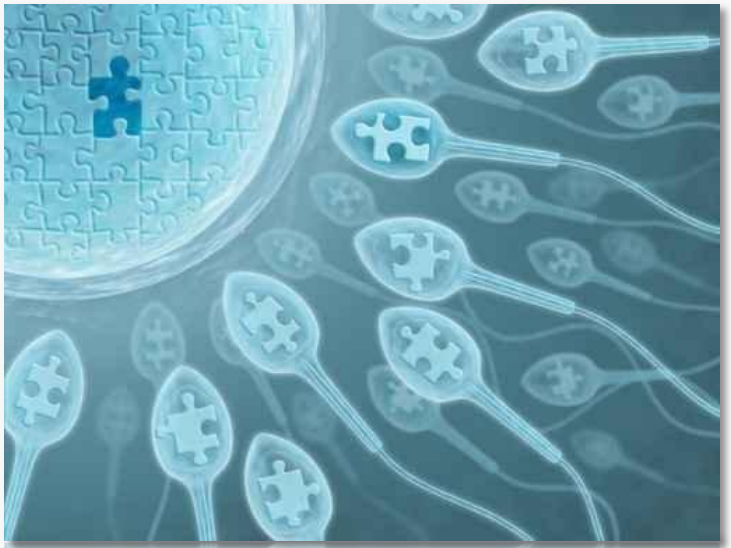
12èmes journées sur l'Assistance Médicale à la Procréation de l'Hôpital Américain de Paris - 24-25 novembre 2011



La protéomique de l'éjaculat

Projet Fertichip™

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Inserm U1085

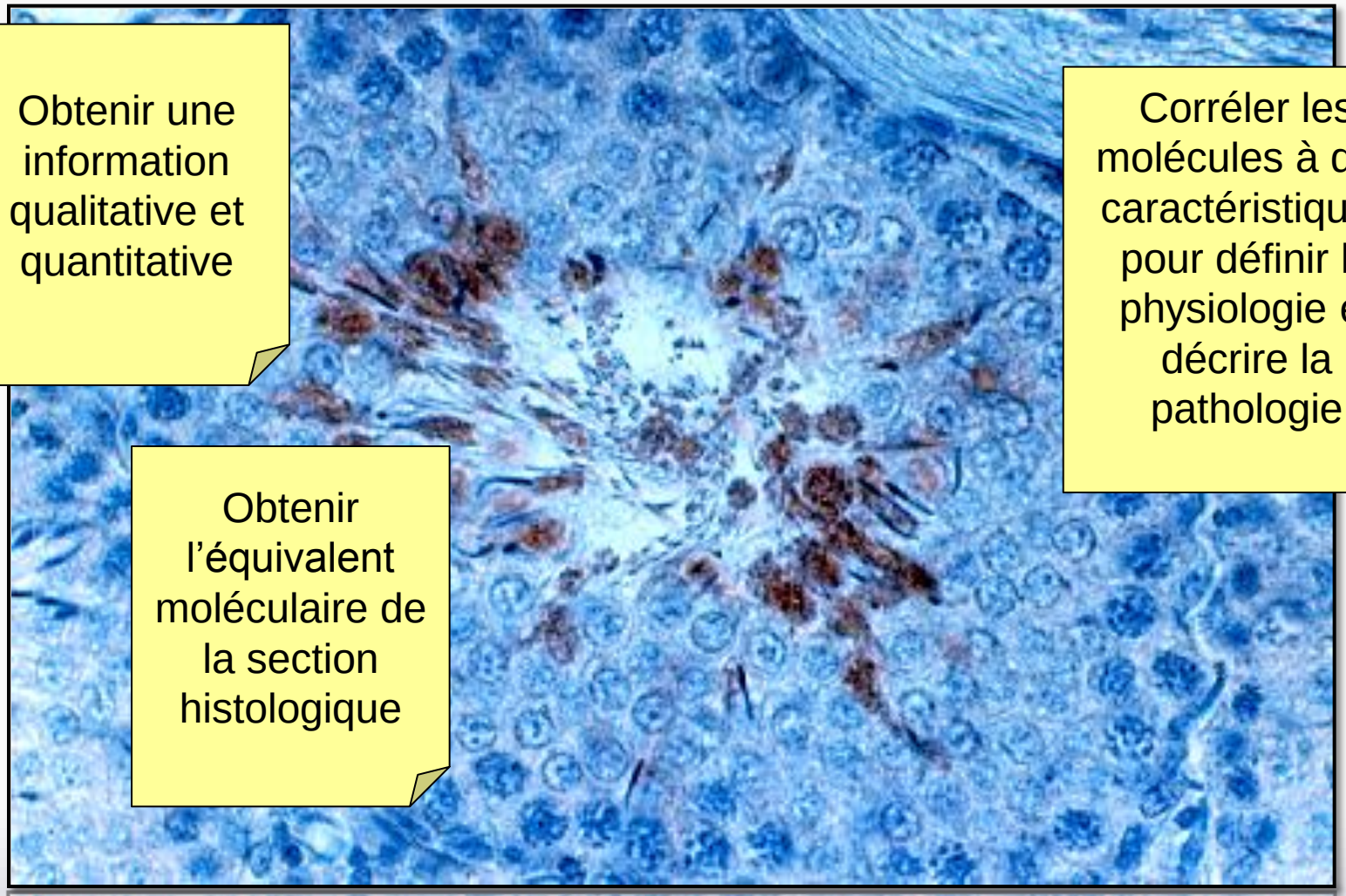
La Protéomique

Etude des protéines à grande échelle par des méthodes de biochimie et de biologie structurale, en relation avec les données du génome

Trois domaines:

- **Cartographie et identification des protéines**
- **Analyse des interactions / recherche de partenaires**
- **Structure / Fonctions**

Pourquoi la protéomique ?



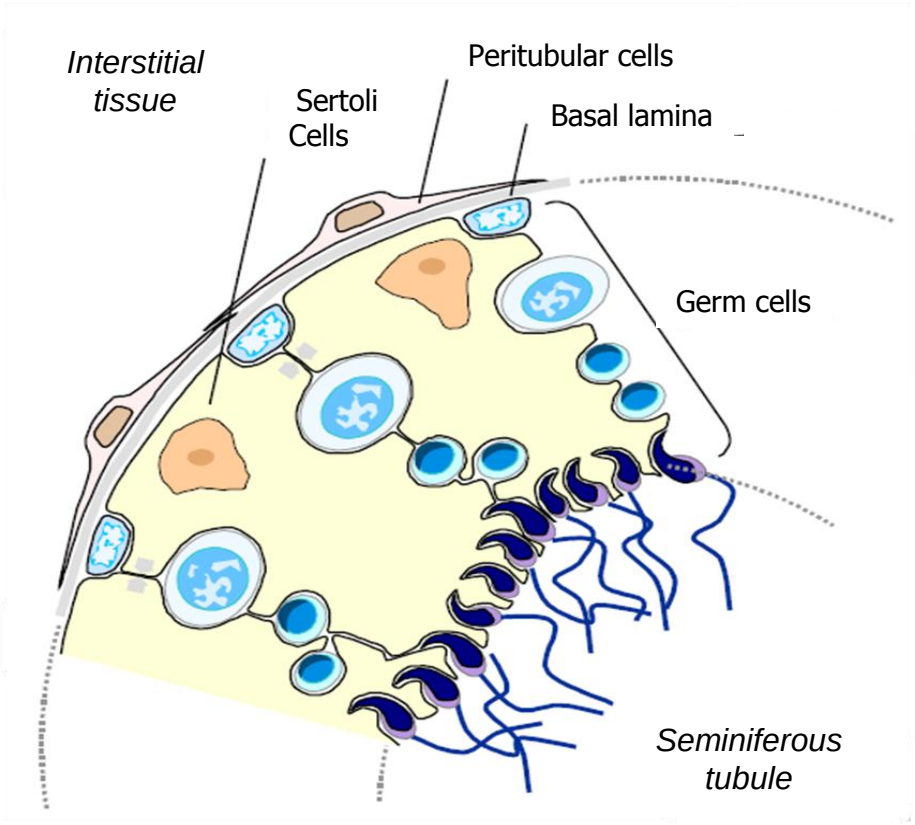
Obtenir une information qualitative et quantitative

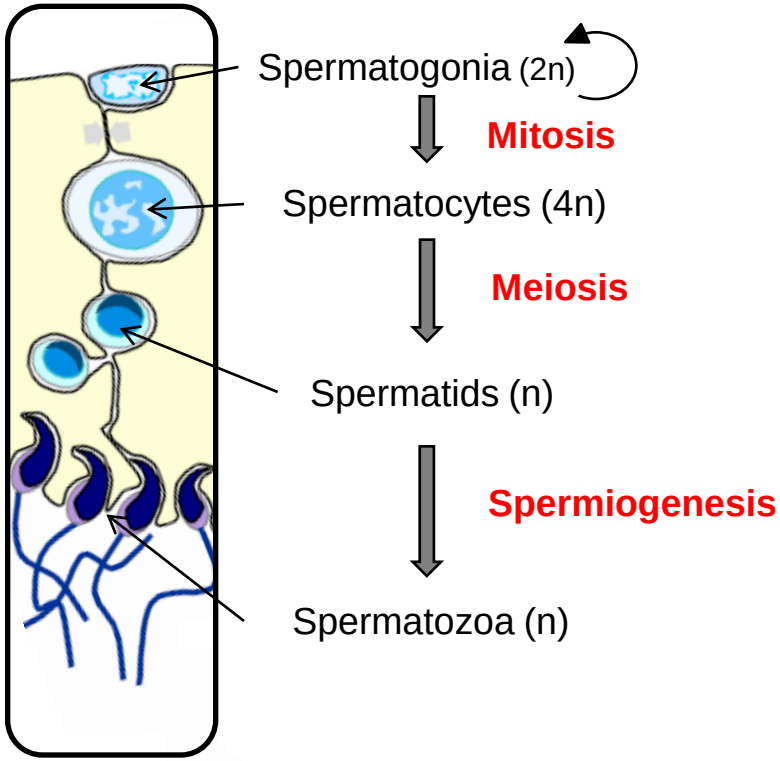
Obtenir l'équivalent moléculaire de la section histologique

Corréler les molécules à des caractéristiques pour définir la physiologie et décrire la pathologie

Approche permettant l'évaluation holistique de l'expression des gènes dans une cellule ou un tissu

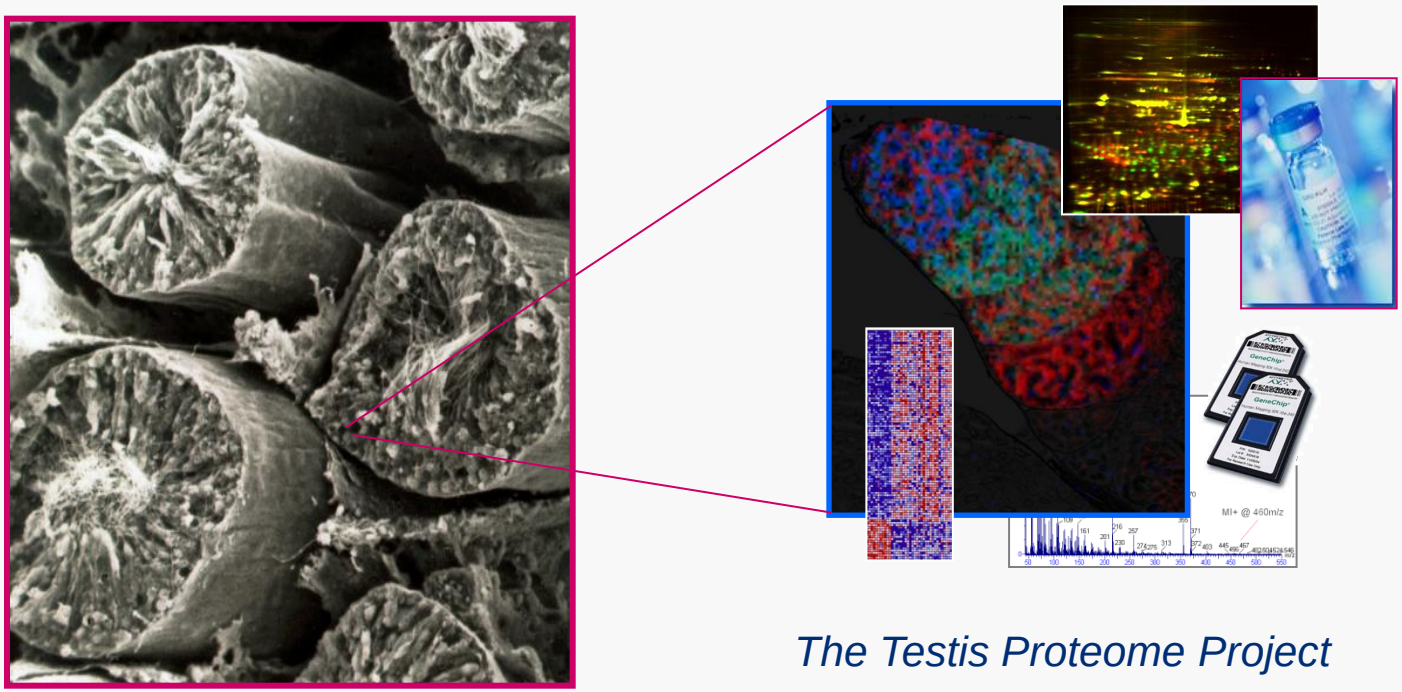
Spermatogenesis in mammals





Our objective:

Decipher the intercellular crosstalks that drive germ cell maturation



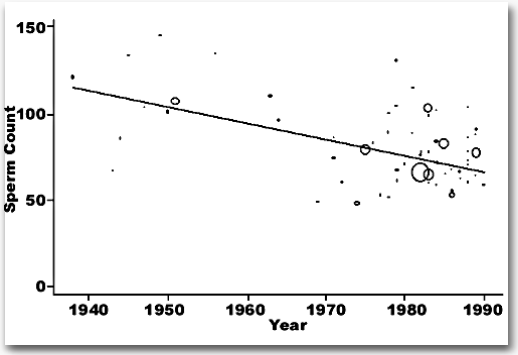
The Testis Proteome Project

- **Establish the repertoire of testicular proteins**
- **Identify key proteins in spermatogenesis**
- **Explore testicular pathologies** *(e.g., sterilities, CIS)*

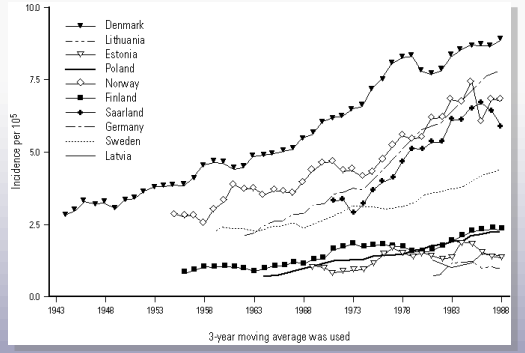
Protein markers for clinical applications

Status of male fertility

- 15% of couples have fertility problems in developed countries
- In a couple, fertility problems are shared equally
- Unknown etiology for 1/3 of cases
- No cure for most of hypofertilities



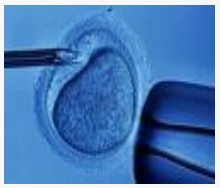
- Sperm decline in developed countries



- Increasing numbers of testicular cancers

Clinical care of male infertility






- **Male infertility:**
  - **Obstructive Azoospermia = secretion cause**
  - **Non Obstructive Azoospermia = excretion causes**

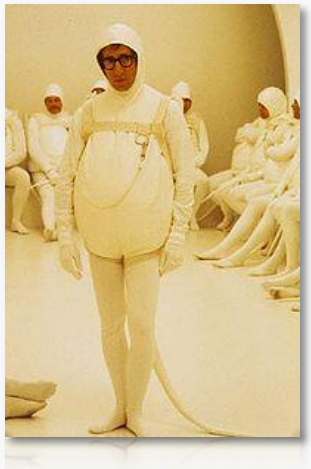
- **Obstructive azoospermia rather easy to diagnose**

- **In vitro fertilization program - NOA within couple**
  1. **Sperm extraction from testicular biopsies (TESE)**
  2. **In vitro fertilization with intracytoplasmic sperm injection (ICSI)**

  Or: Third party reproduction

  Or: Alternative family building

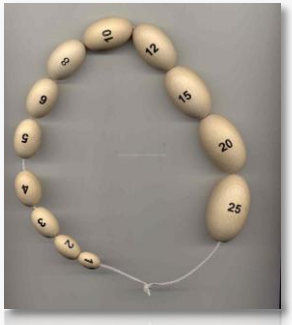
How to predict a positive biopsy outcome?



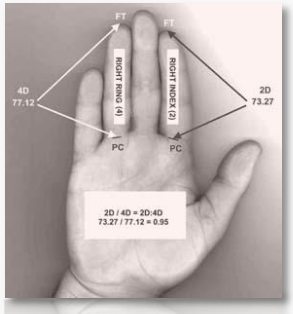
The diagnosis of Non Obstructive Azoospermia

Is there a chance to retrieve live sperm cells from testicular biopsies?

- **Spermogram; sperm biochemistry; plasma FSH, LH, prolactin…**
- **Physical examination parameters:**



Testis size



2D:4D finger length ratio

- **Assays**:

Inhibin A and B, AMH

Indirect tests with very poor predictive power

The market of Assisted Reproductive Technologies

- ✓ **12 millions IVF cycles performed per year worldwide**
- ✓ **>10% involve male infertile patients**

Source: World Health Organization

Number of biopsies performed per year:

France	Italy	Spain	U.K.	Benelux	Brasil	USA
6 000	4 500	8 000	5500	6 000	8500	35 000

~ 60% of testicular biopsies are negative

No consensus test available to predict positive biopsy outcomes

Testicular biopsy is mandatory

The human seminal plasma: a *source for potential biomarkers*

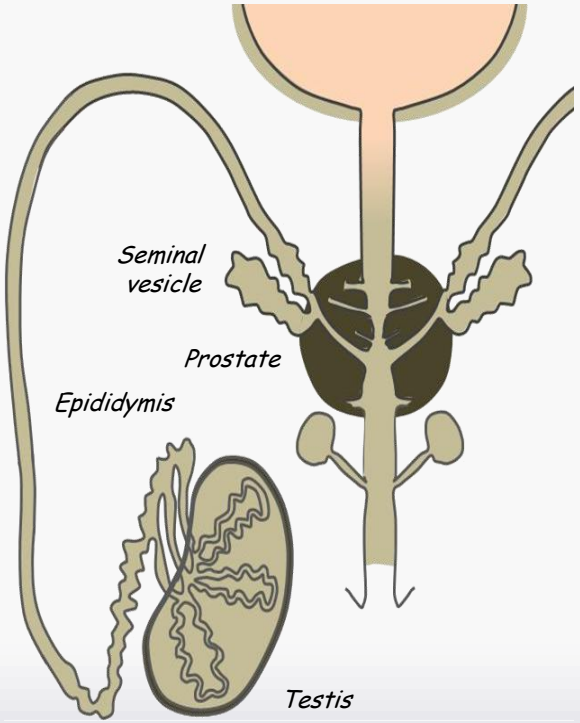
Produced by 4 major organs:

Production of spermatozoa and testicular fluid

Transit and maturation of gametes

- **Energy supplying** (Proteins, Fructose…)
- **Alkaline buffering**

- **Fluidization of ejaculate** (Proteases…)
- **Motility and final maturation of spermatozoa**

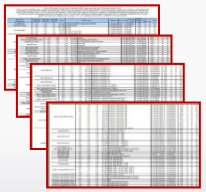


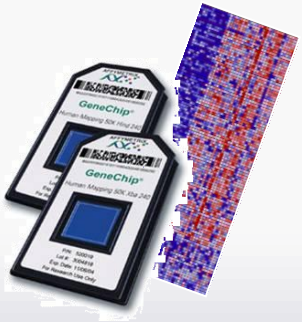
Experimental strategy

Differential analysis

iTRAQ, Label-free, DIGE…

An alternative approach





AMEN: The Annotation, Mapping, Expression and Network suite of tools Chalmel & Primig, BMC Bioinformatics, 2008

The Human seminal Plasma proteome

- > 2000 proteins identified to date
- Relatively poor mining information for male reproduction specialists
- Major effect of coagulation/liquefaction on proteome
- Several known seminal plasma proteins no yet found

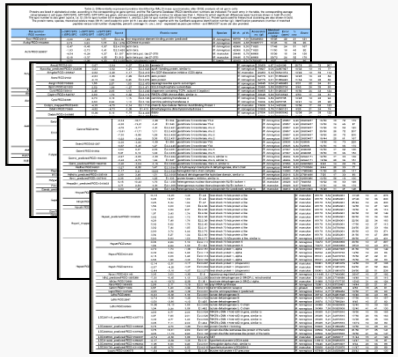
Search for new discrete proteins
Identify organ/function markers

Comparison of human seminal plasma proteomic studies

Studies to be integrated:

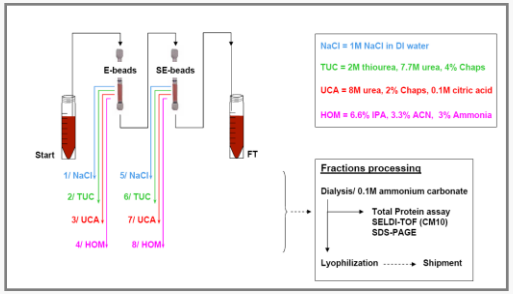
- *Utleg et al., 2003* => **136** proteins
- *Fung et al., 2004* => **46** proteins
- *Pilch & Mann, 2006* => **916** proteins
- *Thimon et al., 2008* => **148** proteins
- *Drake et al., 2009* => **34** proteins
- *Poliakov et al., 2009* => **440** proteins
- *Wang et al., 2009* => **626** proteins
- *Batruch et al., 2011* => **2022** proteins

Identification lists



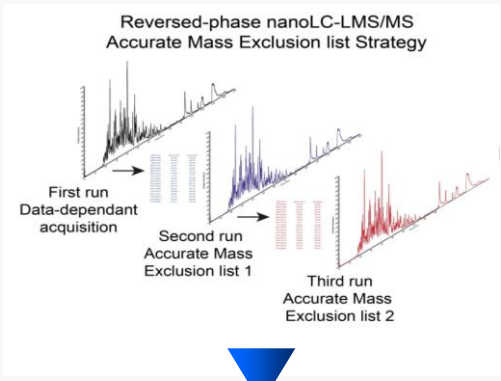
Experimental strategy

Human
seminal plasma

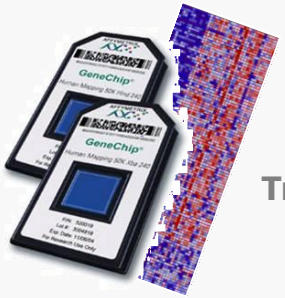


Proteominer™ fractionation

8 Fractions
+ Start
+ Flow Through



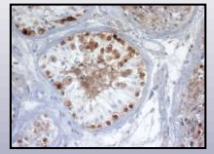
Combined Dataset

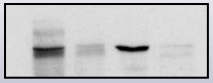


Mining with
Transcriptome datasets

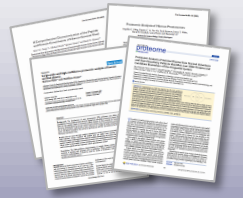
Candidate marker selection

Candidate marker validation



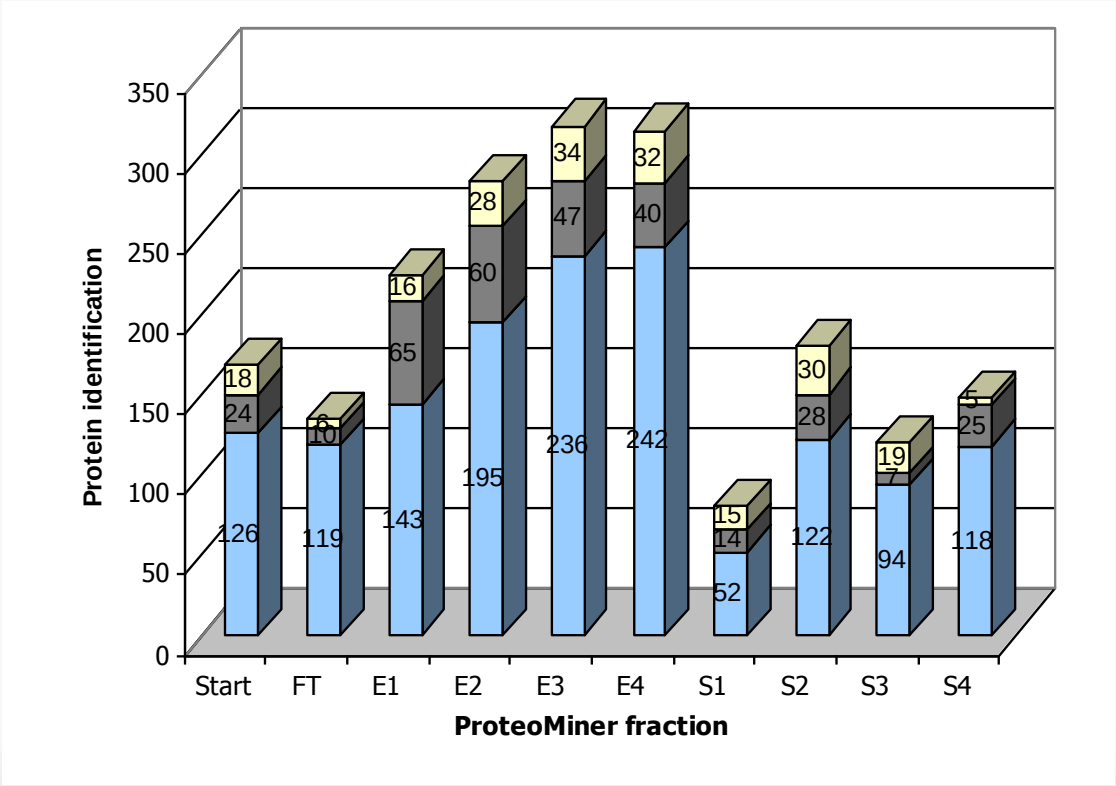


on normal and pathological seminal plasma



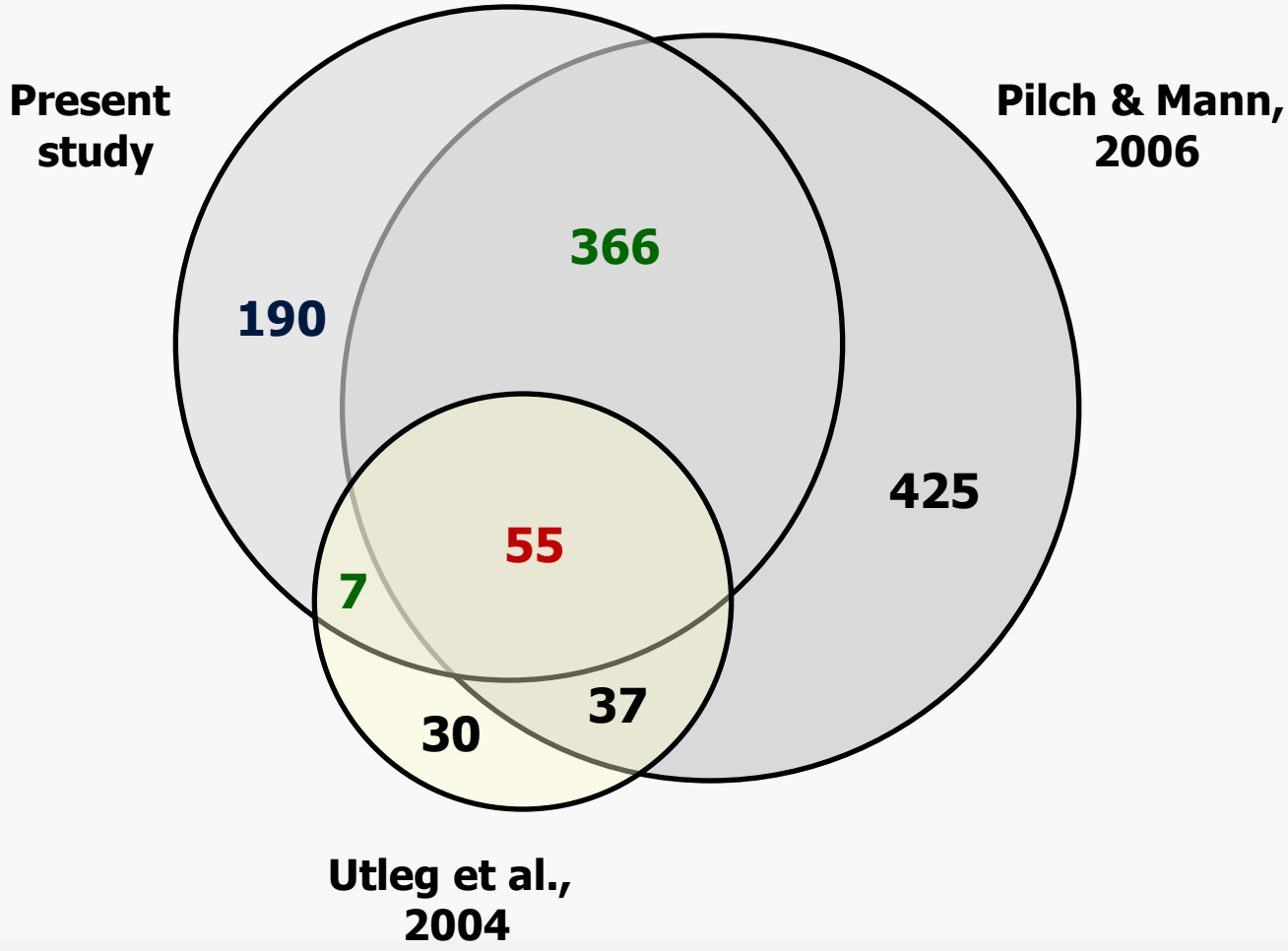
Bibliomics

Number of identified proteins from multiple injections of each fraction and dynamic exclusion of previously identified peptides



~20% more proteins in each fraction

Comparison of 3 proteomic studies on the human seminal plasma

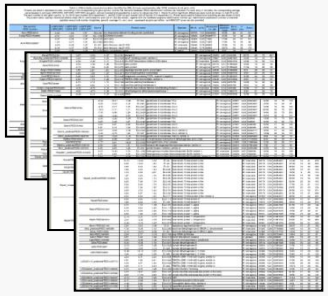


Incomplete covering of the three datasets

Relevant approach in completing the repertoire of proteins

Comparison of human seminal plasma proteomic studies
Mapping of protein identifiers

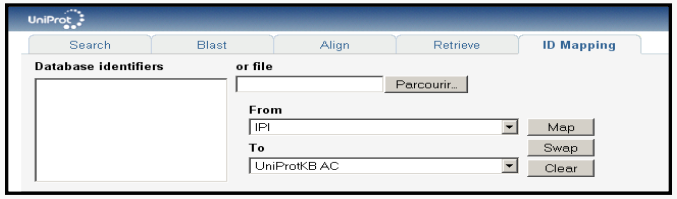
Identification lists

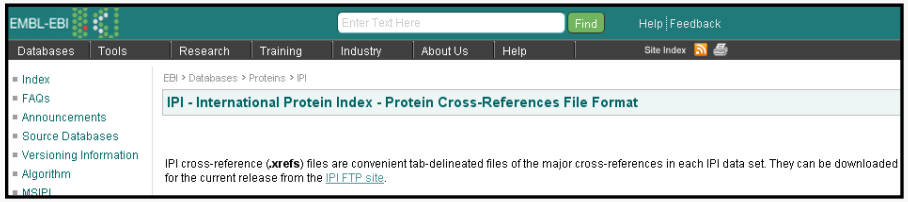


- Uniprot Acc Numbers
- Uniprot Entry Name
- GI numbers
- IPI Identifiers

→ Uniprot Acc Numbers, EntrezGene

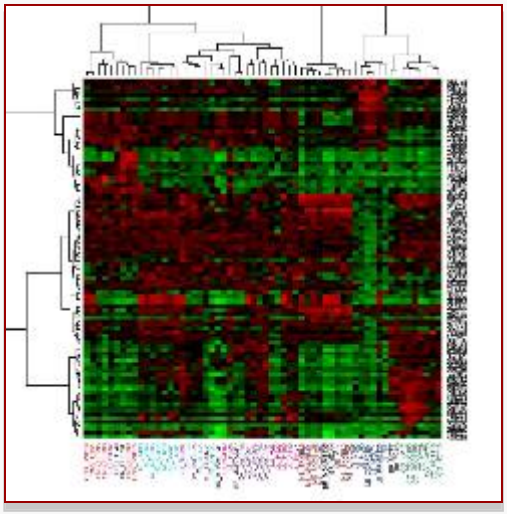
→ GeneOntology, Probeset

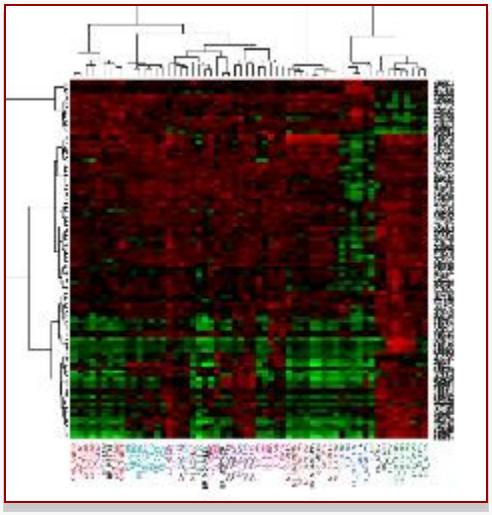


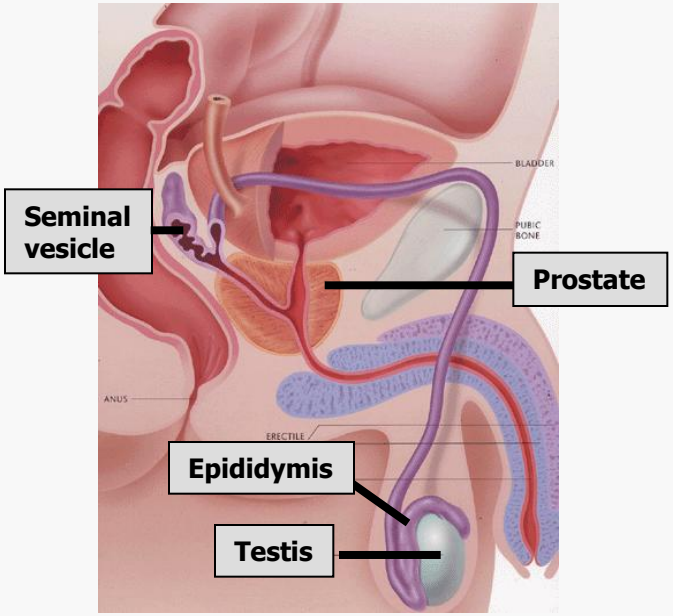


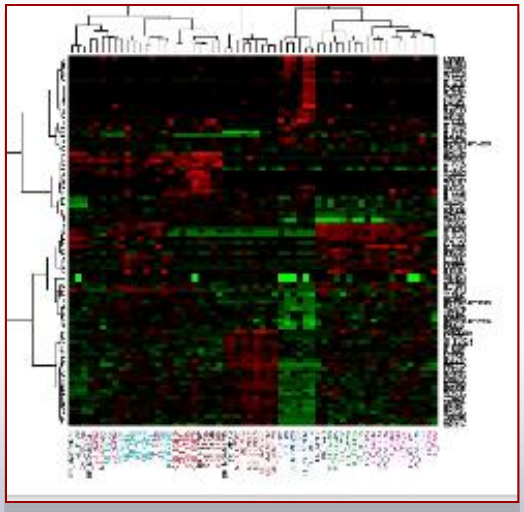
	Original IDs	Uniprot IDs	Entrez Gene IDs	Probe sets
Present study	668	668	676	1462
Pilch & Mann (2006)	916	875	881	1998
Utleg et al. (2003)	136	121	132	347
Fung et al. (2004)	46	48	45	99
Wang et al. (2009)	626	511	450	1024
Drake et al. (2009)	34	35	36	96
Poliakov et al. (2009)	440	443	446	949
Thimon et al. (2008)	148	146	127	298
Batruch et al. (2011)	2022	1970	2083	4771
Overall	-	2567	2331	5341

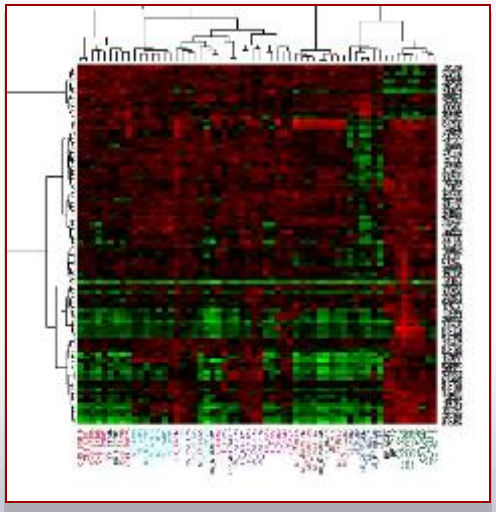
Mining protein identification list with transcriptomics datasets







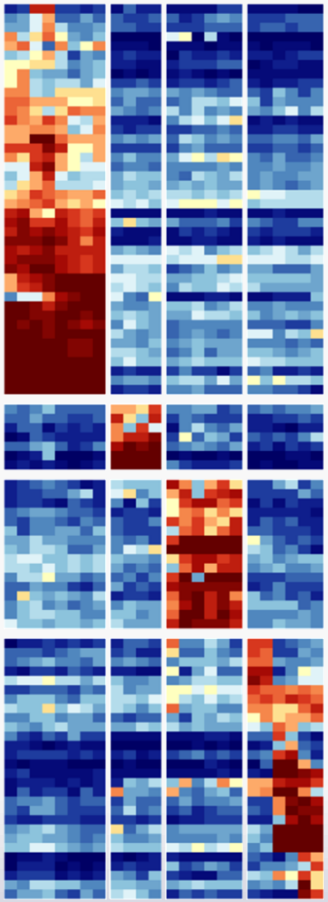




Organs involved in seminal plasma production

Mining protein identification list with transcriptomics datasets

Chalmel & Primig,
BMC Bioinformatics 2008

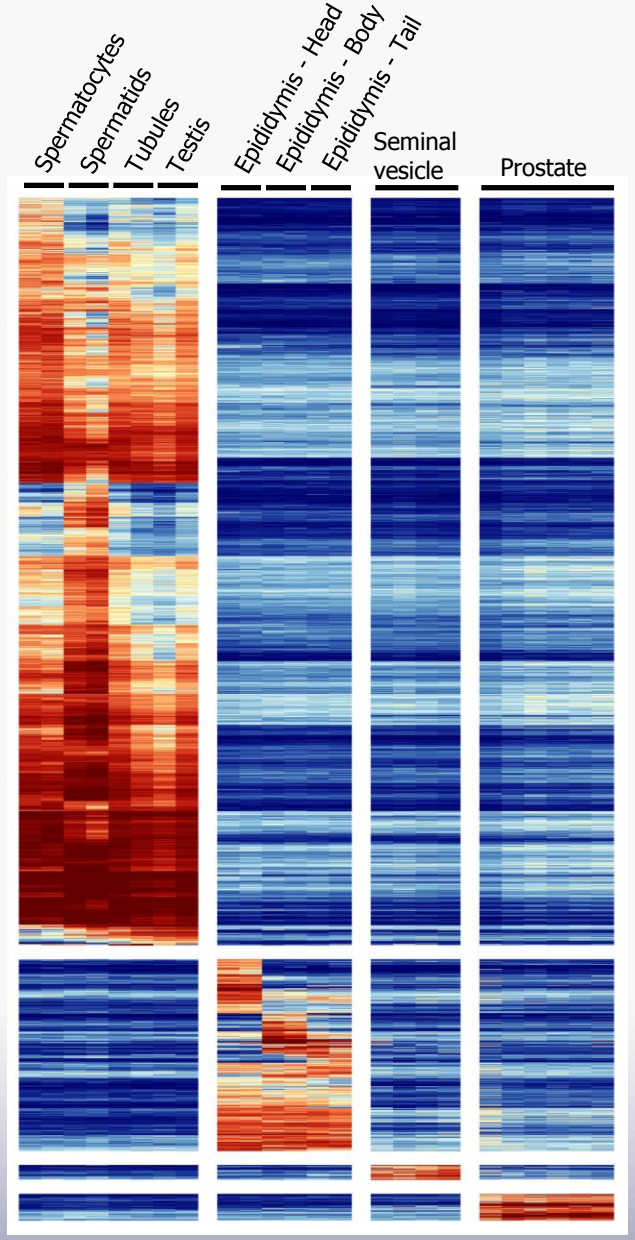


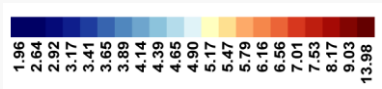
How to identify organ functional markers in the seminal plasma ?

Selection strategy

1. **Identify overexpressed genes in organ datasets**
2. **Verify organ-specificity of candidate genes**
3. **Search for corresponding proteins into the protein list**

Organ-specific gene expression along the human male reproductive tract





Intensity of expression
(Log2)

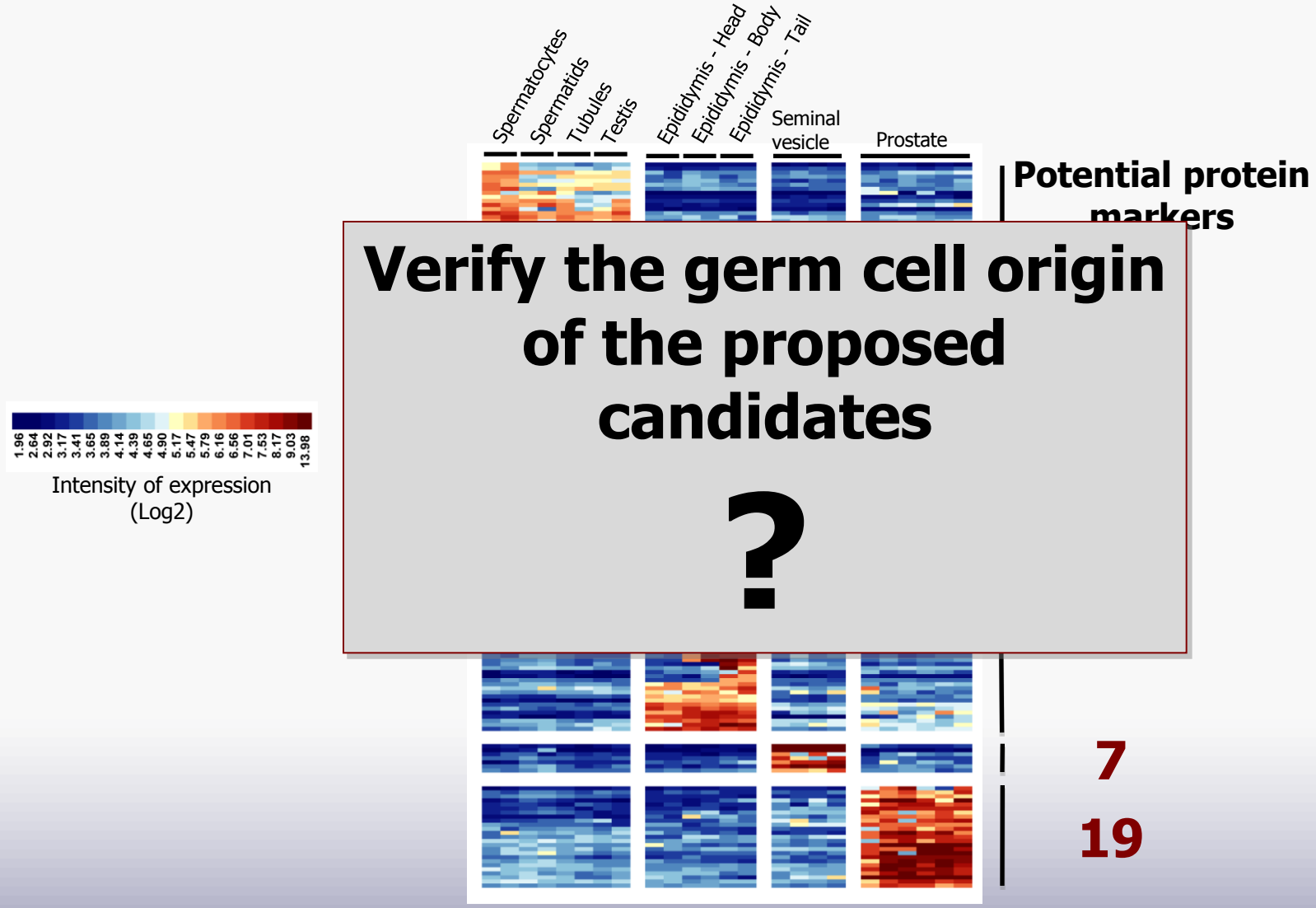
- **Detection Threshold = Mediane**
- **FC > 2**
- **Limma FDR <1%**

1942 genes
(2669 probes)

391 genes
(684 probes)

35 genes (53 probes)
53 genes (95 probes)

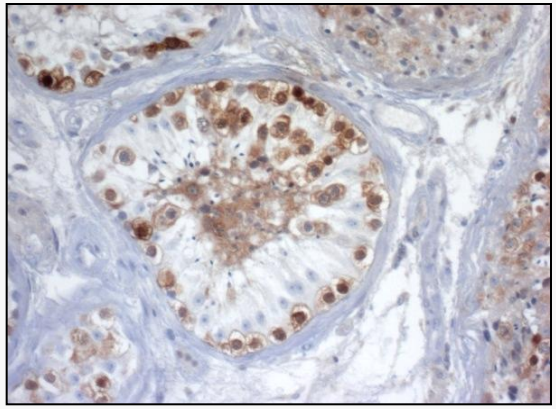
Tissue-specific protein expression along the human male reproductive tract



Verify the germ cell origin of the proposed candidates

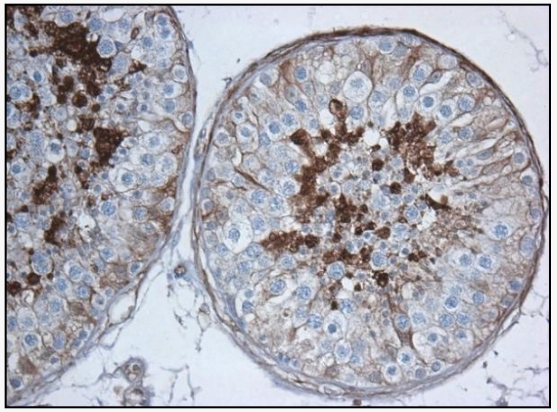
?

Validation of predicted markers of the urogenital tract



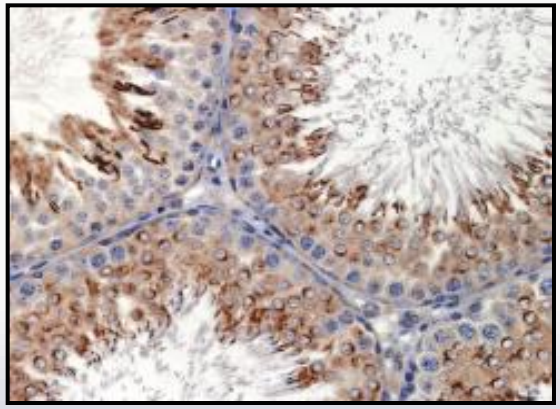
TKTL1

Secreted by spermatids



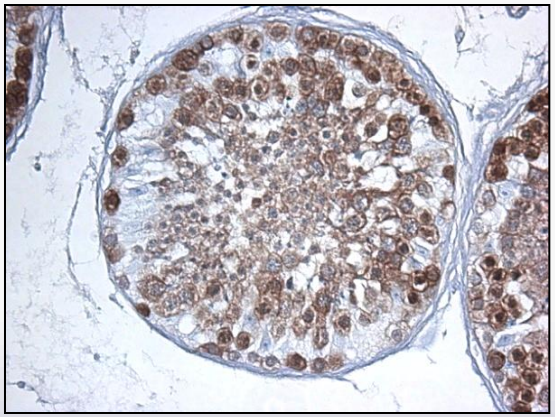
PGK2

Meiotic and post-meiotic



PGAMB

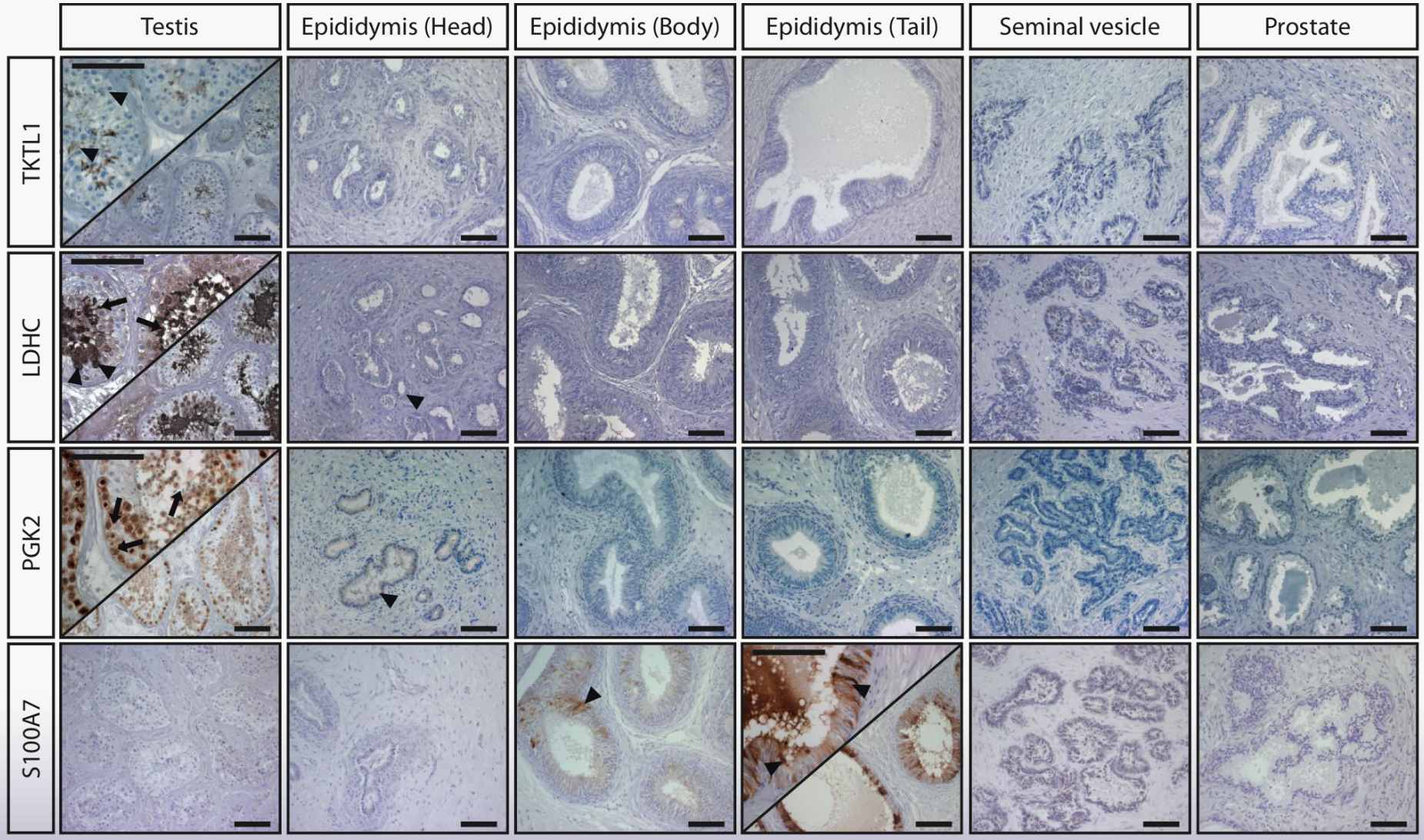
Meiotic and post-meiotic



LDHC

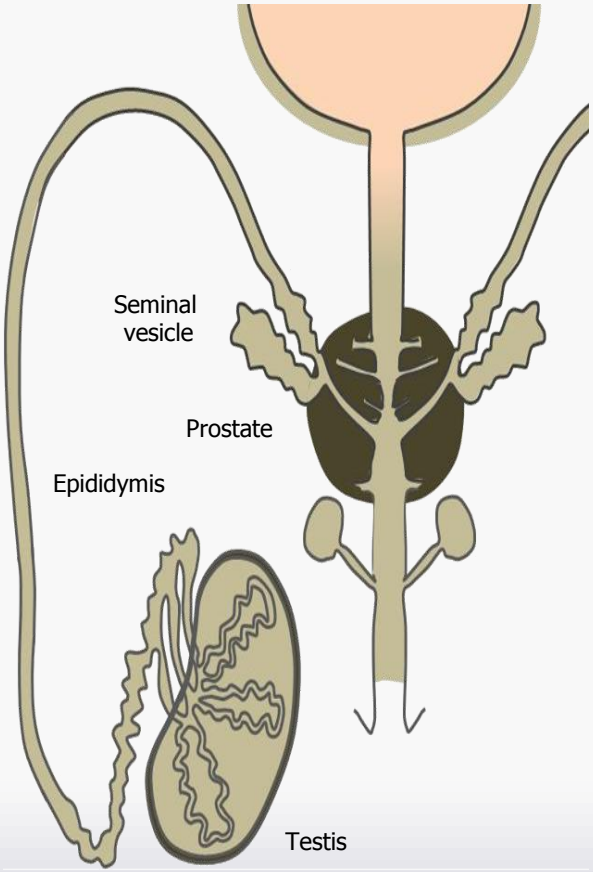
Meiotic and post-meiotic

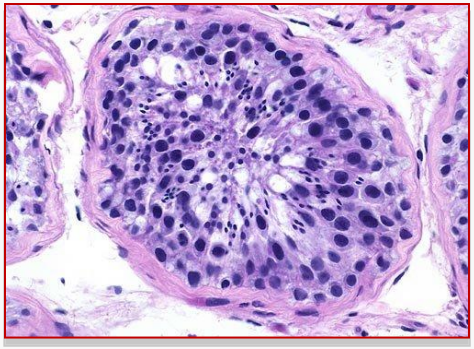
Validation of germ cell line biomarkers



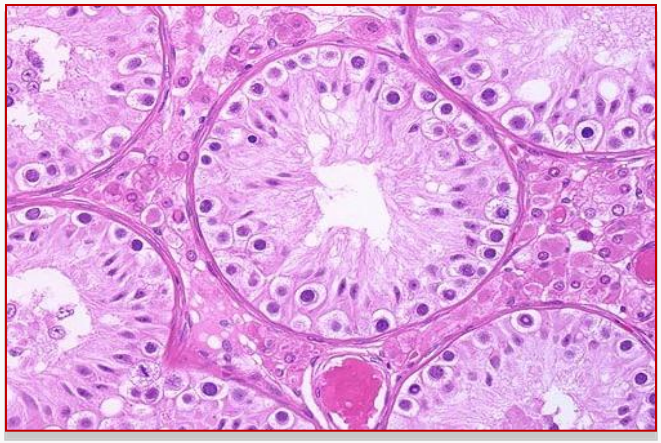
Most frequent Pathological situations

Non Obstructive Azoospermia

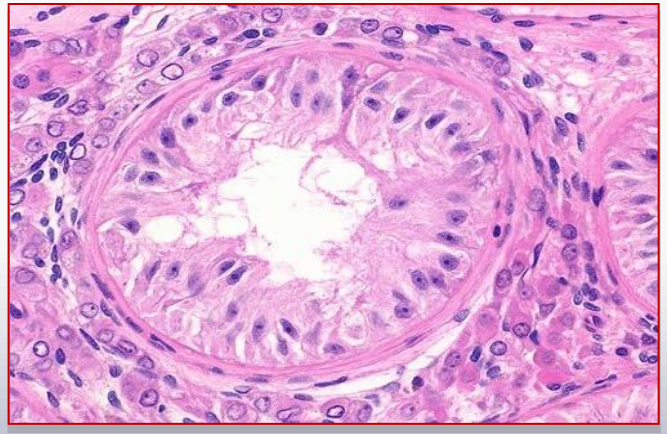




Normal spermatogenesis



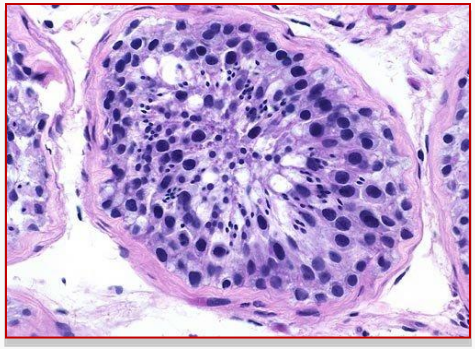
Maturation arrest



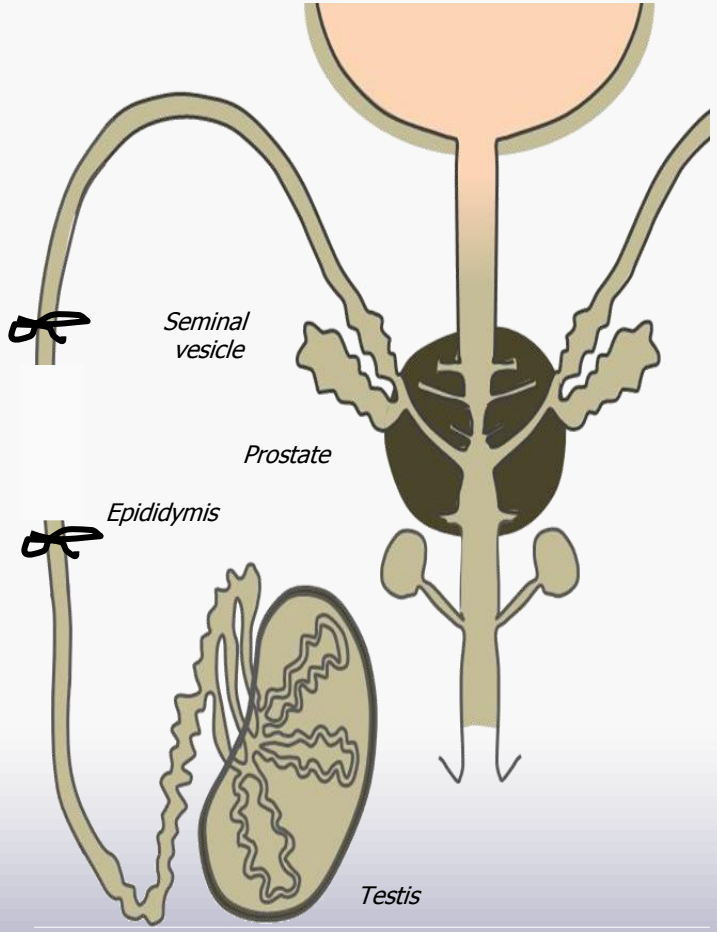
Sertoli-cell-only syndrome
(germ cell aplasia)

Most frequent Pathological situations

Obstructive Azoospermia



Normal spermatogenesis



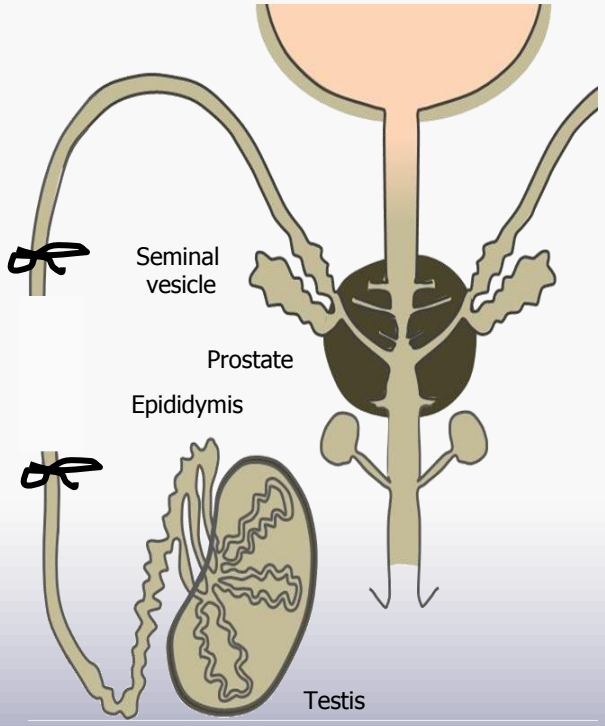
Validation of germ cell line biomarkers

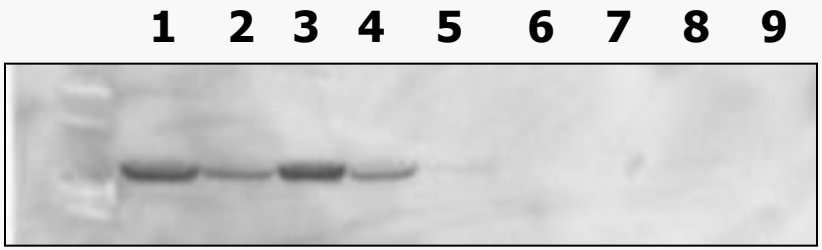
Is it possible to discriminate each type of seminal plasma by assessing the presence or absence of several germ cell-specific biomarkers using ELISA assays?

Example 1:

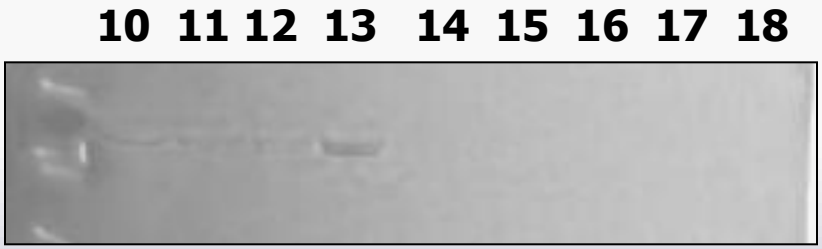
Germ cell-specific TKTL1

protein secreted by spermatids





1- 4: normal
5: unilateral obstruction
6 -7: "CBAVD" (Δf508)
8-9: maturation arrest



10 -13: Normal (set #2)
14-16: Post-vasectomized
17-18: Sertoli-cell-only syndrome

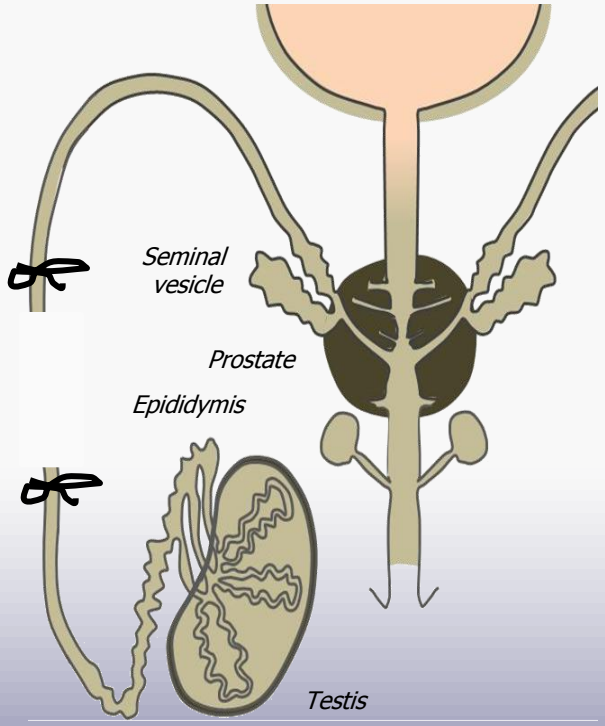
Validation of germ cell line biomarkers
A blind retrospective study

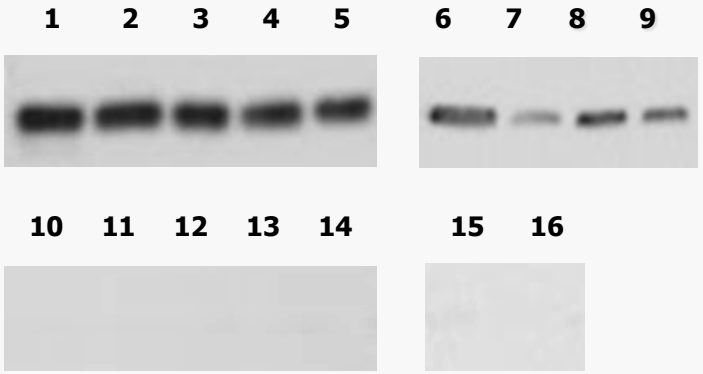
Is it possible to predict the outcome of testicular biopsies?

Example 2:

Germ cell-specific PGK2

Meiotic & post-meiotic





1-9: NOA with positive biopsies

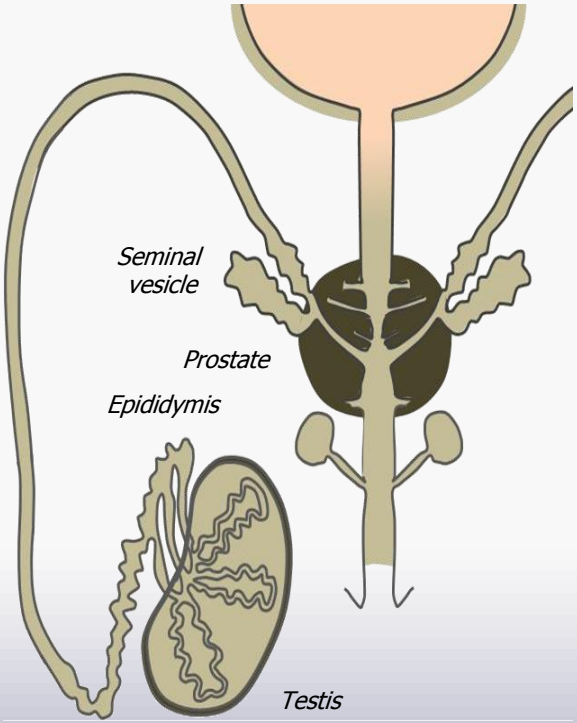
10-14: NOA with negative biopsies

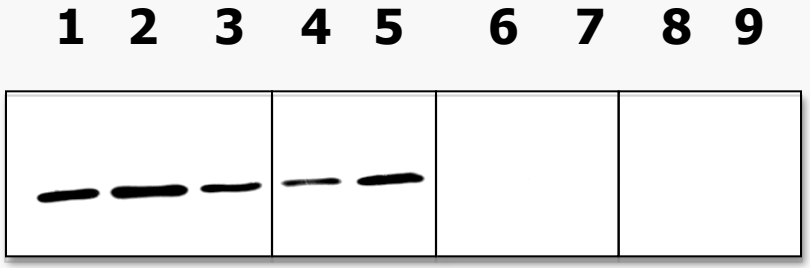
15,16: Post-vasectomy (used as control)

Additional validation of testis biomarkers

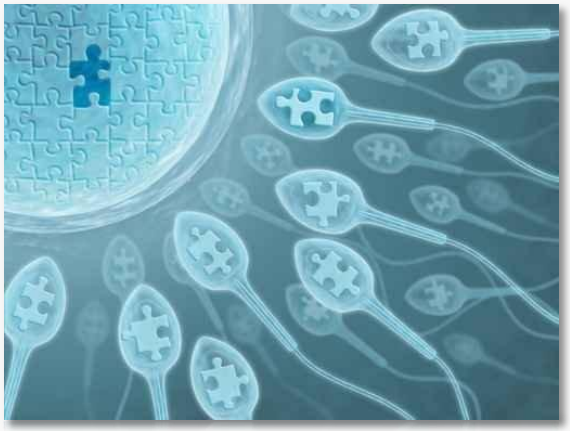
Example 3:

PAEP
Sertoli cell-specific





1- 3: normal
4-5: Sertoli cell-only syndrome
6-7: "CBAVD" (∆f508)
8-10: Post-vasectomy



The Fertichip™ concept

Patent application Inserm Transfert

Objective:
Assess the presence of mature germ cells in the testes of azoospermic patients

Method:
Detect the presence of germ cell-specific proteins in the seminal plasma using specific assays

Output:
Provide a probability score that a testicular biopsy will be positive

The Fertichip™ project

agence de la biomédecine

Selection, cloning of 91 germ cell specific genes and expression of recombinant proteins

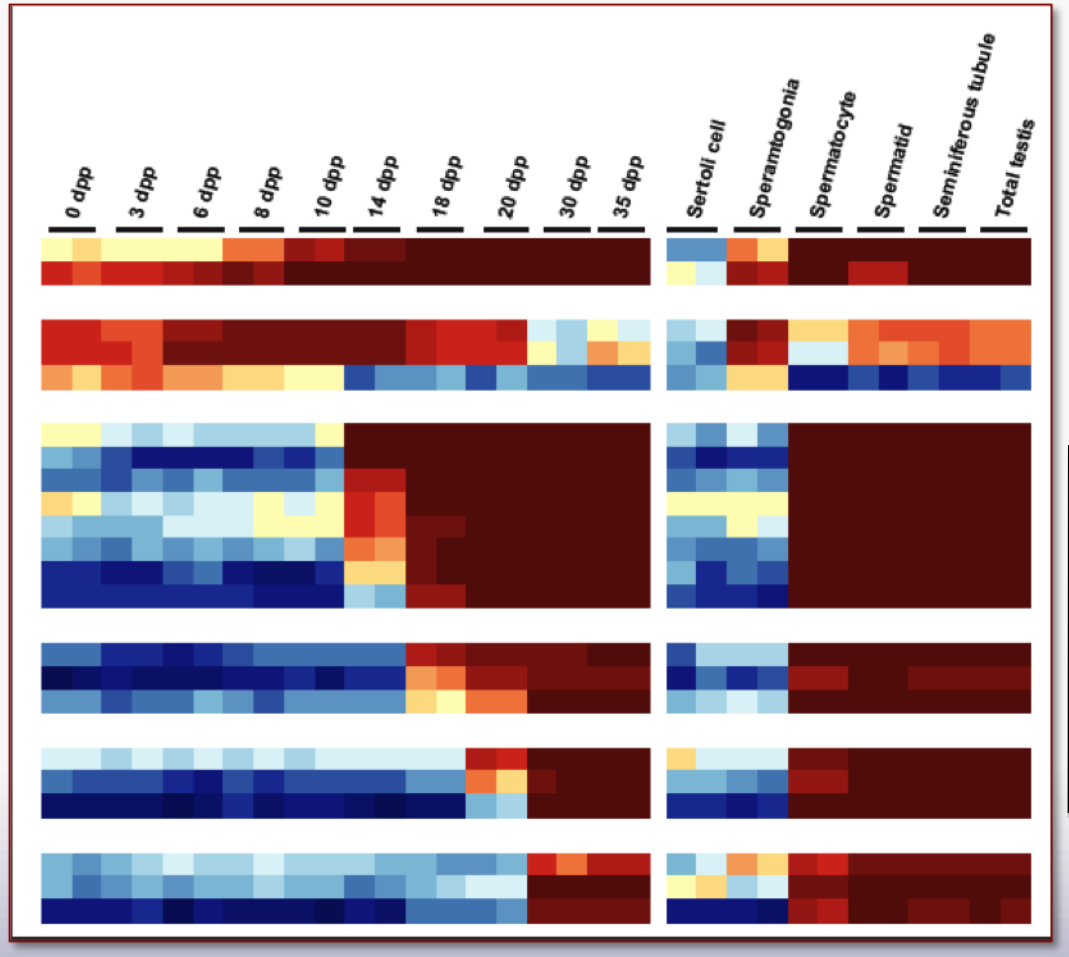
Production and validation of monoclonal antibodies against each candidate protein

Development of sensitive ELISAs

Development of a multiplex suspension bead assay (Luminex™)

Germ-cell specific markers in seminal plasma

Example of 22 top-priority candidates:



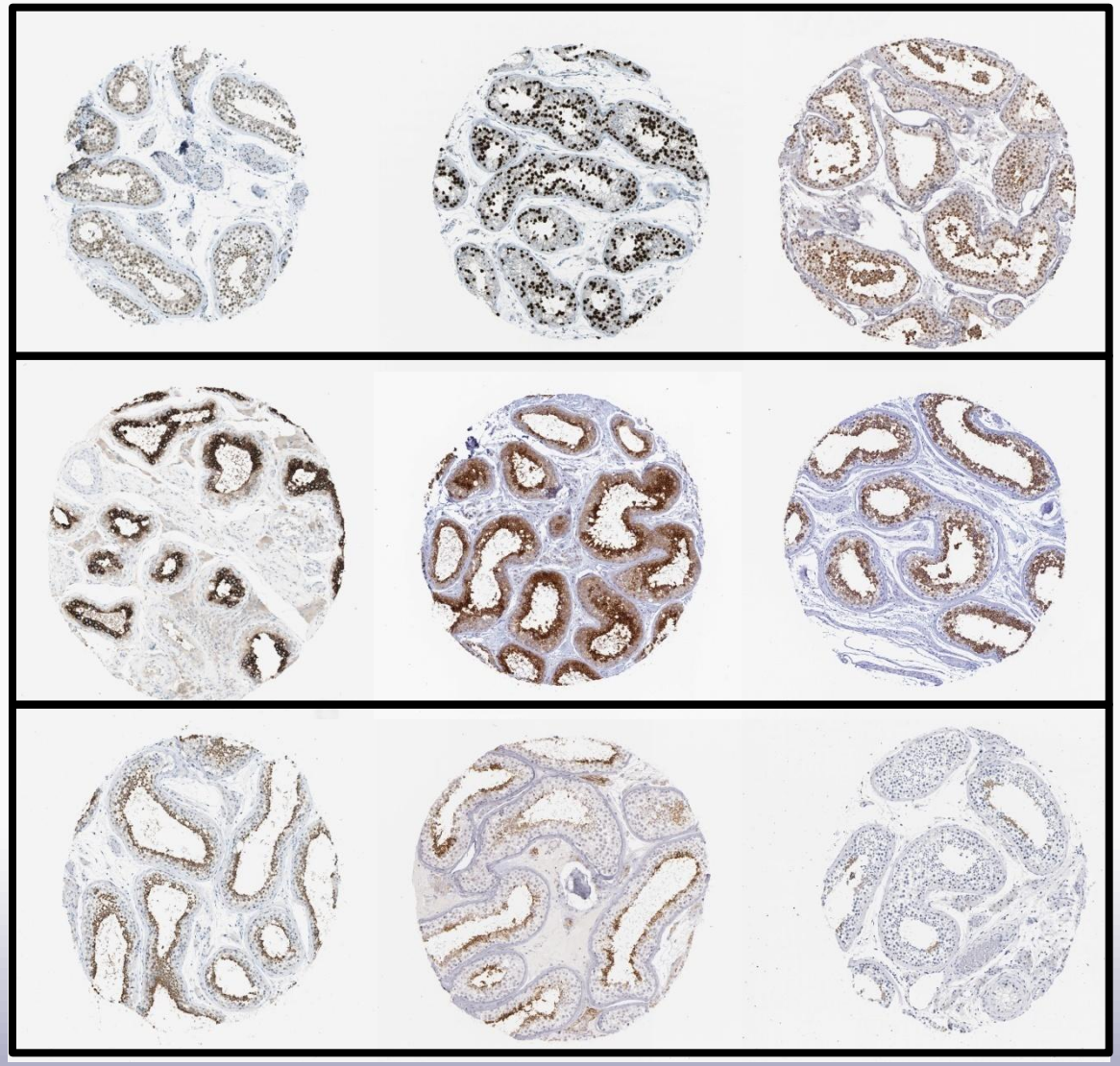
Total GC line

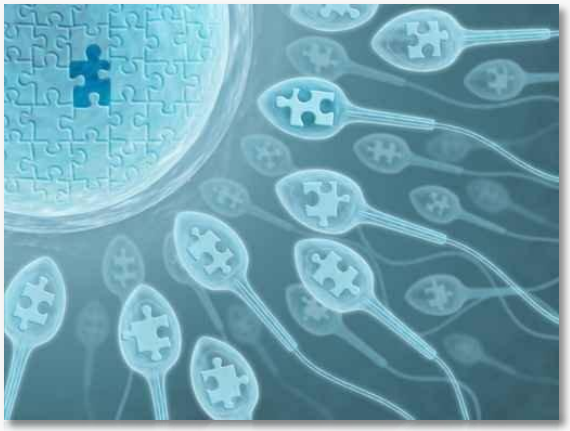
Spermatogonia

Spermatocytes/spermatids (meiosis)

Spermatids

Ongoing: validation of germ-cell specific antibodies





The Fertichip™ assay

Validation step 1

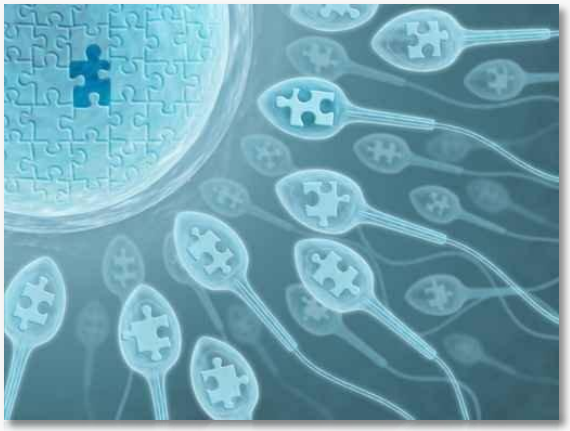
Is it possible to discriminate each type of seminal plasma by assessing the presence or absence of several germ cell-specific biomarkers using ELISA assays?

Cohorts: 400-1200 normal + pathological samples

Validation step 2

Is it possible to predict the outcome of testicular biopsies?

Cohorts: > 600 samples



The Fertichip™ assay

Validation steps

A <u>complement</u> to physical examination by the clinician to:

- ***Avoid unecessary biopsies***
- **Improve patient counselling**
- **Diminish psychological costs**
- **Reduce the financial cost of ART medicine**

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