

# Screening and selection of immunogenic antigens in male ejaculates detectable by antibodies in the sera of infertile women.

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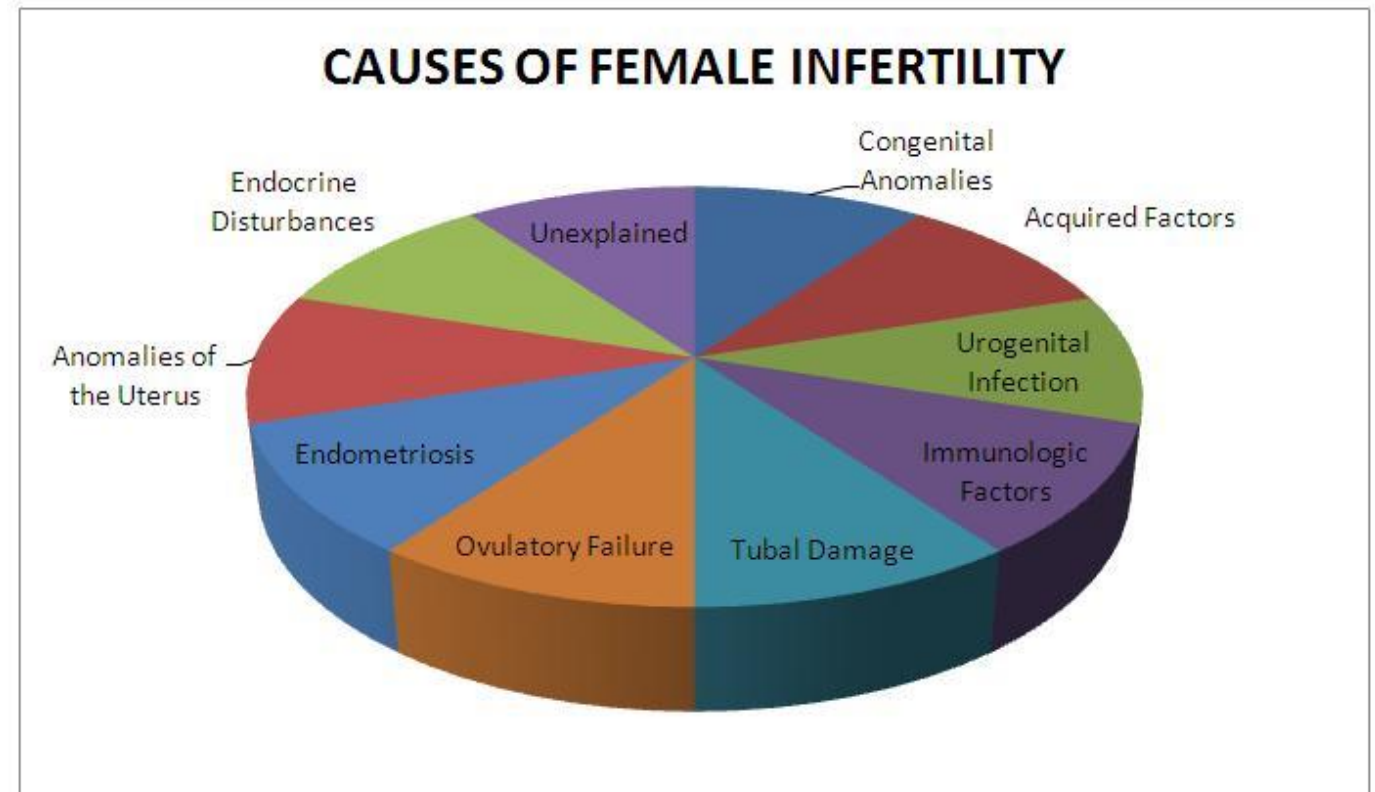
LABORATORY OF REPRODUCTIVE BIOLOGY, INSTITUTE OF BIOTECHNOLOGY, CAS, BIOCEV, VESTEC

26.2.2018

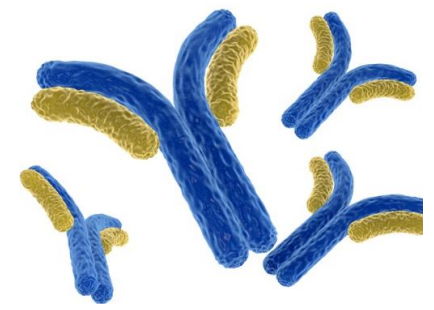
# Infertility

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- Infertility is a disease of the reproductive system
- The decreased fertility is associated with:
  - Other health issues – cancer, renal impairment, infection,...
  - **Immune system**
  - Age
  - Lifestyle and environment



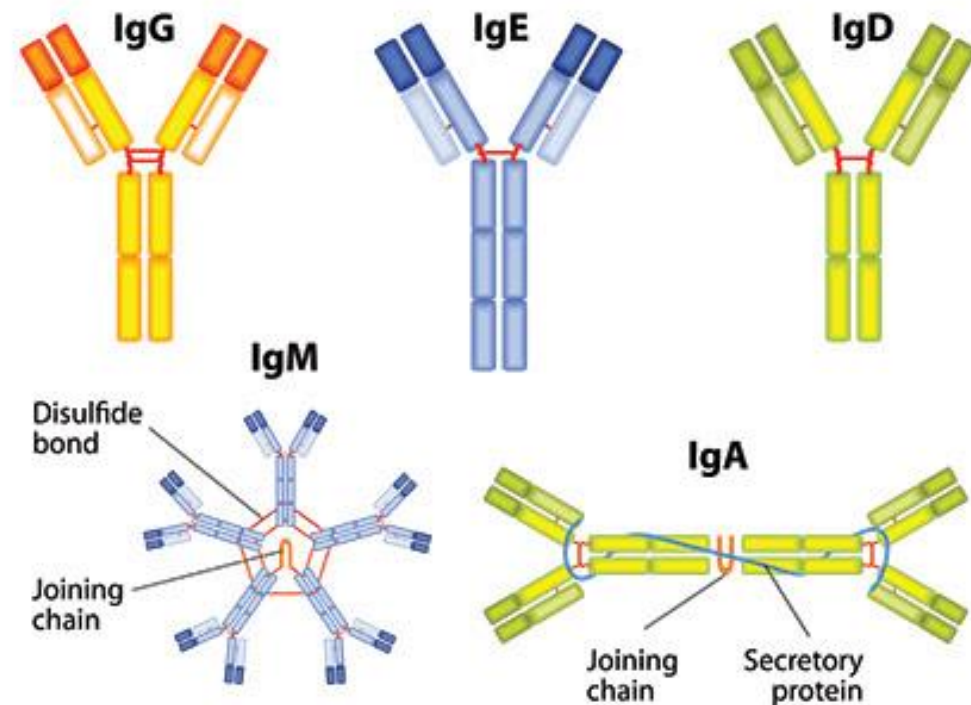
# Immune infertility



- Spontaneously produced antibodies bind to the antigens on gametes

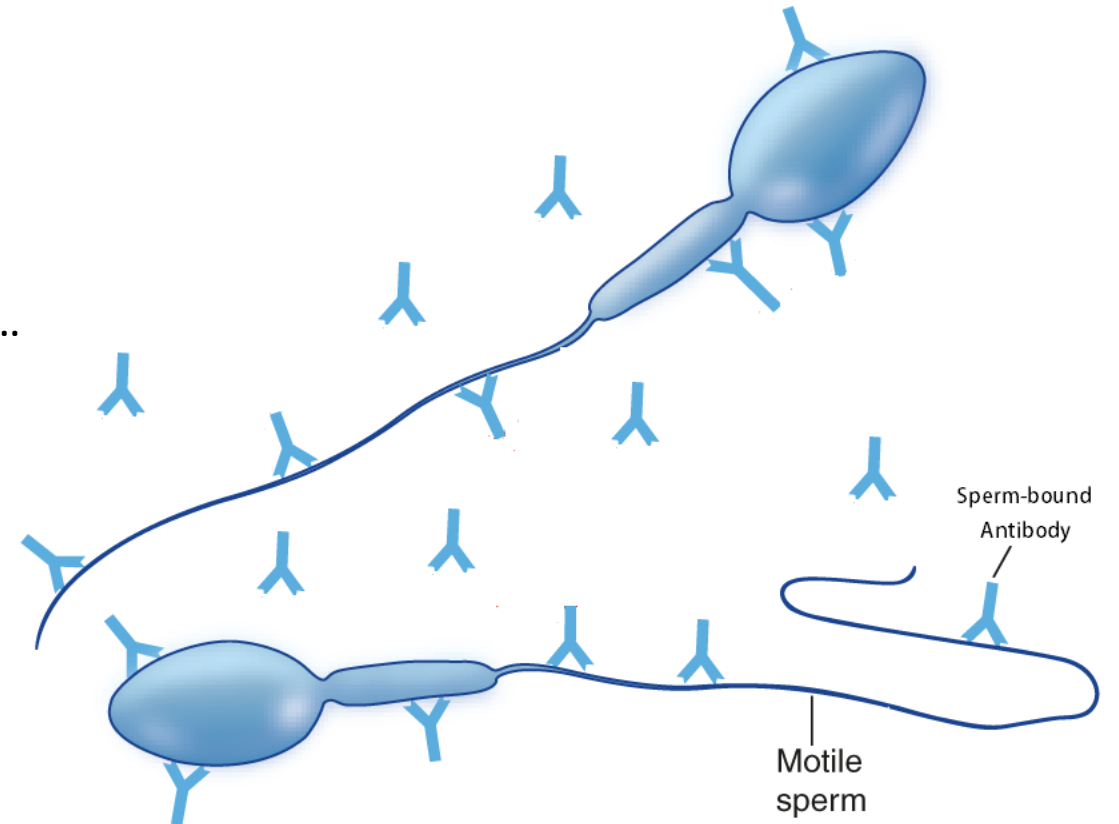
- Antibodies:

- Anti-sperm antibodies
- Anti-oocyte antibodies
- Anti-phospholipid antibodies
- Anti-nuclear antibodies
- Anti-zona pellucida antibodies
- Anti-thyroid antibodies



# Antisperm antibodies

- ♣ Immunoglobulins against a sperm antigen
- ♣ **Functions:** cytotoxic, immobilizing, agglutinating
- ♣ **Influence:** motility of sperm, capacitation, fertilization,...
- ♣ **Occurrence :**
  - Blood
  - Seminal fluid
  - Cervical-vaginal mucus
  - Follicular fluid



# Antisperm Antibodies - Immunoglobulins

IgG



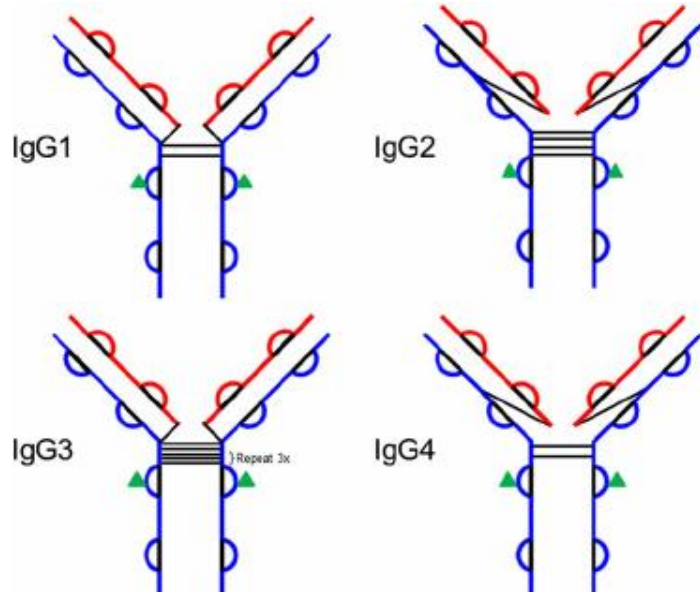
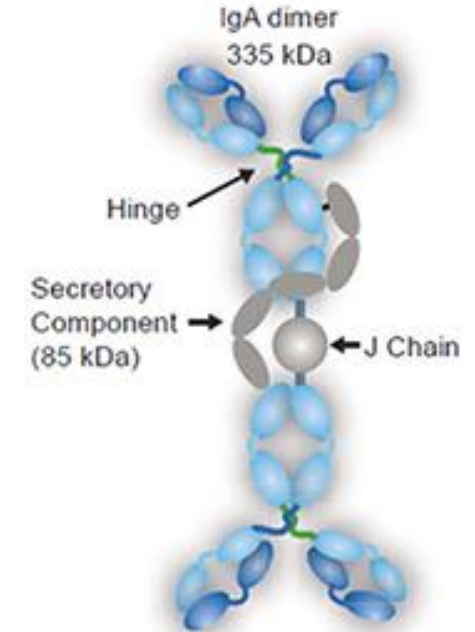
## IgG

- Mainly in blood
- **Subclass:** IgG1, IgG2, IgG3, IgG4

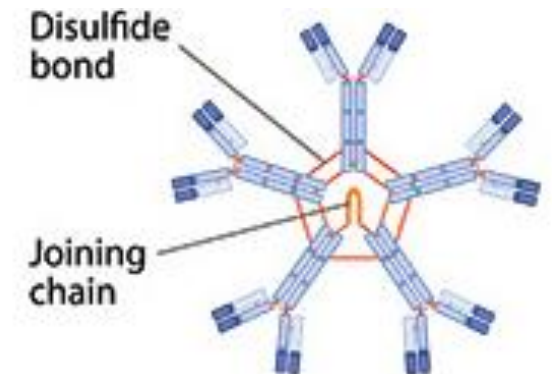
## IgA

- Mucosal antibody
- Monomer or dimer
- **Subclass:**
  - IgA1 – mainly in blood
  - IgA2 – mainly in secretions

IgA



IgM



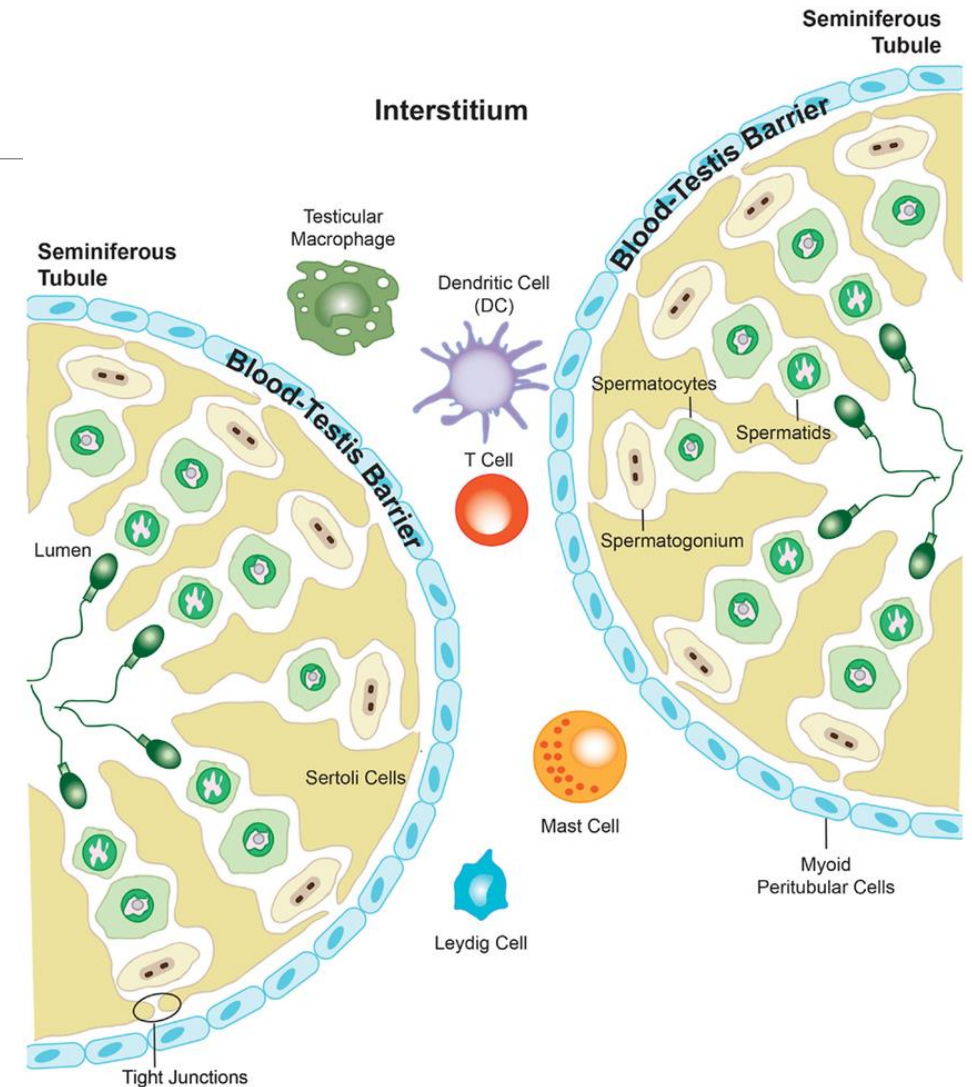
## IgM

- Pentamer and monomer
- It predominates in primary immune responses

# Antisperm Antibodies Production

## Men:

- ♣ Sperm are protected in the testis from an auto-immune attack by the blood-testis barrier; when the barrier is disrupted, auto-antibodies are produced



# Antisperm Antibodies Production

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## Women:

☞ Antibodies are produced:

- when is activated immuno-regulatory mechanism of mucosal immune system
  - Is influenced by the level of antibodies, cytokines and hormones
  - Humoral defense in mucosal tissue provides IgG, IgA and IgM
- The long-term exposure of female to sperm
- Seminal deficiency in immuno-suppressive factors



# Characterization of anti-sperm-antibody antigens

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- Sperm have very heterogeneous antigenic content
  - Improvement of diagnostic method
  - Improvement of therapeutic approaches
  - Understanding of the principles and mechanisms of these antibodies





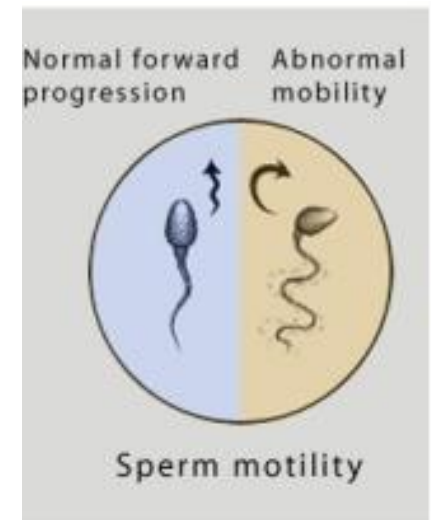
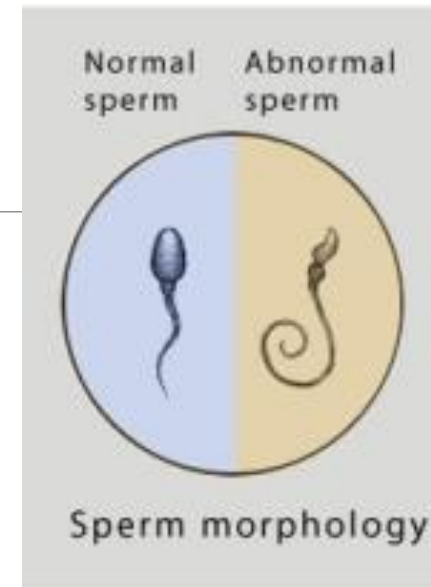
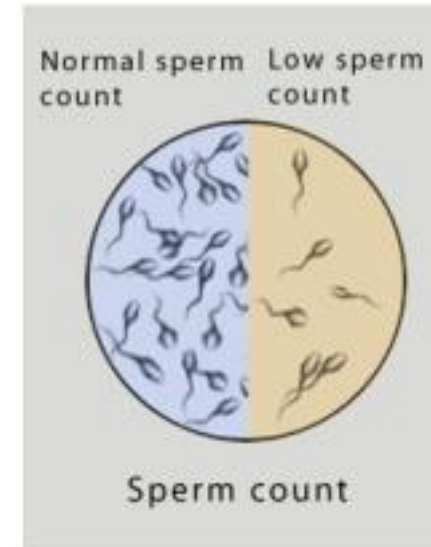
# Samples

## ☞ **Samples of sperm and seminal plasma** – RNDr. Zuzana Krátká, Ph.D.; Ing. Štěpánka Luxová, Gennet

- Normospermia
- Asthenozoospermia - low motility of sperm
- Oligozoospermia - low concentration of sperm
- Oligoasthenozoospermia - low concentration and low motility of sperm
- Oligoteratozoospermia - low concentration and abnormalities in morphology

## ☞ **Samples of sera** – MUDr. Karin Malíčková, 1st Faculty of Medicine, Charles University, Prague; Gennet

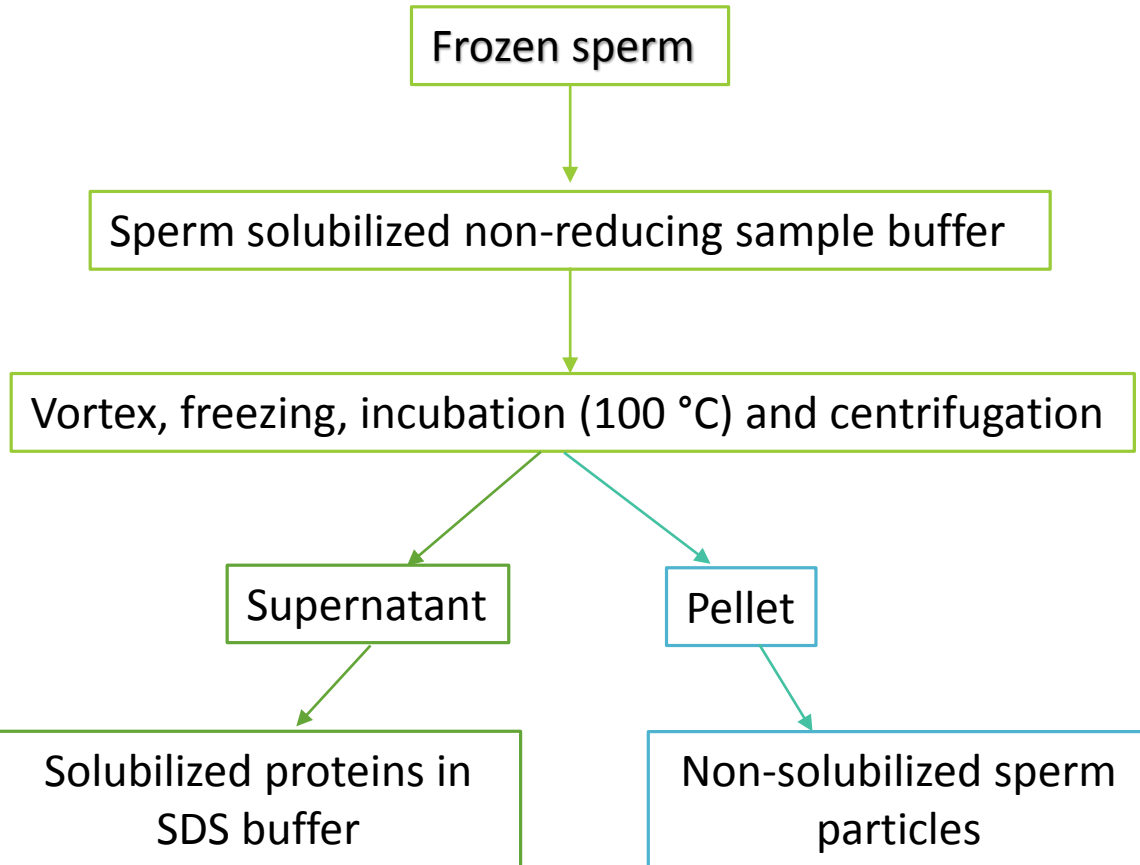
- Women sera from patients with idiopathic infertility



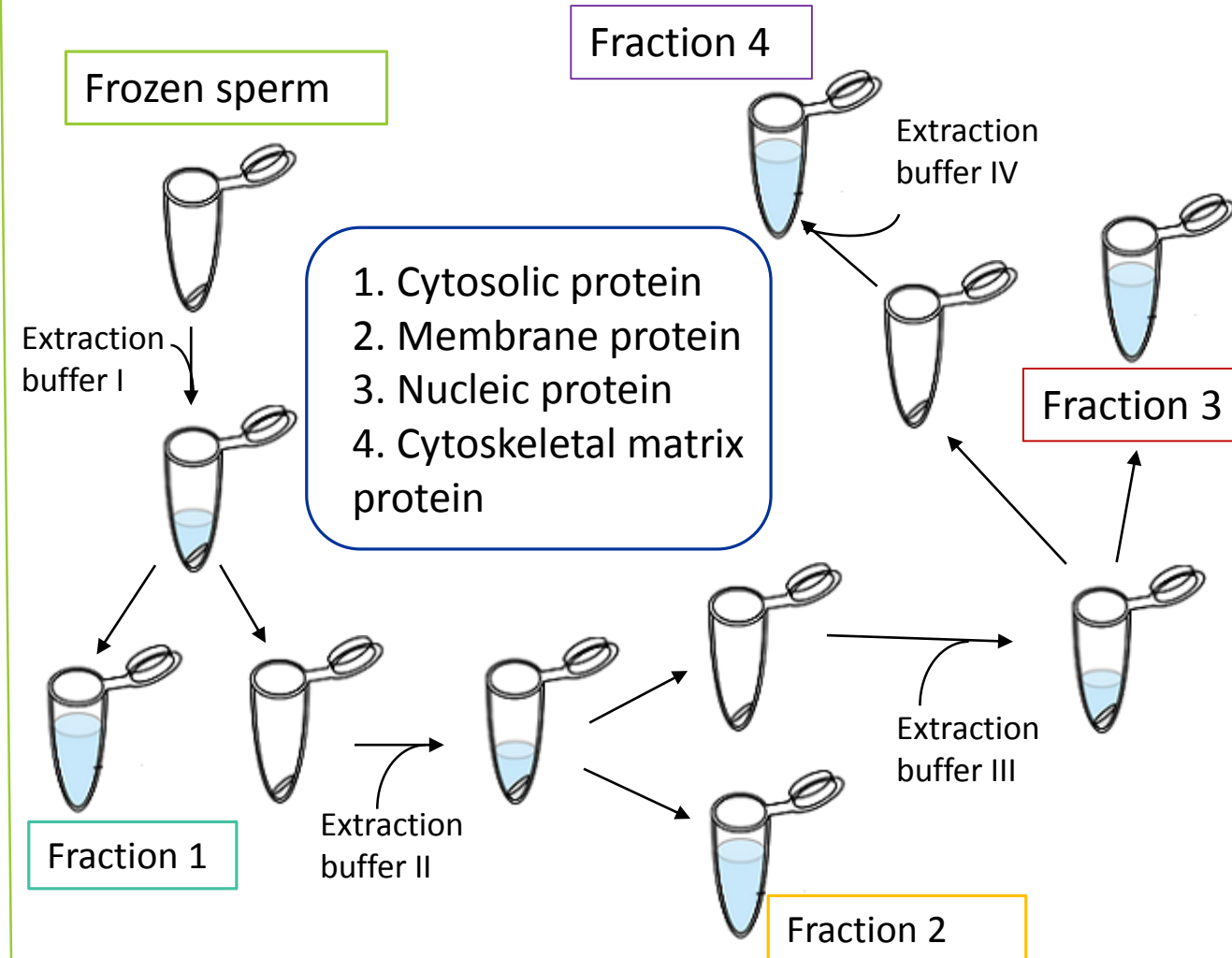
# Methods - Isolation

## 1. Protein extraction from the sperm

- Non-reducing conditions

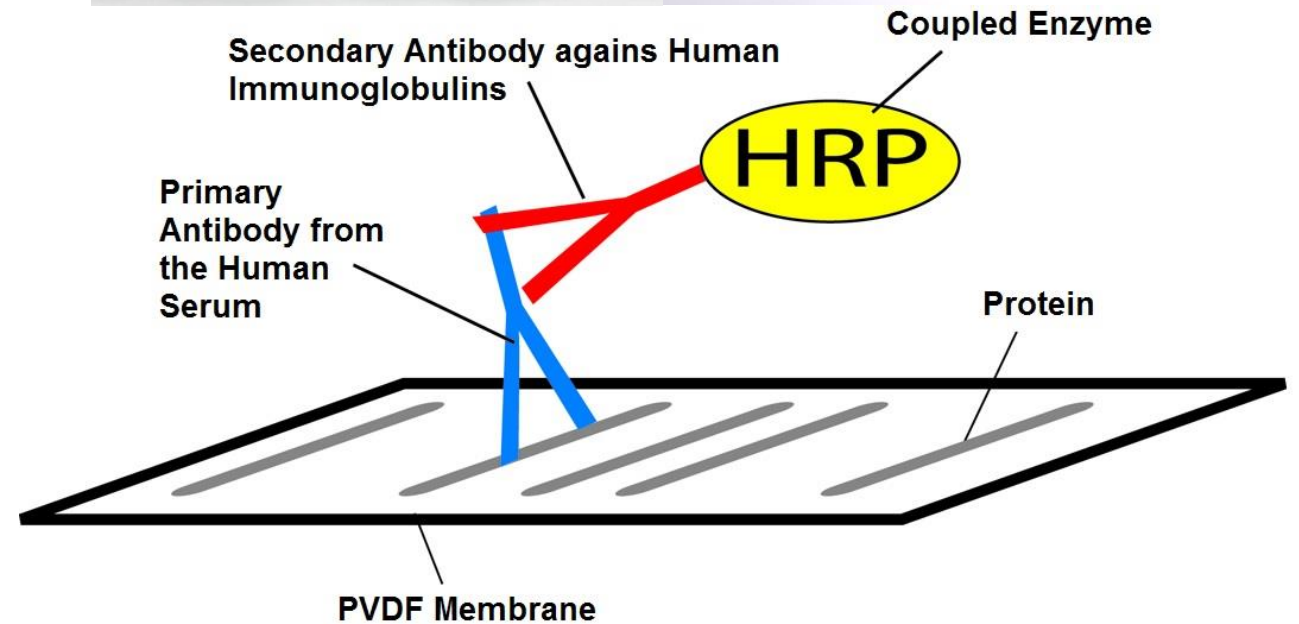
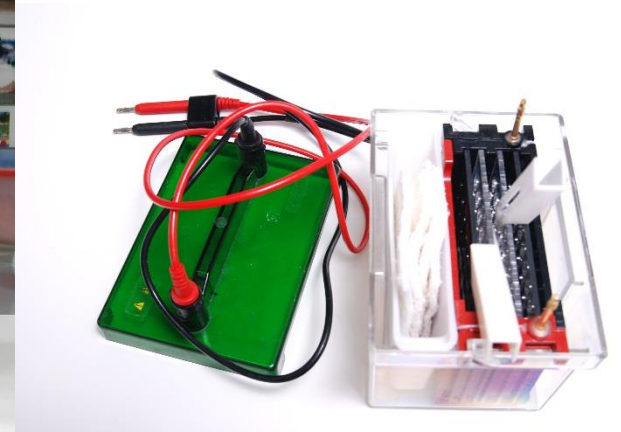
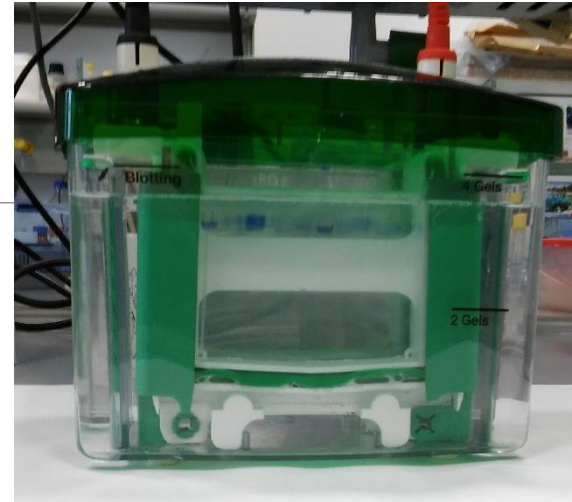


## 2. Subcellular proteome extraction kit (Cat. No. 539790)



# Methods

- SDS-PAGE and Western blot
- Immunodetection
  1. Incubation with serum of women with idiopathic infertility
  2. Incubation with secondary antibody against human immunoglobulins
    - GOAT anti-HUMAN IgG, IgA, IgM



# Results

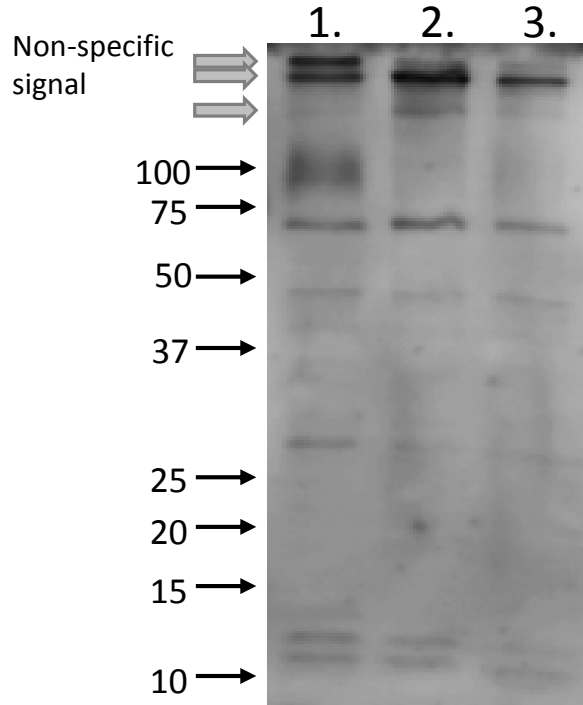
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- 13 women sera
- Tested samples of ejaculates:
  - 6 normospermia
  - 6 asthenozoospermia
  - 2 oligozoospermia
  - 2 oligoasthenozoospermia



# Results - Normospermia

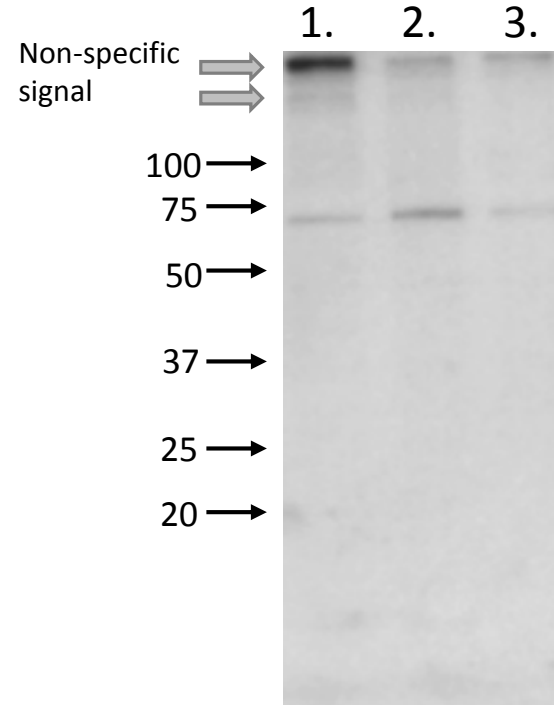
**Serum: 2729**  
**Secondary Ab: IgG**



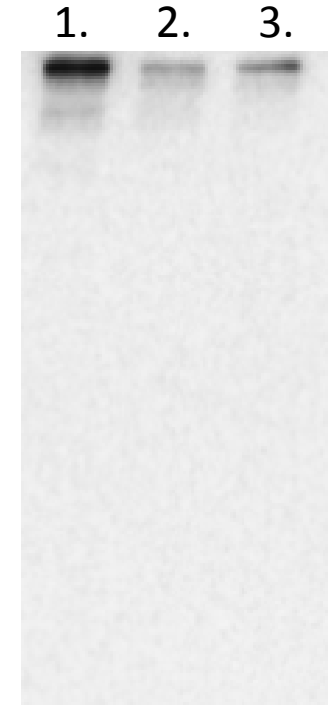
**Negative control**  
only secondary Ab (IgG)



**Serum: 2729**  
**Secondary Ab: IgA**



**Negative control** only  
secondary Ab (IgA)



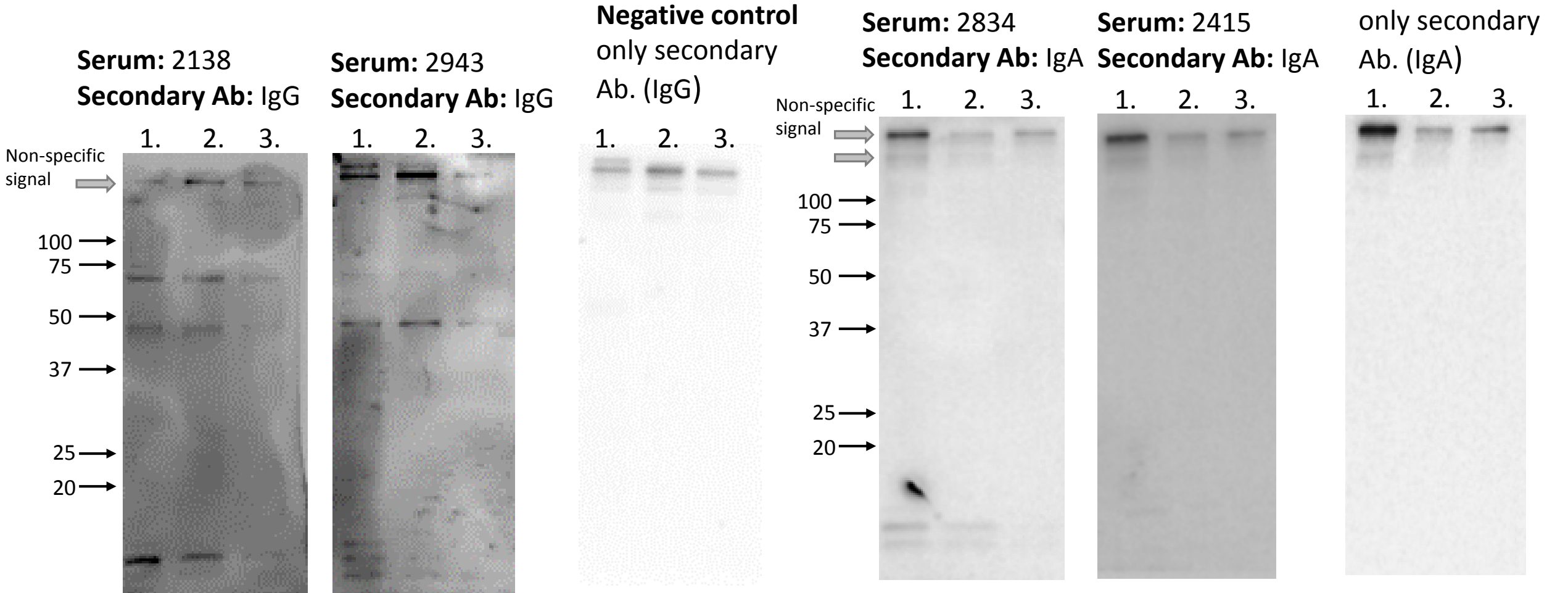
**Sperm samples**

1. 5025
2. 5257
3. 4717

## Sperm samples

1. 5025
2. 5257
3. 4717

# Results - Normospermia

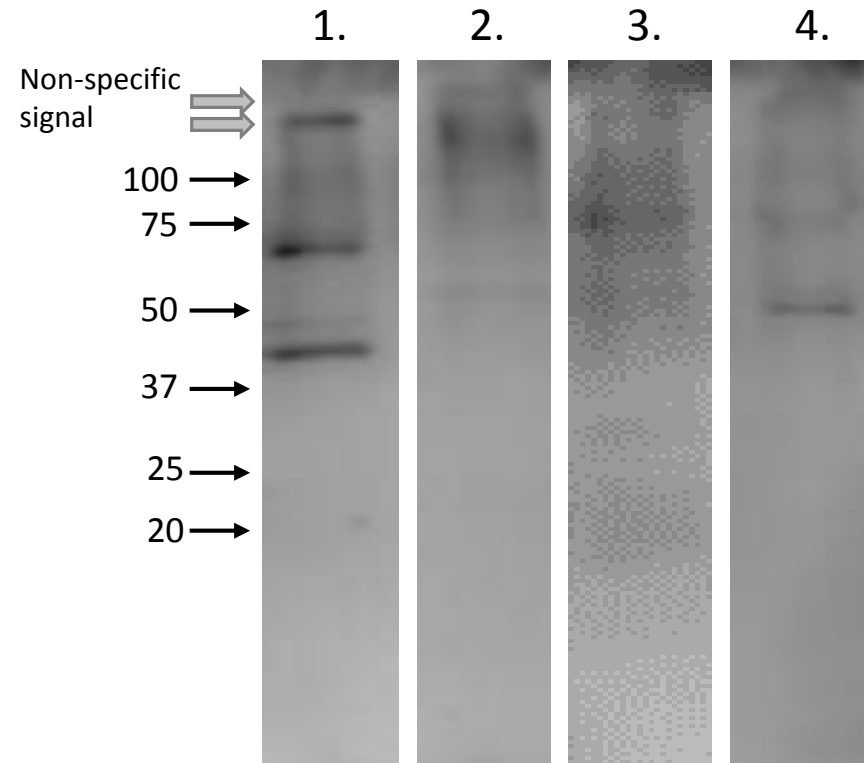


# Results - Normospermia

## Subcellular proteome extraction kit

**Serum:** 2706

**Secondary Ab:** goat anti-  
HUMAN IgG (H+L)



1. 5060 – Cytosolic protein extract
2. 5060 – Membrane protein extract
3. 5060 – Nucleic protein extract
4. 5060 – Cytoskeletal matrix protein

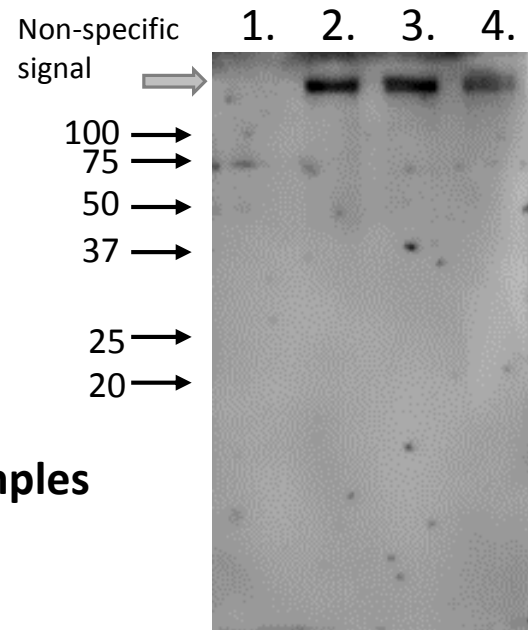
- Cytosolic protein extract also contains proteins from acrosome

# Results - Asthenozoospermia

## Protein extraction from the sperm

Serum: 2729

Secondary Ab: anti-human IgG



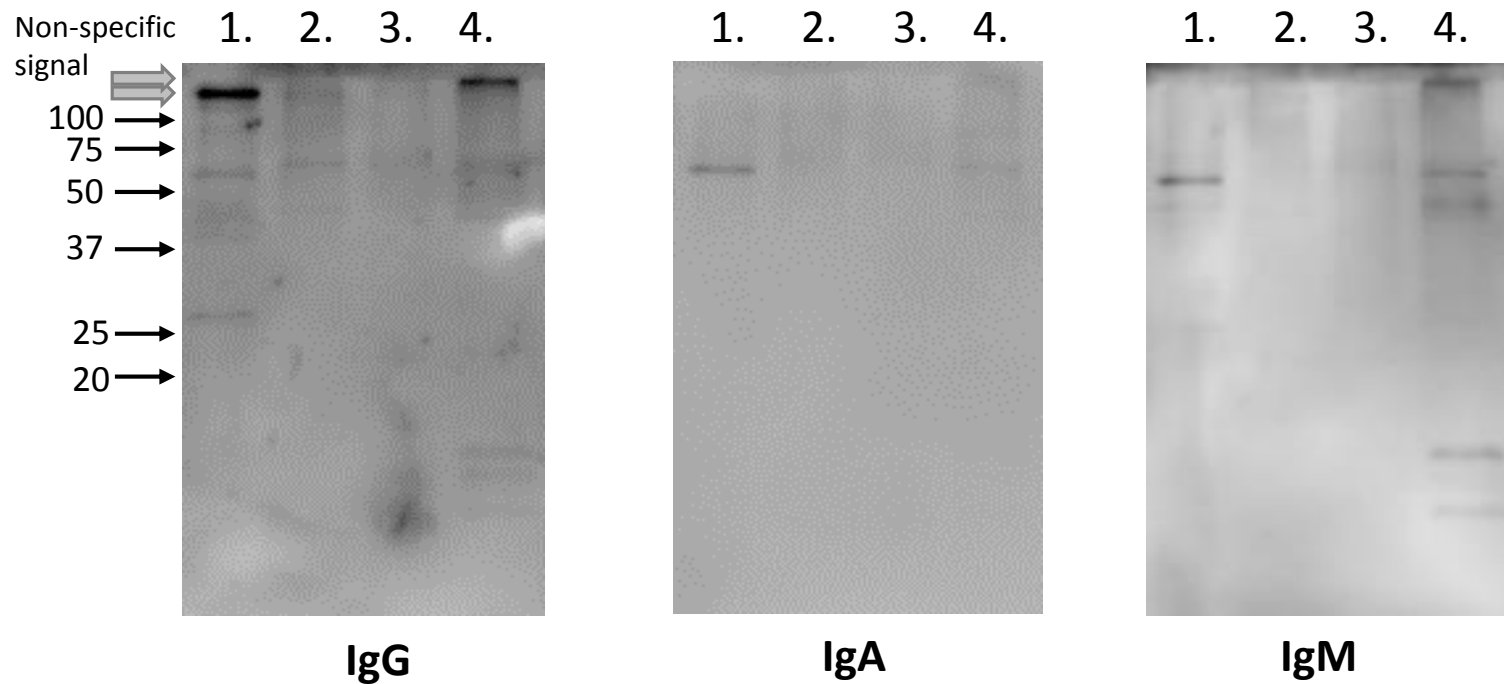
### Sperm Samples

1. 5053
2. 5146
3. 4996
4. 5934

## Subcellular proteome extraction kit

Serum: 2729

Sperm sample: 5373



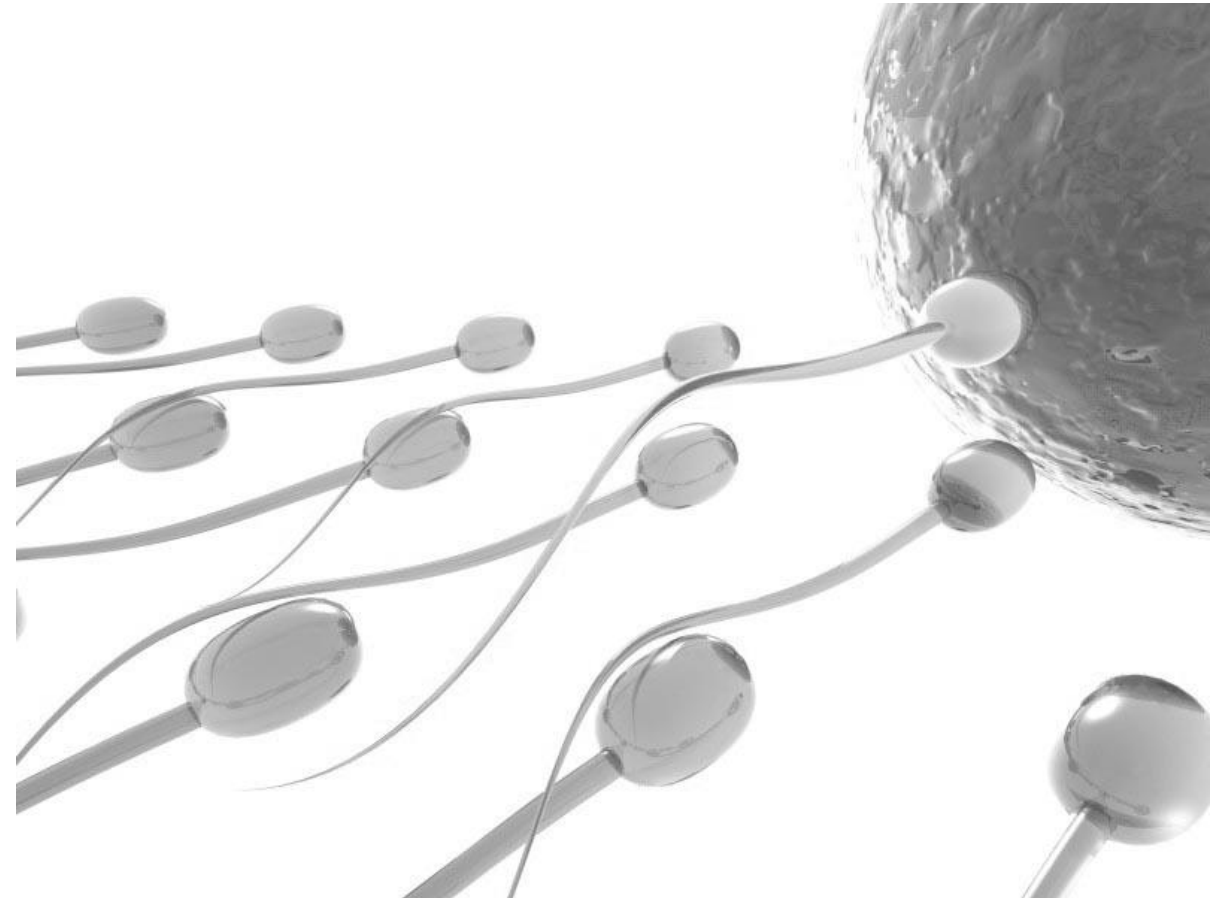
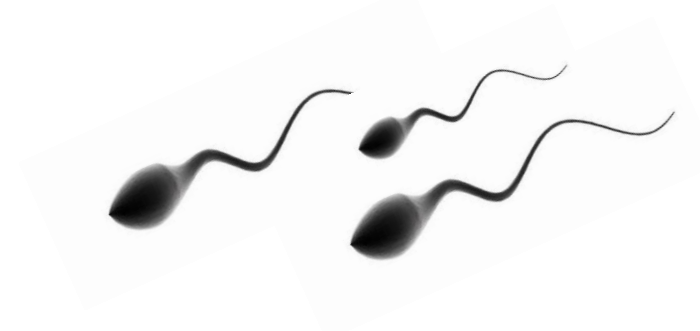
1. Cytosolic proteins
2. Membrane proteins
3. Nucleic proteins
4. Cytoskeletal matrix proteins



# Conclusion

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- All immunogens are still not known
- Serum of each patient contained ASA that gave unique response to each male sample
- It is important to establish a common ASA binding protein for efficient diagnostic tests, which would reduce false-negative results, and subsequently improve therapeutic approaches



# Important

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## Test with cervical mucus!

- Women with negative antibodies in sera may have positive antibody in cervical mucus, mainly IgA

## Test with negative control!

- Serum of women without ASA, who had pregnancy without complications. They have healthy children and they are few years after the baby deliver.
- Serum of young women with menstruation but without sex or with condom protective intercourse.

# Take a look into the future...



- ✍ Testing of the seminal plasma samples
- ✍ Defining of immunogens in the ejaculates
- ✍ Designing of a new kit for the ASA in sera

## ✍ Pair studies:

- Sera of women with idiopathic infertility and ejaculate samples of their partners



Thank you for  
your attention

