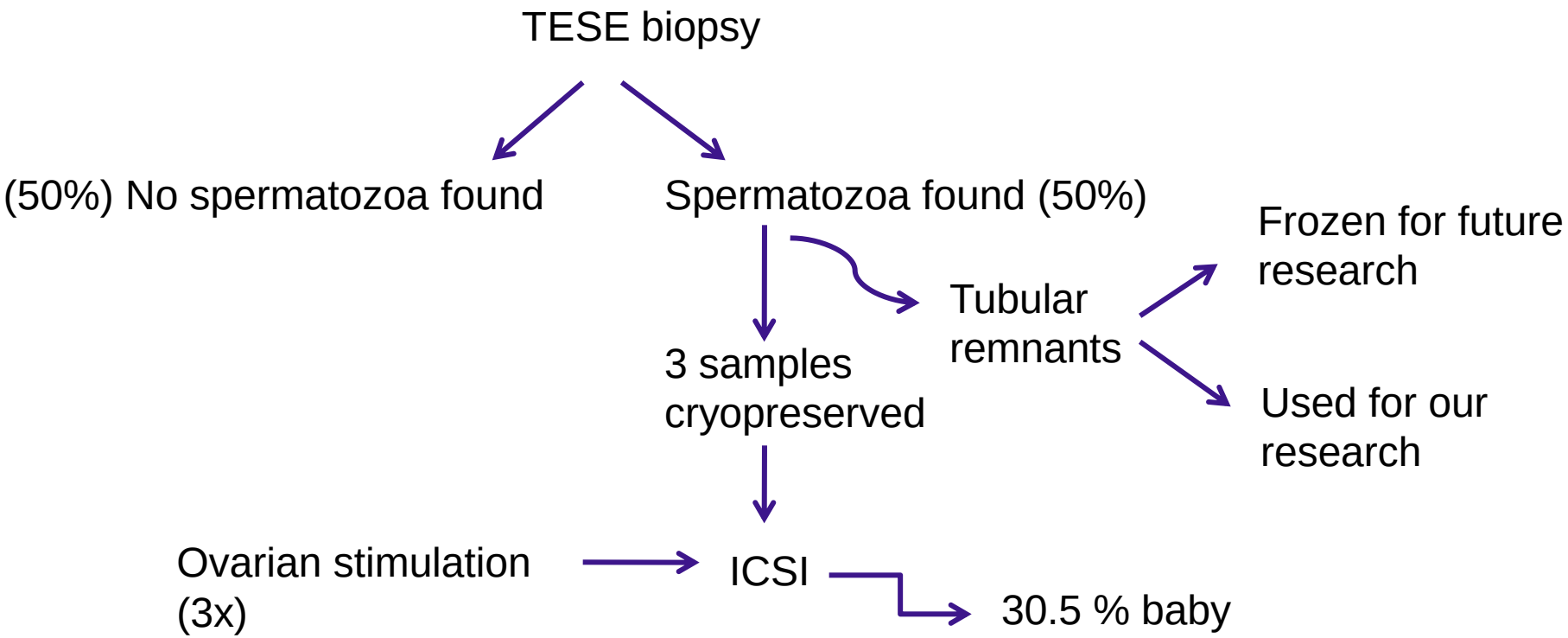


Human spermatogenesis (~ 74 days)



Heller and Clermont, Recent Prog Horm Res 1964; 20:545-75

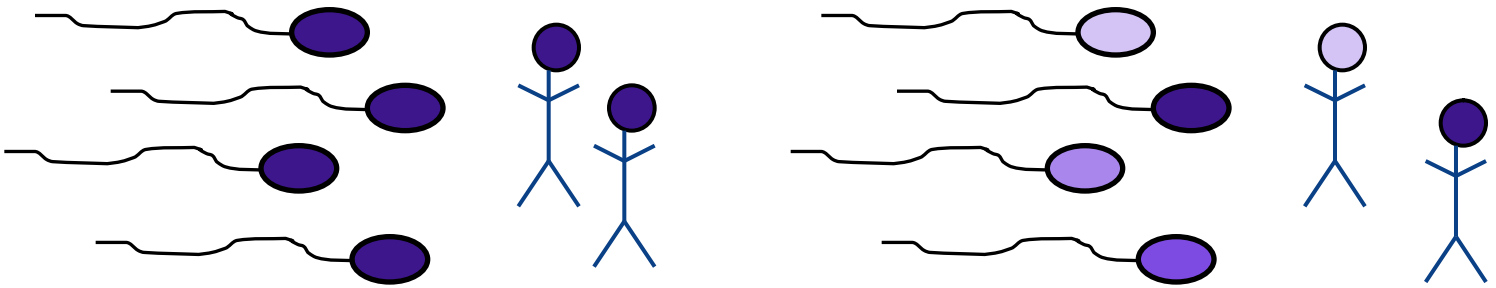
Derivation of research material



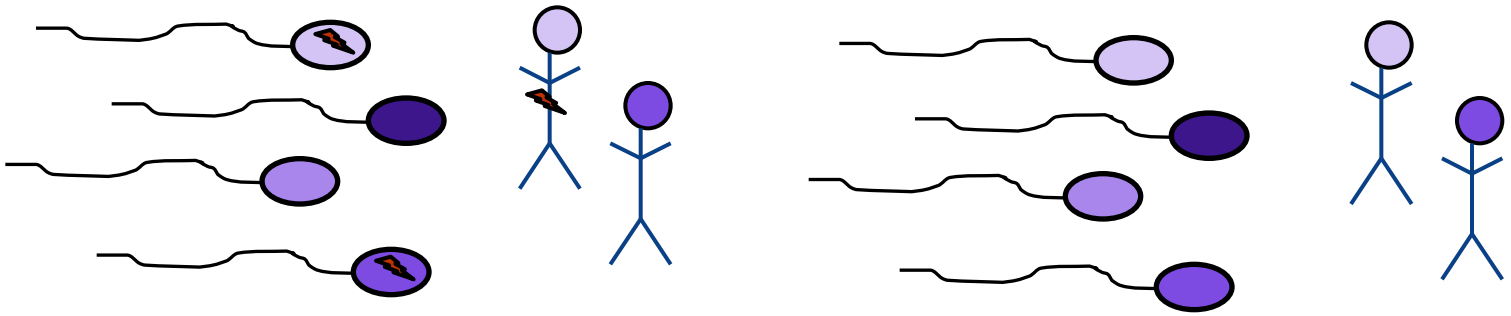
The goal of spermatogenesis

- Transmitting genetic material (to generate healthy offspring)

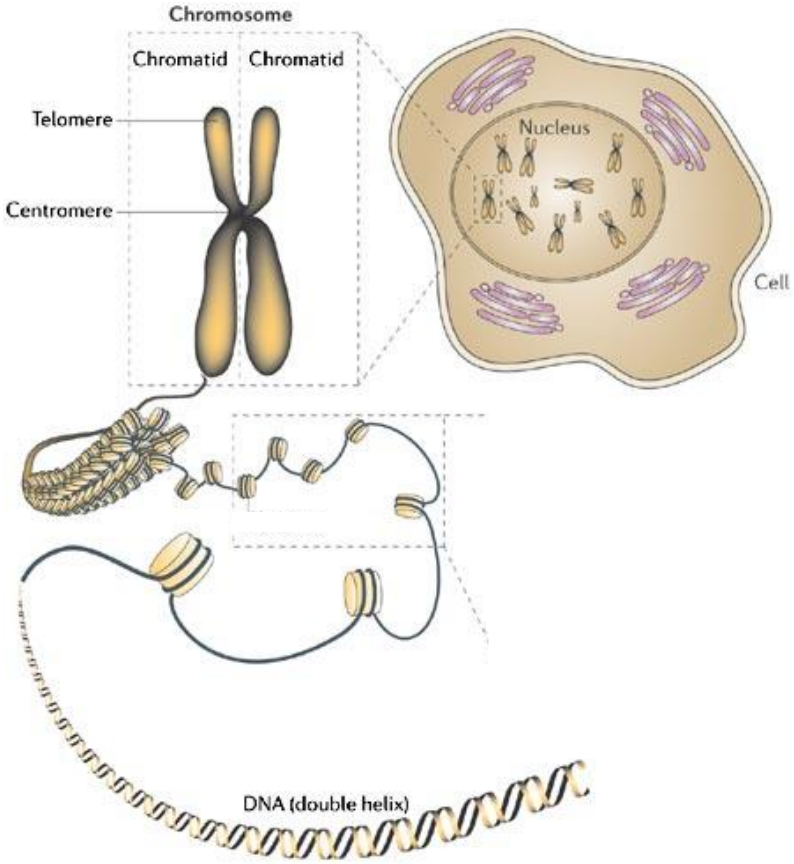
  → Create unique genetic content (meiosis)

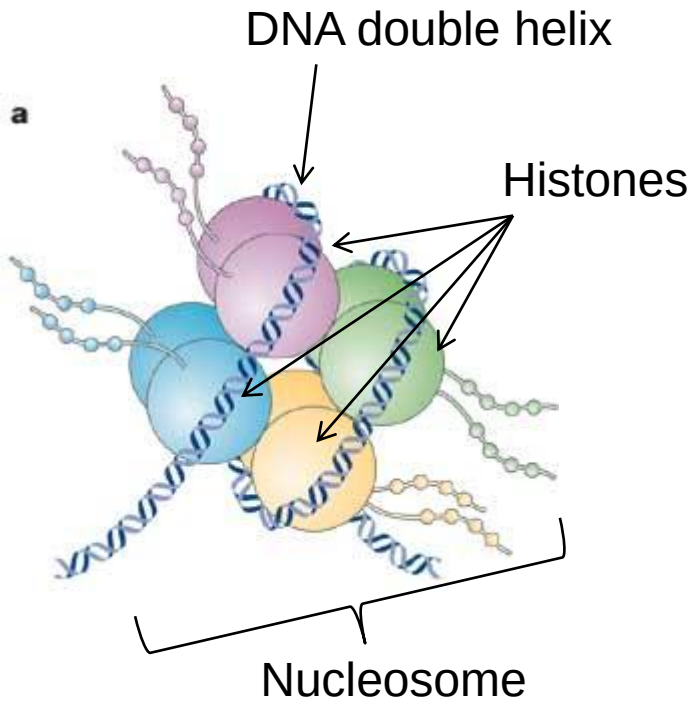


→ Conserve genetic integrity (Spermiogenesis)



DNA, histones, nucleosomes, chromatin

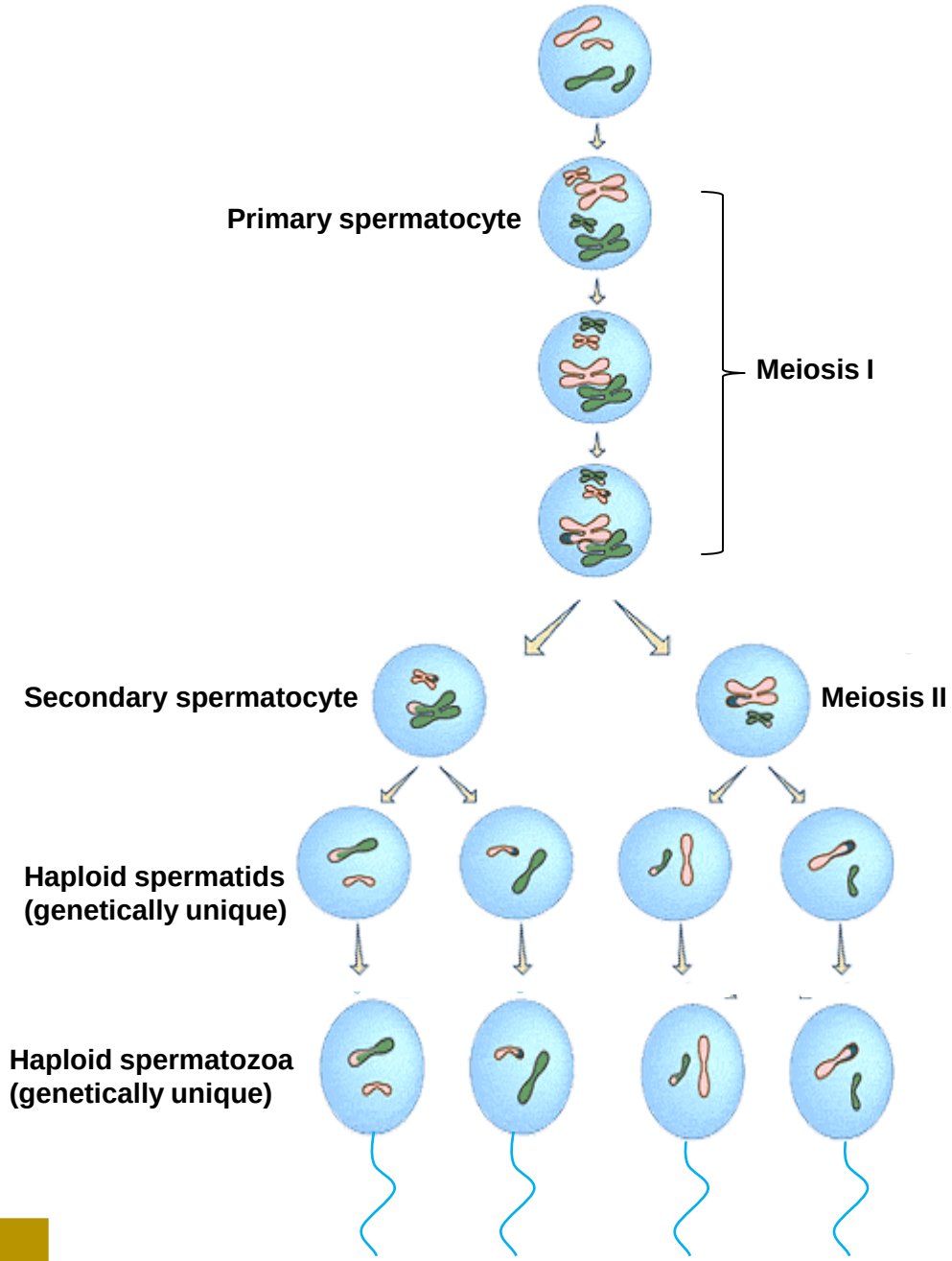




Meiosis

- ~ 35 % of spermatogenesis (25 days) in human
- Several stages → prophase I is the most important

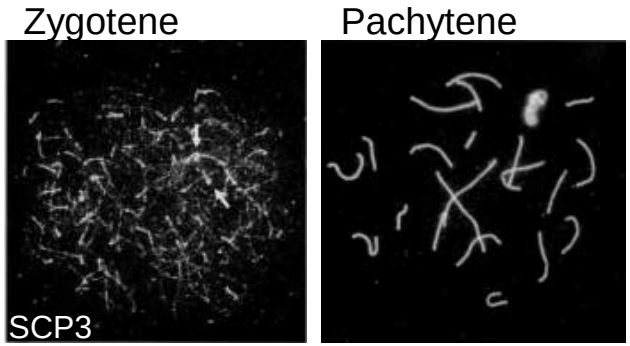
  (24 days)



http://fhs-bio-wiki.pbworks.com/w/page/17008730/meiosis

Meiosis

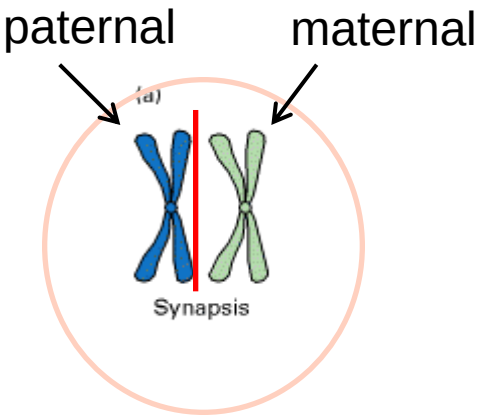
- How do we study prophase I of meiosis?
- Stage specific appearance of the synaptonemal complex stained with the SCP3 antibody

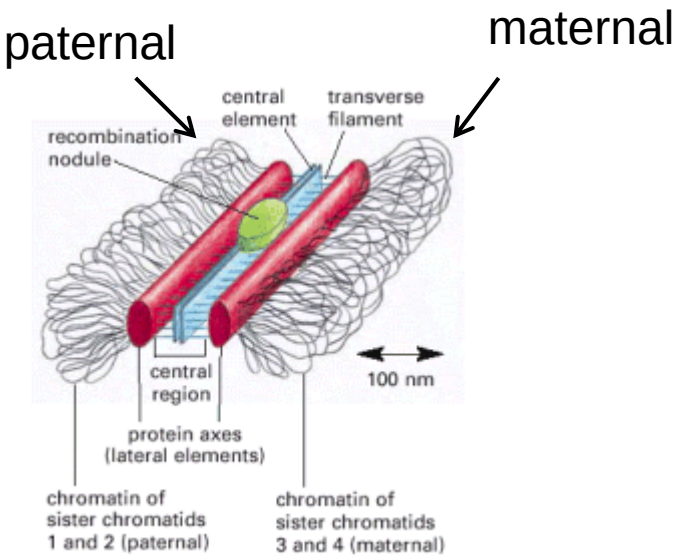


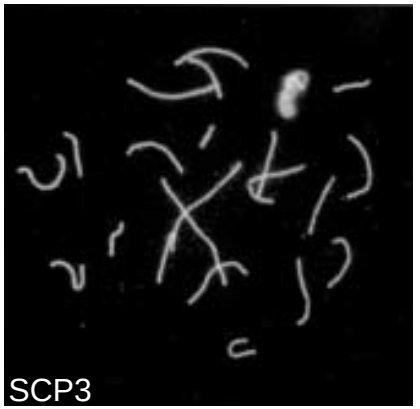
SCP3 = synaptonemal complex protein 3

Boer P. de, *Cytogenet.Genome Res. 105 (1):36-46, 2004*

What is the synaptonemal complex?



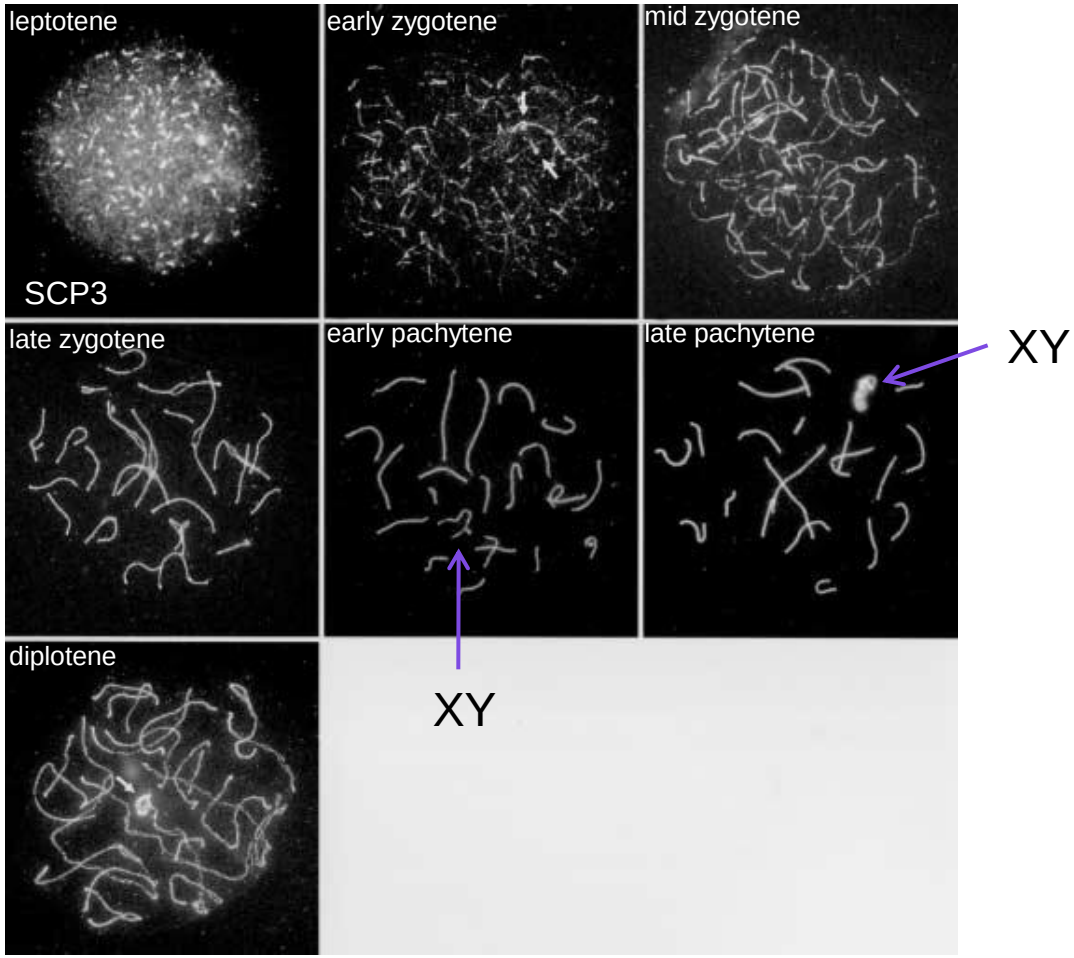




- Protein structure that connects homologous paternally and maternally derived chromosomes

Molecular Biology of the Cell 4th edition. Alberts B, et al. New York: Garland Science; 2002.
Boer P. de, *Cytogenet.Genome Res. 105 (1):36-46, 2004*

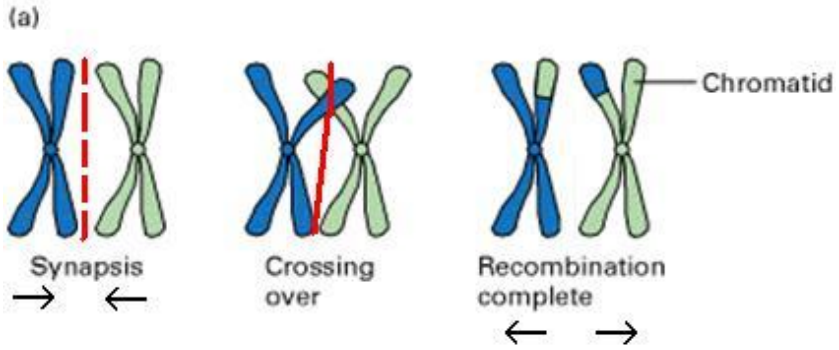
The synaptonemal complex forms gradually during prophase I = synapsis



Boer P. de, *Cytogenet.Genome Res. 105 (1):36-46, 2004*

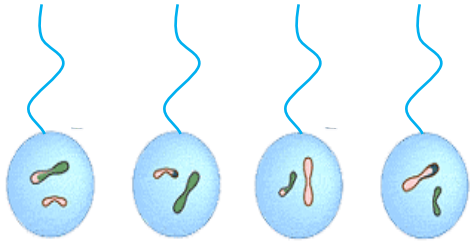
Why is the synaptonemal complex formed?

- To properly align paternally and maternally derived chromosomes → exchange of genetic material

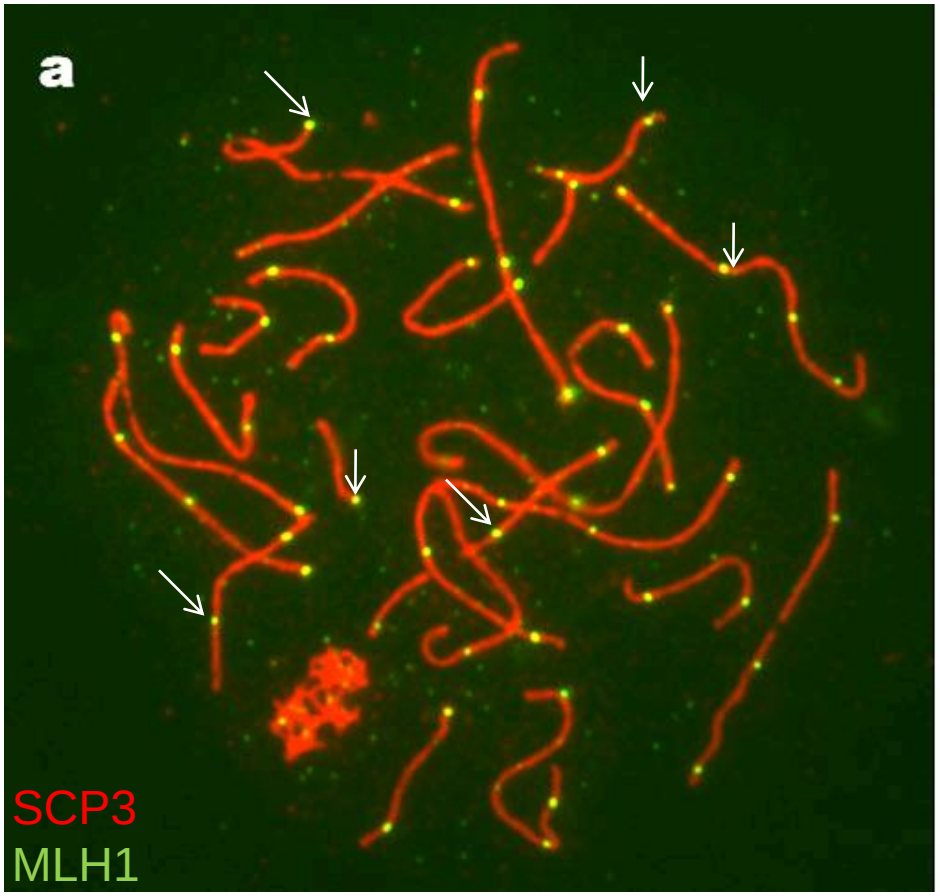


= Recombination + segregation

- Leads to the generation of unique genetic content in spermatozoa



Recombination sites in a late pachytene nucleus

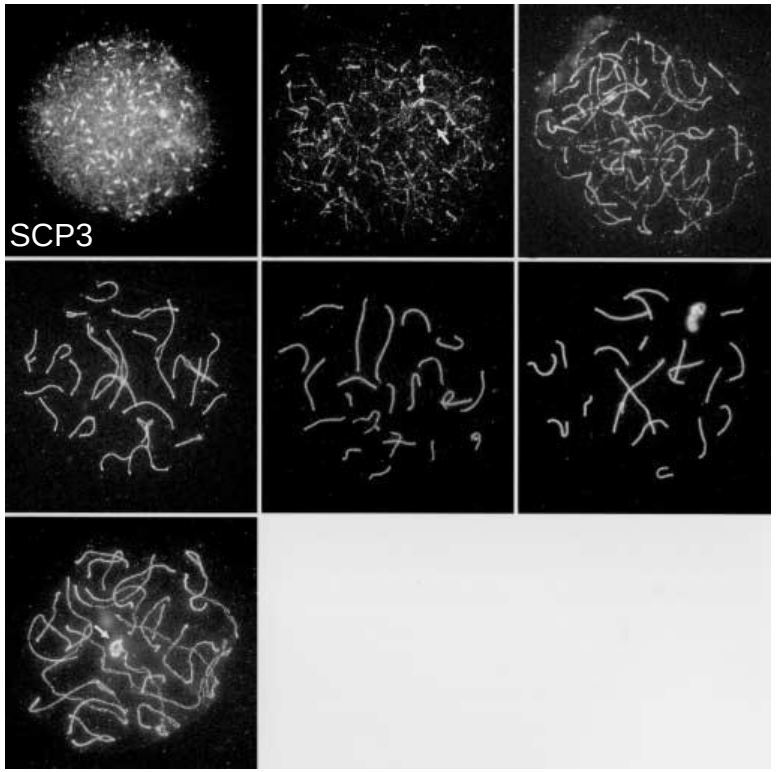


MLH1 is a marker for recombination sites

- Recombination is important for proper segregation of homologous chromosomes at the end of meiosis I
- Improper recombination → non-disjunction

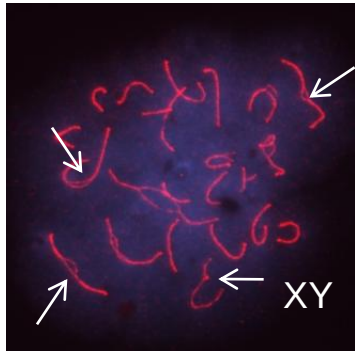
Synaptonemal Complexes are very useful for studying meiosis

Normal

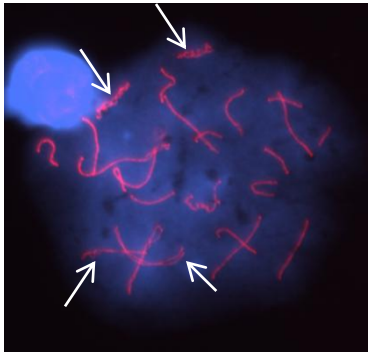


Abnormal: asynapsis

zygotene



early pachytene

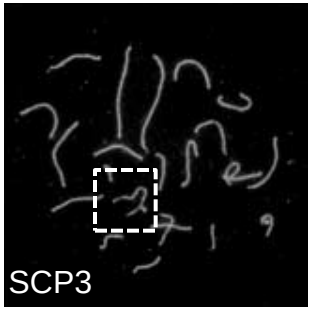


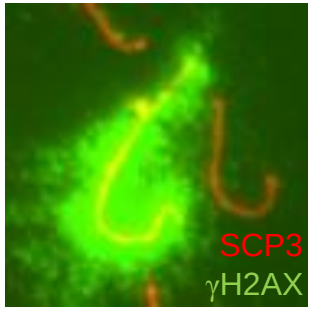
early pachytene

- A-synaptic sites hamper recombination

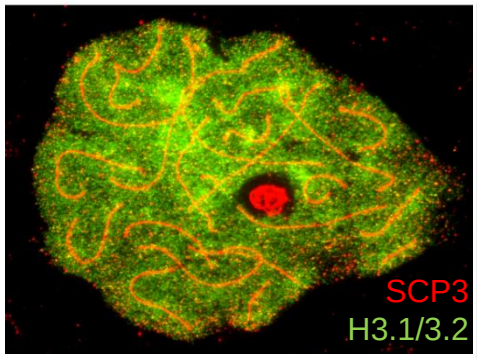
Boer P. de, *Cytogenet.Genome Res. 105 (1):36-46, 2004*

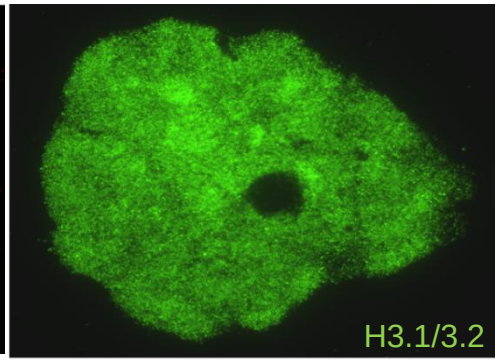
The X and Y chromosome during meiosis

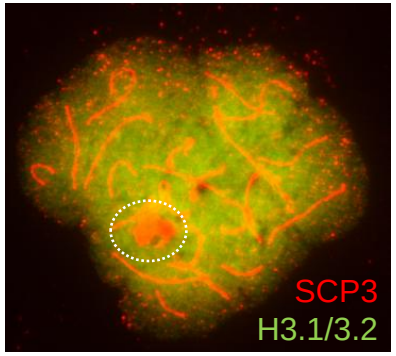


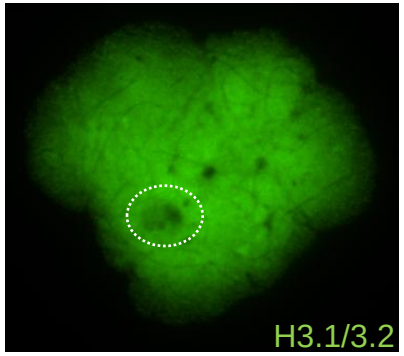


- Marked by γH2AX (non-synapsed) → gene silencing



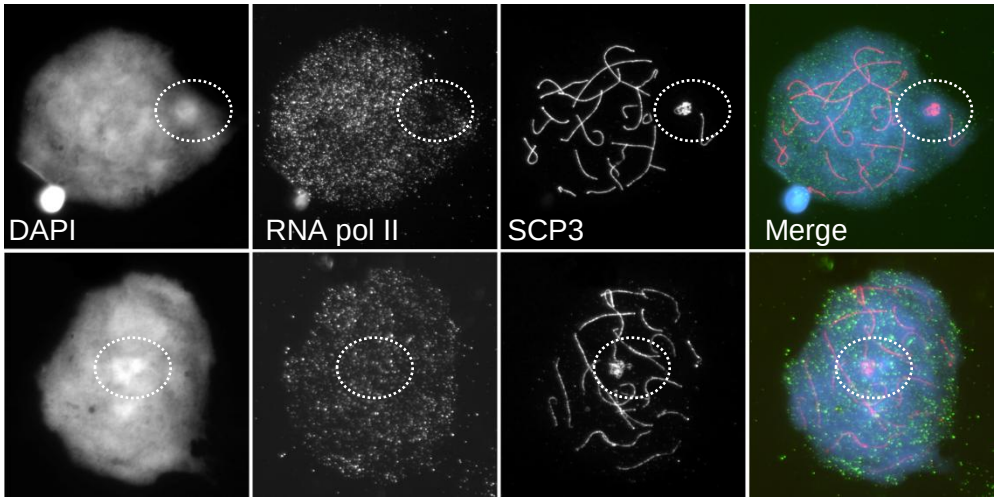




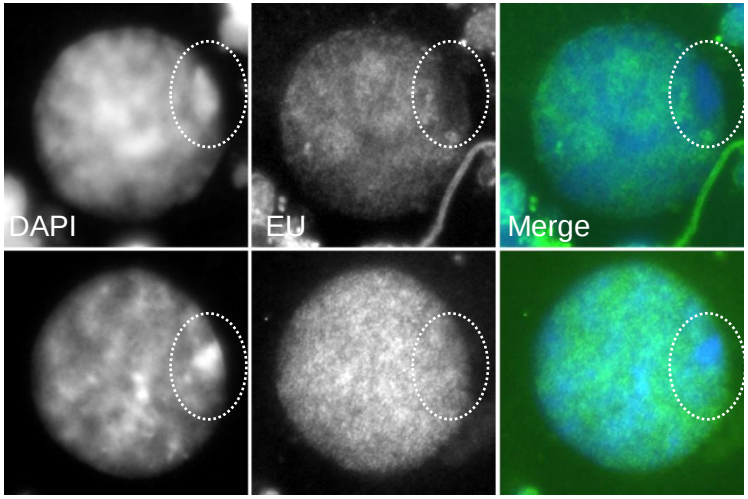


- Removal of histone 3.1/3.2

Meiotic sex chromosome silencing in human late pachytene is variable



Indirect transcription marker:
RNA polymerase II

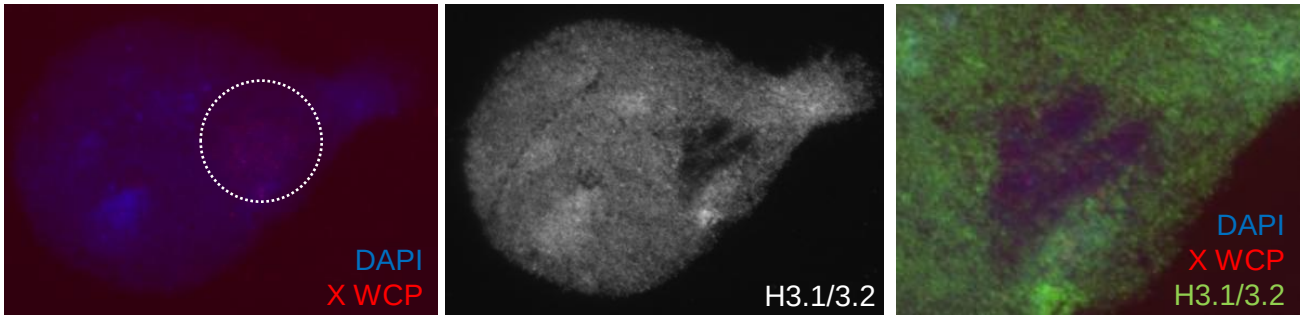


Direct transcription marker:
EU incorporation into newly transcribed RNA

Meiotic sex chromosome silencing in human is variable

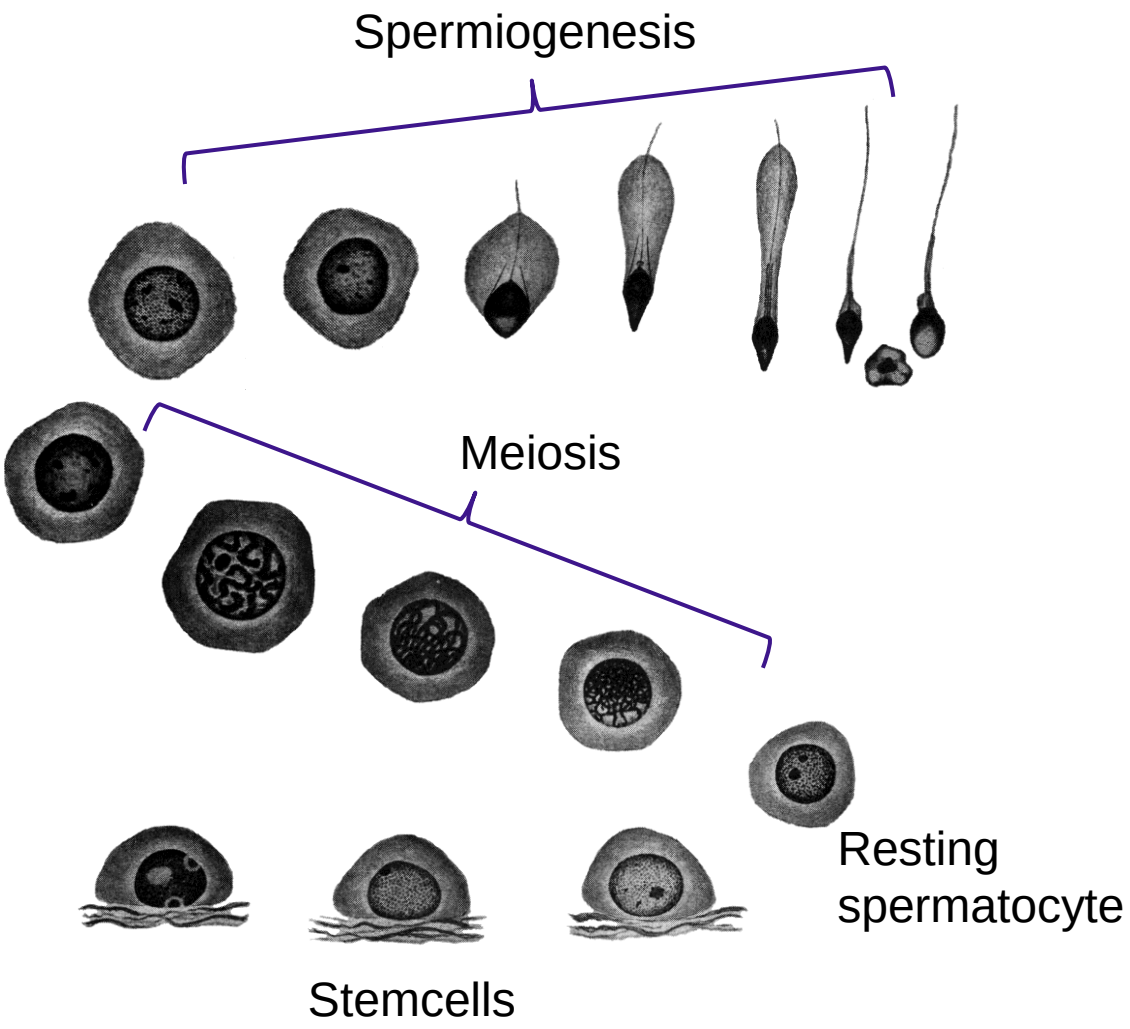
- Improper sex chromosome silencing → harmful gene expression
- In mouse these cells die (apoptosis) or lead to weak/non-fit spermatozoa
- In human they probably proceed with spermatogenesis:

Round spermatid showing incomplete H3.1/3.2 nucleosome remodeling



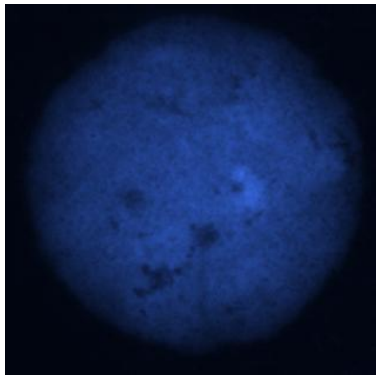
- Does this lead to weak/non-fit spermatozoa?

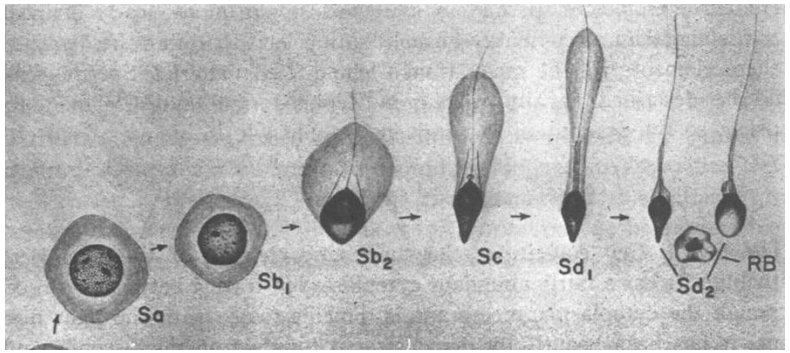
Human spermatogenesis



Heller and Clermont, Recent Prog Horm Res 1964; 20:545-75

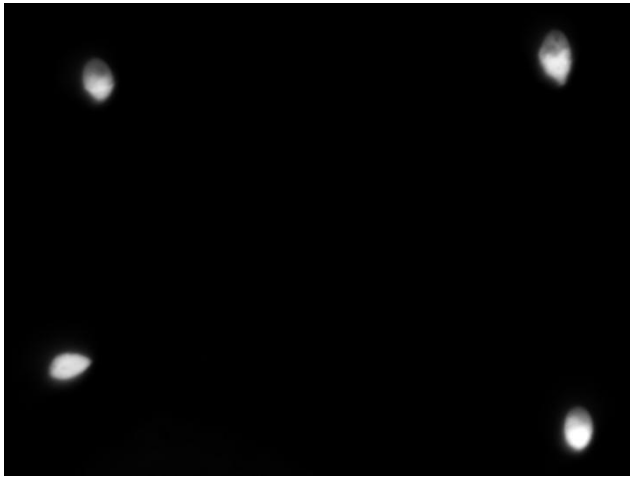
round spermatid





?

spermatozoa

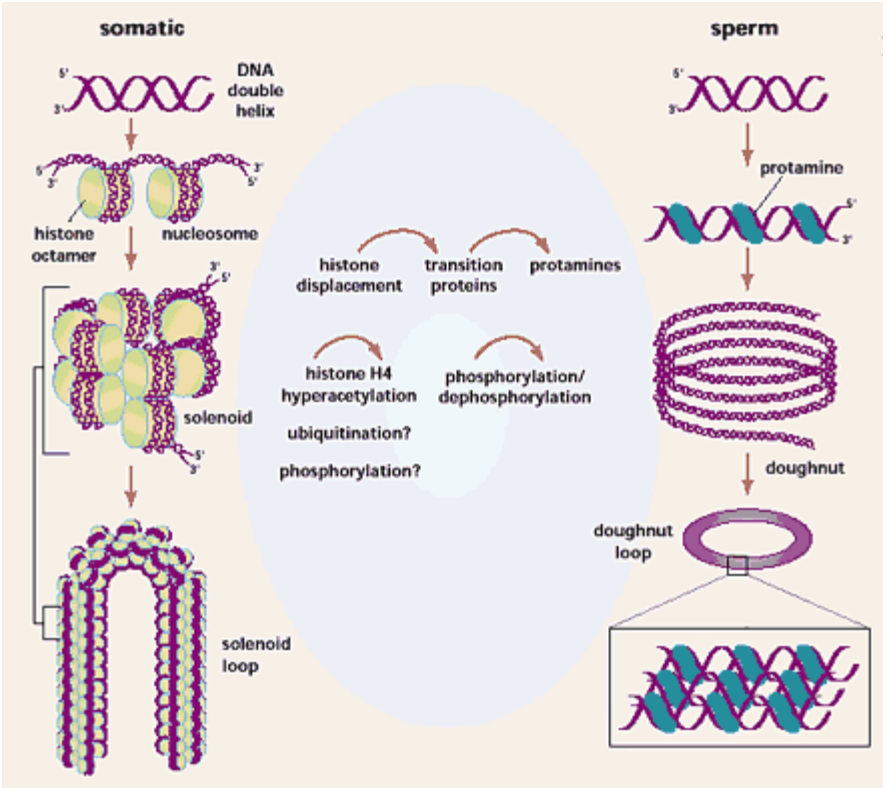


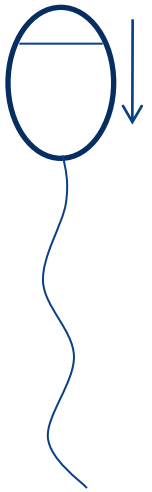
a.o. 2 types of processes:

Occurring <u>outside</u> the nucleus (Acrosome and manchette development)

Occuring <u>inside</u> the nucleus (Chromatin remodeling process; DNA condensation by repackaging)

Shaping process of the spermatid head – chromatin remodelling

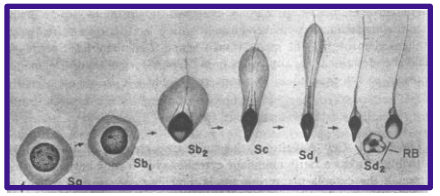




- Replacement of histones/nucleosomes by transition proteins and later on by protamines
- In human 85 % of the DNA will be packed in protamines
- Nuclear condensation starts at the apical side of the spermatozoon and gradually moves downwards

Adapted from Braun, Nat Genet, 28, 2001

Development of a ‘doughnut like’ structure



1

DAPI

DAPI

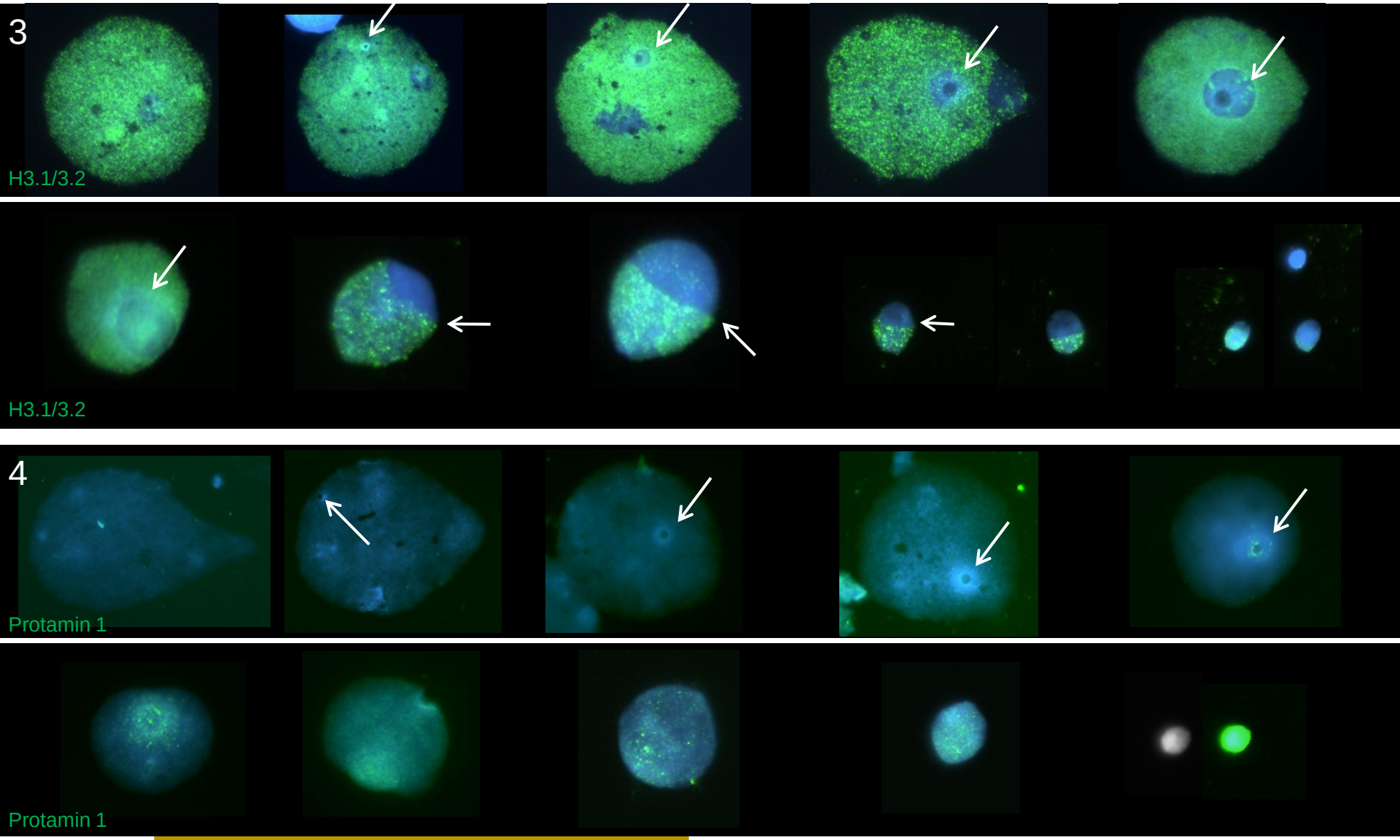
2

Acrosin

Acrosin

UMC St Radboud

Chromatin remodeling starts at the ‘doughnut like’ structure



Importance of protamination

- Difference in protamination between normospermia and OAT sperm samples
- Less protamines in OAT → incomplete nucleus maturity
  - Less compact: more vulnerable for DNA damage
  - Less hydrodynamic: slower spermatozoa
  - More DNA packed in histones: histones carry epigenetic messages (transmission to oocyte and influencing embryo development?)

Ramos et al. Hum Repr, 2008

Summary

Two important processes during spermatogenesis are:

- Generation of unique genetic content of spermatozoa. Takes place at meiosis I during the process of recombination and segregation
- Conservation of genetic integrity is helped by protamination. This leads to nucleus condensation during spermiogenesis → protects against a.o. DNA damaging factors
- Studying these processes will shed some light on the quality of spermatogenesis in (NOA patients) human

Acknowledgements

- Jan Kremer, Peter de Boer, Liliana Ramos, dept. of Obstetrics and Gynaecology, Radboud University Nijmegen Medical Centre
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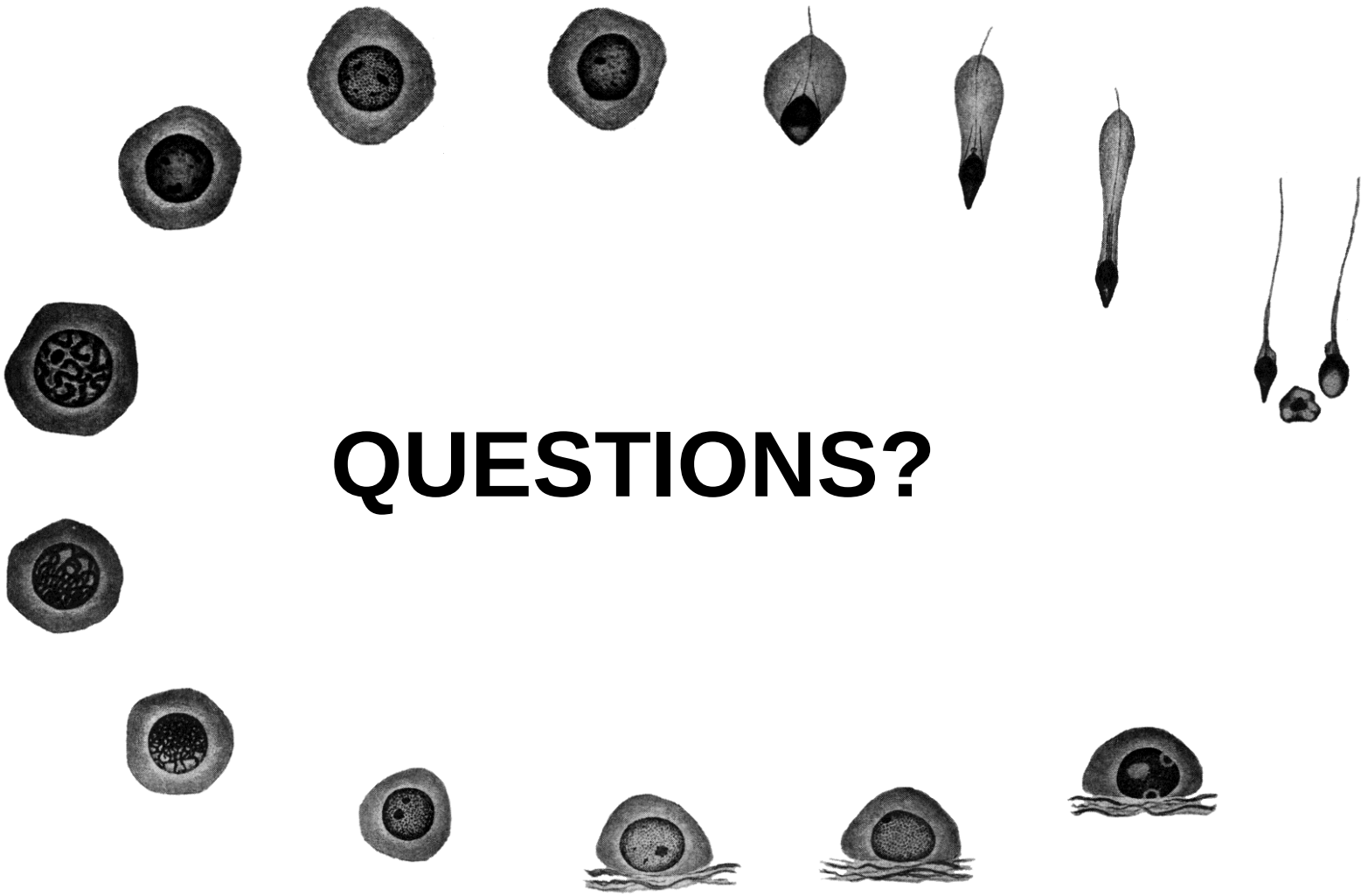
- Willy Baarends, dept. of Reproduction and Development, Erasmus MC, Rotterdam
- Johan van der Vlag, dept. of Experimental Urology, Nijmegen Centre for Molecular Life Sciences



QUESTIONS?