Male Infertility

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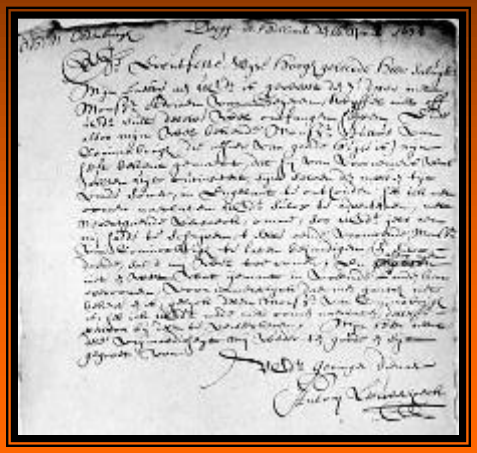
Anton Leeuwenhoek (1632-1723)

- Self constructed microscope
- 270x magnification
- Royal Medical Society
- **„In tenui labor, at tenuis non gloria”**

(Vergil Georgica 4.th book – much work on a little topic: but no little glory.



Andrology

- **1891- Section of Andrology in the Congress of American Physicians and Surgeons**

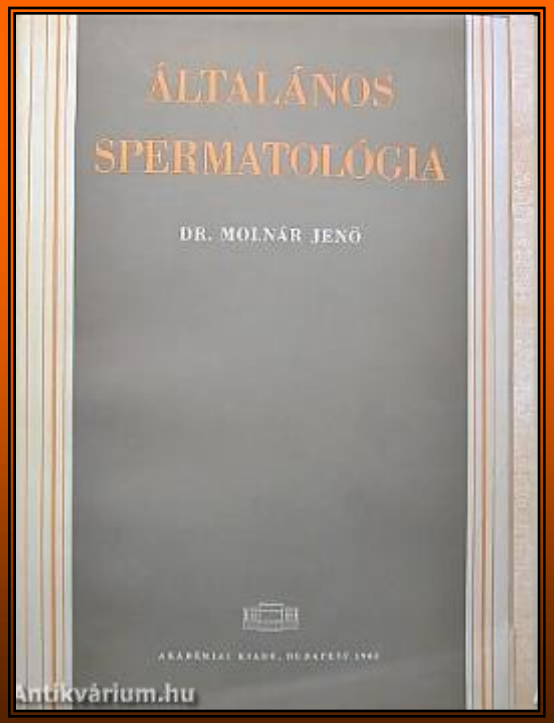
Dr. Jack Phillips, Dr. Richmond of Edna, Dr. Hugh Walker Gates, Dr. August Saltman, Daniel Page Redwine, Dr. J.C. "Coon" Davidson, Dr. Judson Montgomery Andrews and Dr. Addison Lysander Linecum.

Historical data

- **1891** – Congress of American Physicians and Surgeons
- **1951** – Harald Siebke
- Semmelweis University

Dept. of Urology – oldest Urology Dept. in Europe **1920**

- **1947** – Jenő Molnár



Male infertility - causes

- **About 15% of couples do not achieve pregnancy within 1 year and seek medical treatment for infertility.Eventually 5% remain unwillingly childless.**
- **Infertility affects both men and women. In 50% of involuntarily childless couples a male infertility associated factor is found together with abnormal semen parameters.**

  (A fertile partner may compensate for the fertility problem of the men and thus infertility usually becomes manifest if both partners have reduced fertility)

Idiopathic male infertility	31 %
Maldescended testes	7.8 %
Urogenital infection	8.0 %
Disturbances of semen deposition and sexual factors	5.9 %
General and systemic disease	3.1 %
Varicocele	15.6 %
Hypogonadism	8.9 %
Immunological factors	4.5 %
Obstructions	1.7 %
Other abnormalities	5.5 %

Diagnosis of male infertility

- History
- Phyisical examination
- Sperm analysis
- Infections
- Biochemical and immunological markers
- Hormones
- Imaging techniques
- Genetic examinations
- Operative diagnostics

History

- cryptorchidism;
- testicular torsion;
- genito-urinary infection;
- testicular trauma;
- exposure to environmental toxin(s);
- gonadotoxic medication;
- exposure to radiation or chemical(s);
- testicular cancer

Physical examination

- absence of testes;
- cryptorchidism;
- abnormal testicular volume and/or consistency;
- abnormal secondary sexual characteristics;
- epidydimes
- vas deferens
- penis
- gynaecomastia
- varicocele
- (prostate)

Sperm analysis

- If the results of semen analysis are normal according to WHO criteria, one test should be sufficient.
- If the results are abnormal in at least two tests, further andrological investigation is indicated.
- It is important to distinguish between the following:
  - Oligozoospermia: < 15 million spermatozoa/mL.
  - Asthenozoospermia: < 32% motile spermatozoa.
  - Teratozoospermia: < 4% normal forms.
  - Quite often, all three pathologies occur simultaneously as **OAT syndrome**.

WHO Reference levels

Parameter	Lower reference limit
Semen volume (mL)	1.5 (1.4–1.7)
Total sperm number (106 per ejaculate)	39 (33–46)
Sperm concentration (106 per mL)	15 (12–16)
Total motility (PR+NP, %)	40 (38–42)
Progressive motility (PR, %)	32 (31–34)
Vitality (live spermatozoa, %)	58 (55–63)
Sperm morphology (normal forms, %)	4 (3.0–4.0)
Other consensus threshold values	
pH	≥ 7.2
Peroxidase-positive leukocytes (106 per mL)	< 1.0
MAR test (motile spermatozoa with bound particles, %)	< 50
Immunobead test (motile spermatozoa with bound beads, %)	< 50
Seminal zinc (µmol/ejaculate)	≥ 2.4
Seminal fructose (µmol/ejaculate)	≥ 13
Seminal neutral glucosidase (mU/ejaculate)	≥ 20

Percentiles

- 15 M/ml., 32%, 4%.

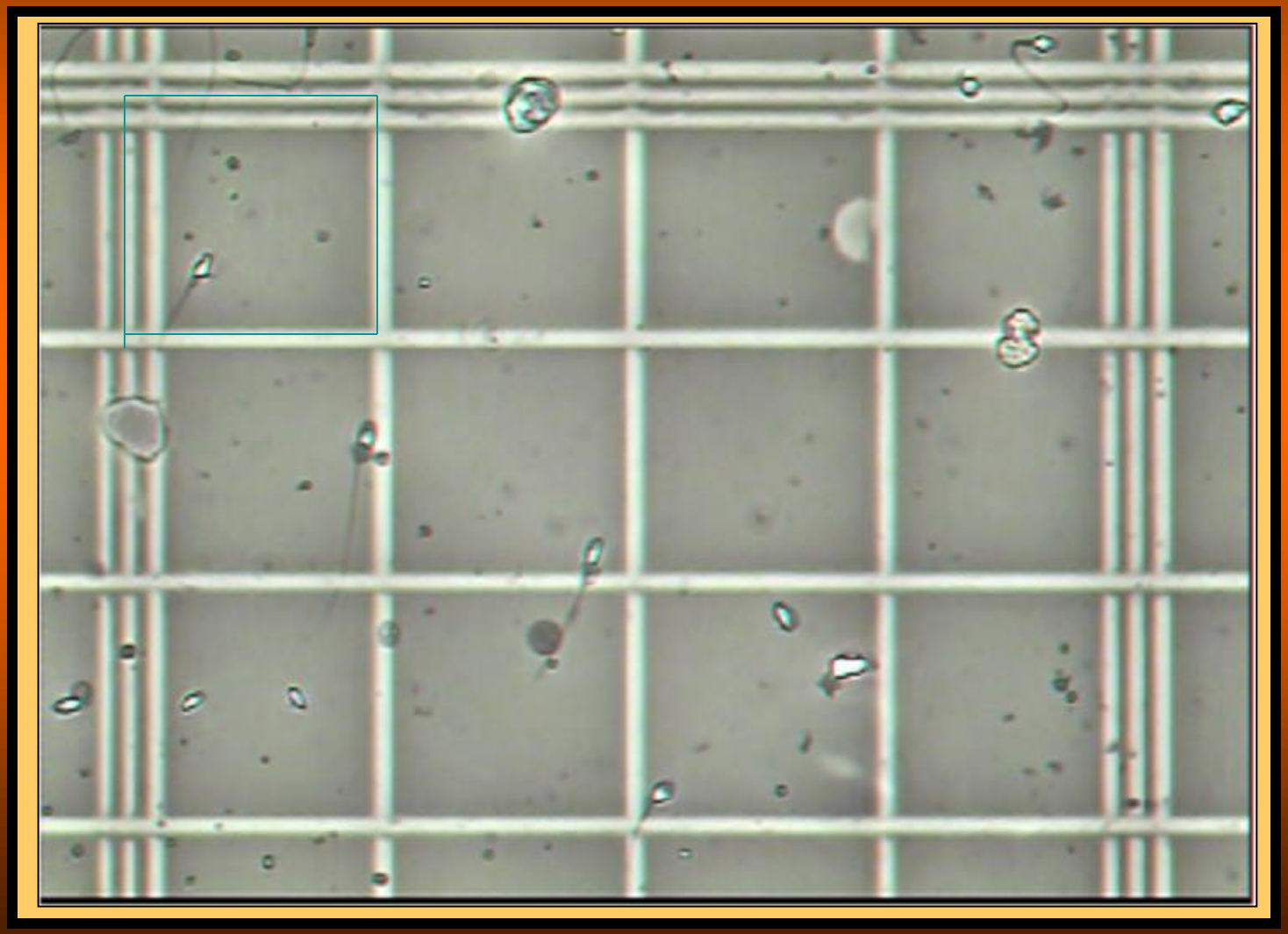
Parameter (units)	N	Centile								
		2.5	5	10	25	50	75	90	95	97.5
Semen volume (ml)	1941	1.2	1.5	2.0	2.7	3.7	4.8	6.0	6.8	7.6
Total sperm number (10^6 per ejaculate)	1859	23	39	69	142	255	422	647	802	928
Sperm concentration (10^6 per ml)	1859	9	15	22	41	73	116	169	213	259
Total motility (PR + NP, %)	1781	34	40	45	53	61	69	75	78	81
Progressive motility (PR,%)	1780	28	32	39	47	55	62	69	72	75
Non-progressive motility (NP, %)	1778	1	1	2	3	5	9	15	18	22
Immotile spermatozoa (IM, %)	1863	19	22	25	31	39	46	54	59	65
Vitality (%)	428	53	58	64	72	79	84	88	91	92
Normal forms (%)	1851	3	4	5.5	9	15	24.5	36	44	48

Source: Cooper et al., 2010.

WHO

Concentration
Neubauer-chamber

5 cubes / 2
Field / 10



Motility

WHO categories:

- **progressive**
- **non progressive, motile**
- **immotile (v < 2 µm/s)**

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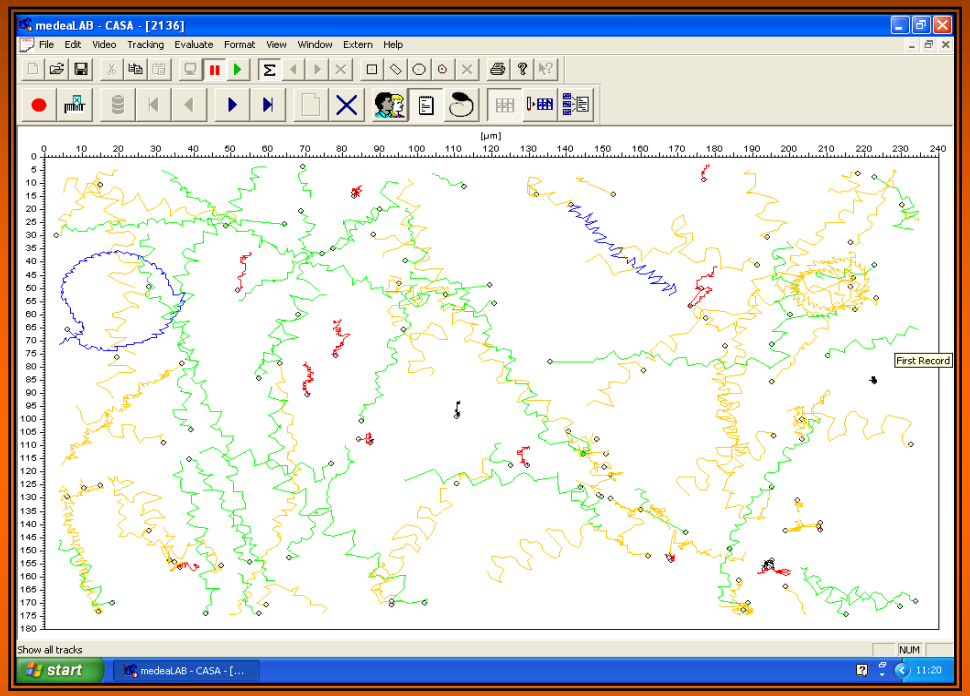
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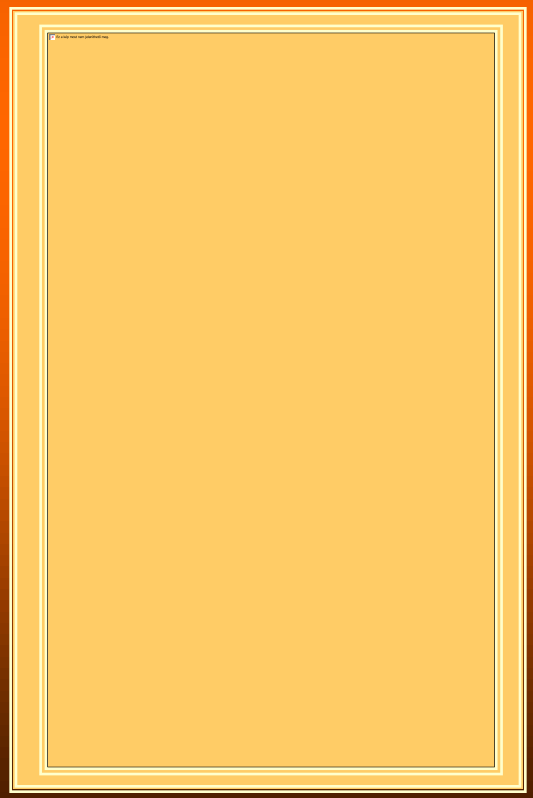
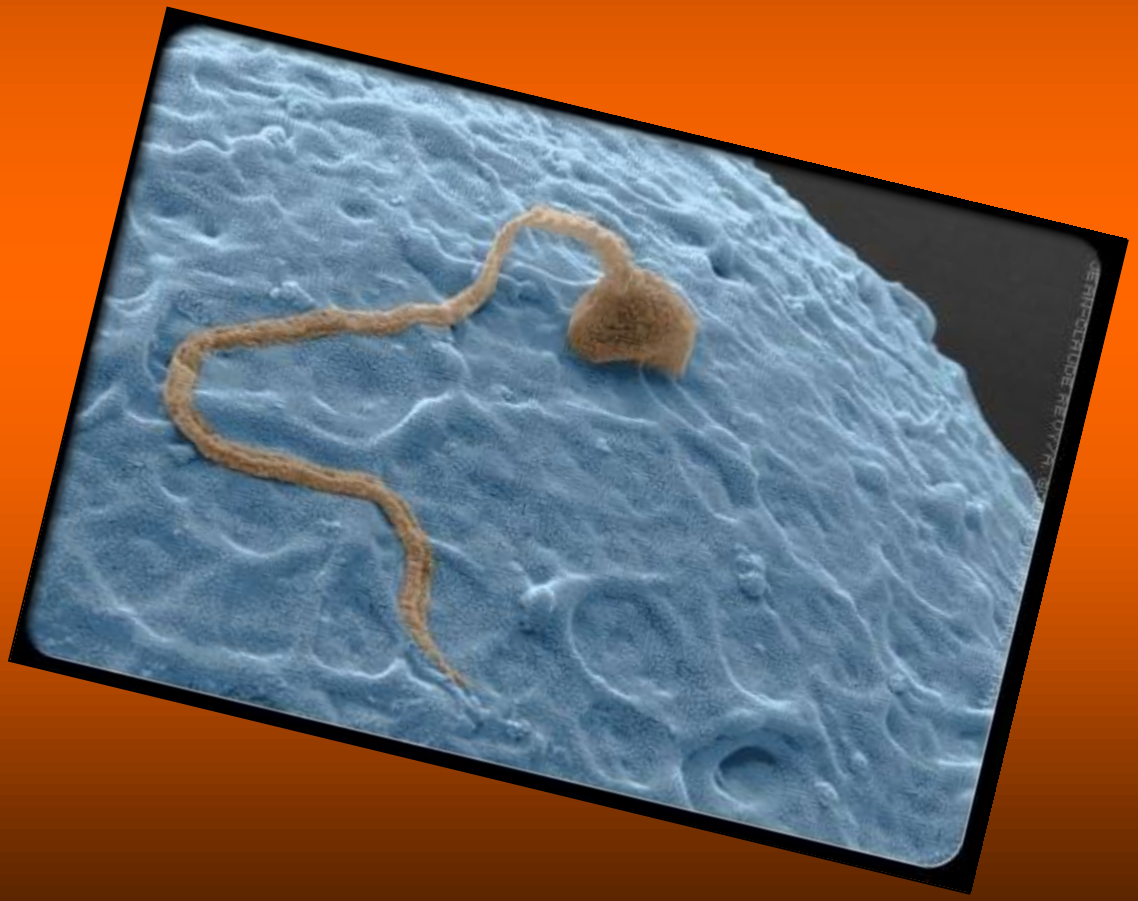
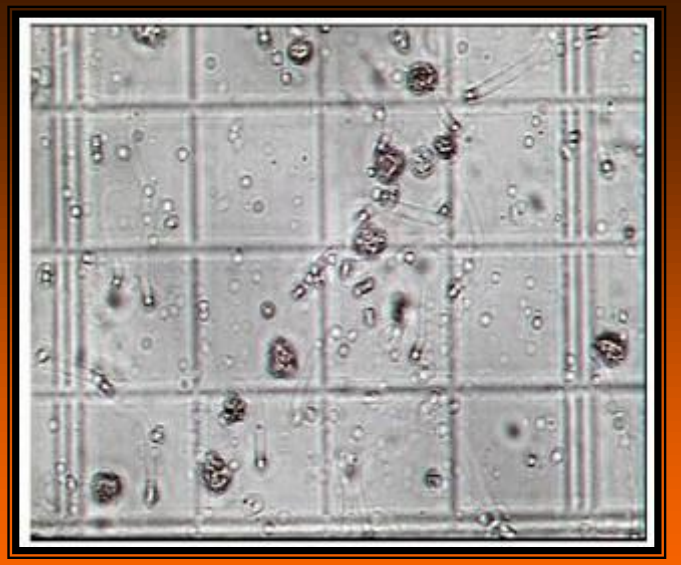


Normal morphology

Sperm functional tests



Infection - facts



- classical sperm parameters unchanged
  - Motility
  - Morphology (?)
- impairment of spermatozoa function
- inflammation is accompanied by chemical reactions
- determination of standard ejaculate parameters is not sufficient for diagnosis
- determination of biochemical markers are needed

The damage of spermatozoa function

- **neutrophilic granulocytes releasing reactive oxygen species (ROS)**
- **invade the lipid membrane of the spermatozoa**
- **30-40% of the ejaculates from infertile men generate excessive levels of ROS**
  - **Oxidative stress**
  - **Sperm membrane destruction**
  - **DNA fragmentation**

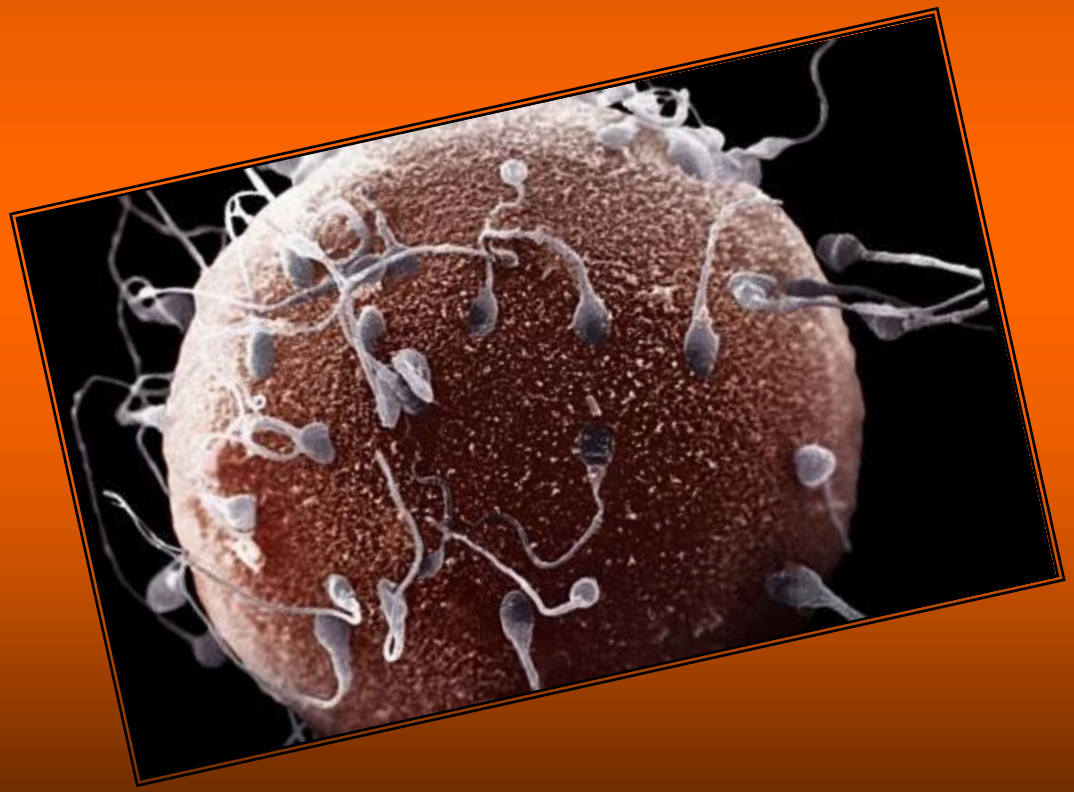
Infection markers

- markers can be classified by their biological properties
  - round cells < 5 M/ml (WHO)
  - leukocytes < 1 M/ml (WHO)
  - **oxidative stress – real function damaging factor**
  - **Main biochemical infection markers:**
    - **Enzymes**
      - Granulocyte elastase
      - Alpha glucosidase
      - Acrosin
      - Gamma glutamil transferase
    - **small molecules**
      - Malondialdehyd
      - Isoprostane
      - Fructose
    - **proteins and immune proteins**
    - **DNA fragmentation and chromatin condensation**
    - **Prostatic gland secretions**

Sperm DNA fragmentation, chromatin condensation

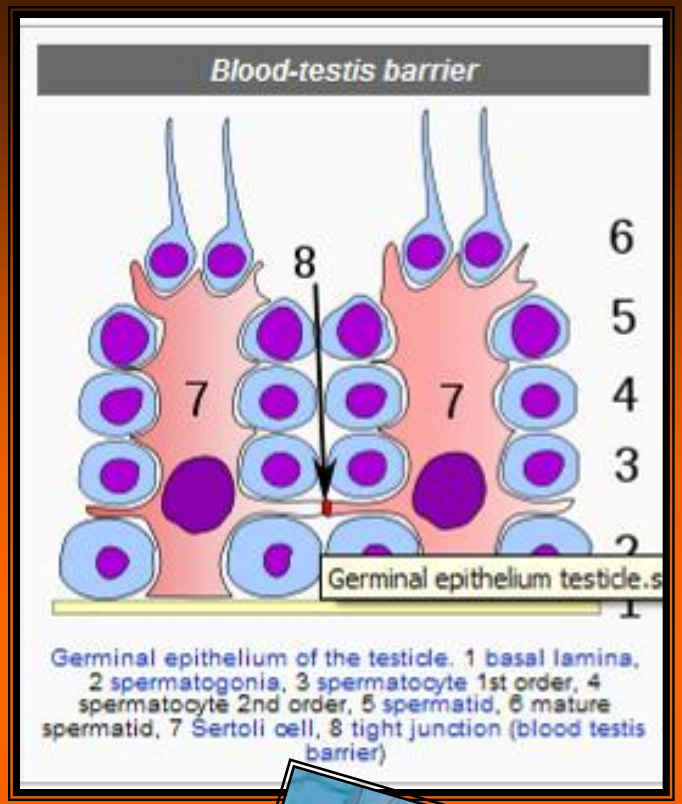
- sperm chromatin (DNA and nuclear proteins) is a double nucleoprotamine-nucleohistone structure
- tightly packaged by protamines
- up to 15% of the DNA remains packaged by histones
- infertile men have an increased sperm histone/protamine ratio
- normal chromatin condensation is mandatory to induce fertilization
- disturbed chromatin condensation is often combined with defect of the acrosome
- with high percentage of DNA damage the natural fertility rate is very low
- associated with high levels of reactive oxygen species
- markers of sperm maturity

Immunological tests



Blood-testis barrier

- Infection
- Aging
- Toxins
- Varicocele
- Iatrogenic



MAR test
Immunobead test
ASA test

Hormones

- **FSH**
  - **Spermatogenesis**
- **LH**
  - **Testosterone**
- **Prolaktin**
  - **Hyper-prolactinaemia**
- **Testosterone**

- Usually, in men with testicular deficiency hypergonadotrophic hypogonadism is present
- The levels of follicle-stimulating hormone (FSH) correlate with the number of spermatogonia
- When spermatogonia are absent or markedly diminished, FSH hormone values are usually elevated.
- When the number of spermatogonia is normal, but spermatocyte or spermatid blockage is complete, FSH values are within normal range.
- Data indicate a stronger correlation between low inhibin B level and spermatogenic damage.

Chromosomal abnormalities

- Chromosome abnormalities can be numerical (e.g. trisomy) or structural (e.g. inversions or translocations)
- The incidence of chromosomal abnormalities is 5.8%
- Of these, sex chromosome abnormalities accounted for 4.2% and autosomal abnormalities for 1.5%.
- The more severe is the testicular deficiency the higher is the frequency of chromosomal abnormalities
- Patients with less than 10 millions spermatozoa/mL show already a 10 times higher incidence (4%) of mainly autosomal structural abnormalities in respect to the general population

- karyotype analysis should be indicated in azoospermic men and in oligozoospermic men with < 10 millions spermatozoa/mL
- In case of a family history of recurrent abortions, malformations, mental retardation karyotype analysis should be requested regardless of the sperm concentration.

Chromosomal abnormalities

- **Sperm chromosomal abnormalities**
  - Using multicolour **fluorescent in situ hybridisation (FISH)** analysis sperm can be examined for chromosomal normality.
    Techniques are needed to **separate** populations of **genetically abnormal sperm** from normal sperm or to safely screen individual spermatozoa before IVF IVF: in vitro fertilisationand ICSI.
- **Sex chromosome abnormalities (Klinefelter's syndrome and variants [47,XXY; 46,XY/ 47,XXY mosaicism])**
  - Klinefelter's syndrome is the most frequent sex chromosome abnormality
    Adult men with Klinefelter's syndrome have small firm testicles devoid of germ cells..
  - Testosterone levels may be normal or low, oestradiol levels normal or elevated and FSH levels increased.
    Follow up (possibly yearly) of men with Klinefelter's syndrome is required and androgen replacement therapy should be started when testosteron level is in the range of hypoandrogenism.
- **Autosomal abnormalities**
  - Genetic counselling should be offered to all couples seeking fertility treatment (including IVF/ICSI) where the male partner is known, or found to have, autosomal karyotype abnormality.
- **Translocations**
  - The most frequently found autosomal karyotype abnormalities are Robertsonian translocations, reciprocal translocations, paracentric inversions and marker chromosomes. The importance of the detection of these structural chromosomal anomalies is related to the increased risk of aneuploidy or unbalanced chromosomal complements in the fetus.

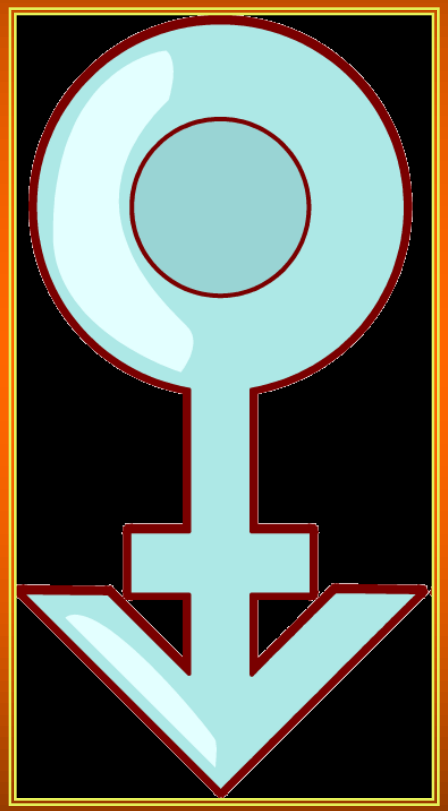
Y Chromosome microdeletions

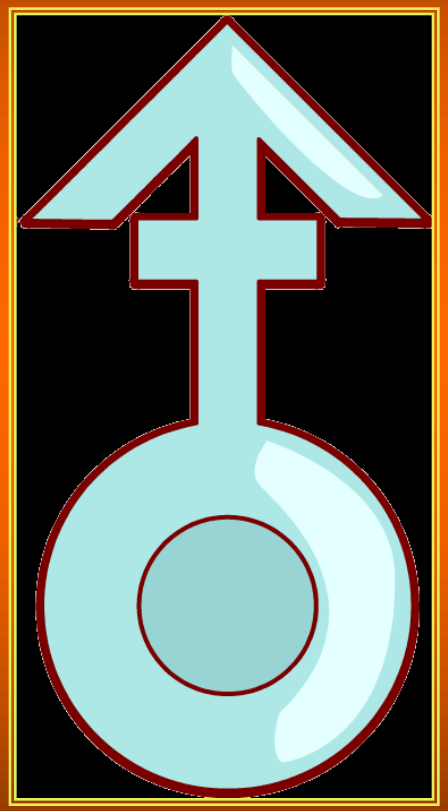
- For men with severely damaged spermatogenesis (with < 5 million spermatozoa/mL) Yq microdeletion testing is advised.
- If complete AZFa or AZFb microdeletions are detected, micro-testicular sperm extraction is not worthwhile because the chance of finding sperm is virtually zero.
- If a man with microdeletion and his partner wish to proceed with ICSI, should be advised that microdeletions will be passed to sons, but not to daughters.
- A son who inherits a microdeletion will not have normal spermatogenesis since complete AZF deletions were not reported in normozoospermic men.

Cystic fibrosis

- Cystic fibrosis (CF)CF: cystic fibrosis, a fatal autosomal-recessive disorder, is the most common genetic disease; 4%
- The gene is located on the short arm of chromosome 7
- Congenital bilateral absence of the vas deferens is associated with CFTR (cystic fibrosis transmembrane conductance regulator gene) mutations and was found in approximately 2% of men with OA

Treatment





Treatment – Primary Hypogonadism

- **Testosterone substitution**
  - total testosterone level above 12 nmol / L (350 ng / dL) does not require substitution
  - serum total testosterone levels below 8 nmol / L (230 ng / dL) will usually benefit from testosterone treatment
  - If the serum total testosterone level is between 8 and 12 nmol / L testosterone supplementation is based on the occurrence of symptoms. The measurement of total testosterone with sex hormone binding globulin (SHBG) to calculate free testosterone or measurement of free testosterone may be helpful for decision making
  - Injectable, oral and transdermal testosterone preparations are available for clinical use
- **Never forget:**
  - The patient who is given any form of testosterone replacement will suffer a progressive decline in the function of the testicles, as the exogenous testosterone is **a powerful inhibitor of the feedback loop that governs spermatogenesis** and testicular testosterone production.

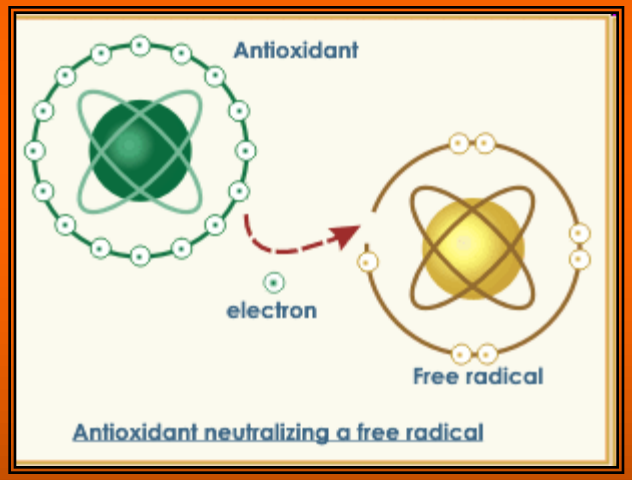
Treatment – Secondary Hypogonadism

- Normal androgen levels and subsequent development of secondary sex characteristics (in cases of onset of hypogonadism before puberty) and eugonadal state can be achieved by **androgen replacement** alone
- However, stimulation of sperm production requires treatment with **human chorionic gonadotrophin (hCG)hCGcombined with recombinant FSH.**
- If hypogonadotropic hypogonadism is hypothalamic in origin, an alternative to hCG treatment is therapy with **pulsatile GnRH**
- In patients who have developed hypogonadism before puberty and have not been treated with gonadotropins or GnRH, 1-2 years of therapy may be needed to achieve sperm production
- Once pregnancy has been established, patients can return to testosterone substitution.

Treatment - OAT

- **Causal**
  - **Antibiotics**
    infection
  - **Antiphlogistics**
    infection
  - **Alfa sympathomimetics**
    transport aspermia
    retrograde ejaculation
  - ***Z*inc**
    prostatic secretion
- **Empirical**
  - **Kallikreine**
  - **Pentoxifilline**
  - **Amino acids**
  - **Vitamines**
  - **Nucleotids**
  - **Mast cell blockers**

Antioxidant treatment



Treatment – idiopathic OAT

- Androgens
- hCG/human menopausal gonadotropin
- Bromocriptine
- α-blockers
- systemic corticosteroids
- magnesium supplementation

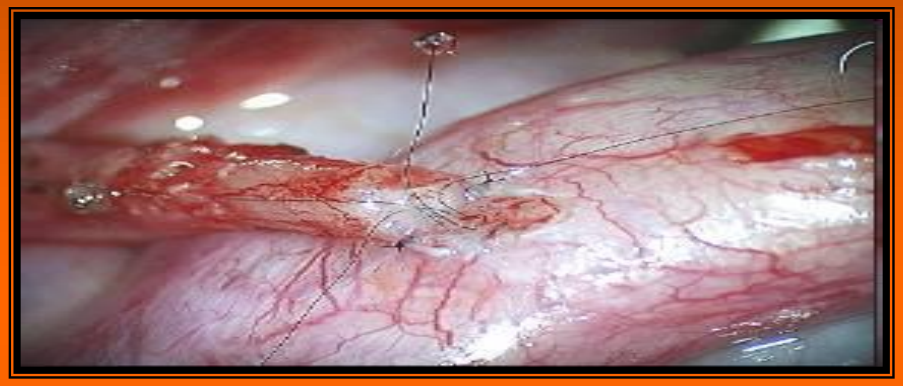
are not effective in the treatment of OAT syndrome - ART

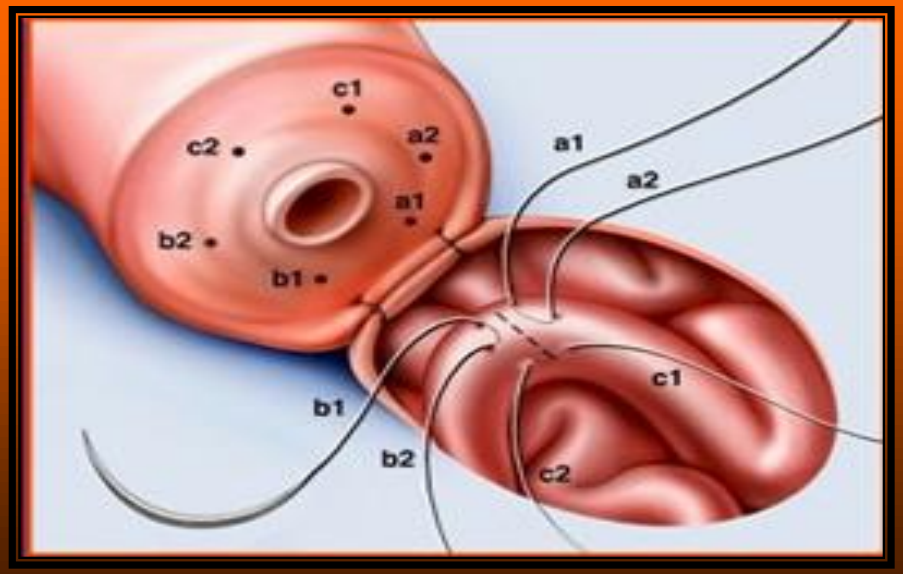
Medical treatment of male infertility is recommended only for cases of hypogonadotropic hypogonadism.

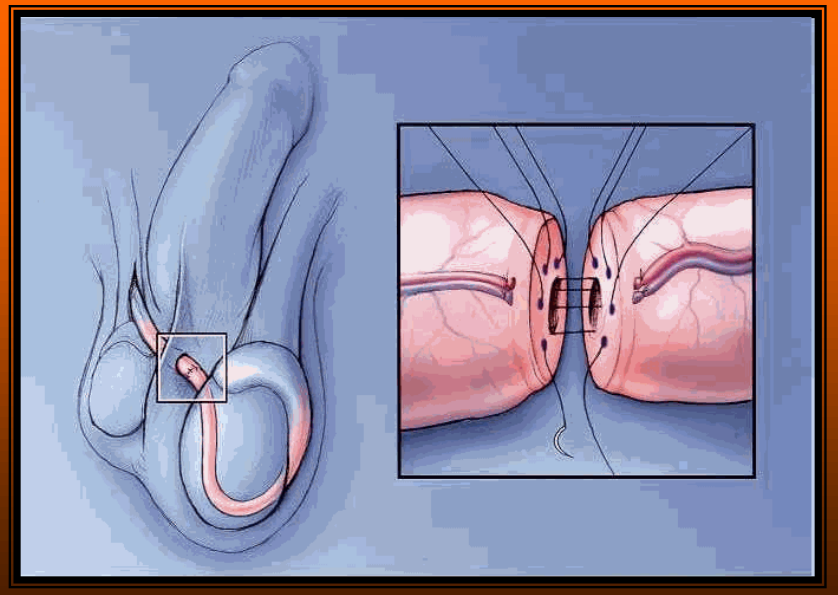
Treatment – OA

- **Intratesticular obstruction**
  - At this level seminal duct recanalisation is impossible
  - **TESE** is therefore recommended. The spermatozoa retrieved may be used immediately for ICSI
  - Both TESE and fine-needle aspiration allow sperm retrieval
- **Epididymal obstruction**
  - **Microsurgical epididymal sperm aspiration** is indicated in men with CBAVD.
  - Acquired epididymal obstruction, end-to-end or end-to-side microsurgical epididymo-vasostomy is recommended.
  - **bilateral reconstruction**
- **Proximal vas obstruction**
  - Proximal vas obstruction after vasectomy requires **microsurgical vasectomy reversal**
  - Vaso-vasostomy is also required in the rare cases of proximal vasal obstructions (iatrogenic, post-traumatic, post-inflammatory).
- **Distal vas deferens obstruction**
  - It is usually impossible to correct, large bilateral vas defects resulting from involuntary vas excision during hernia surgery in early childhood or previous orchidopexy
  - **TESE/MESA** can be used for cryopreservation for future ICSI
  - large mono-lateral vas defects associated with contralateral testicular atrophy, the vas of the atrophic testis can be used for a crossover **vaso-vasostomy or vaso-epididymostomy**.
- **Ejaculatory duct obstruction**
  - Treatment of ejaculatory duct obstruction depends on the aetiology.
  - transurethral resection of the ejaculatory ducts
  - **MESA**, MESA: microsurgical epididymal sperm aspirationTESE, proximal vas deferens sperm aspiration, seminal vesicle ultrasonically guided aspiration and direct cyst aspiration.
  - antegrade seminal tract washout
  - Spermatozoa retrieved by any of the aforementioned surgical techniques should always be cryopreserved for assisted reproductive procedures.

Microsurgical VEA, Vaso-vasostomia





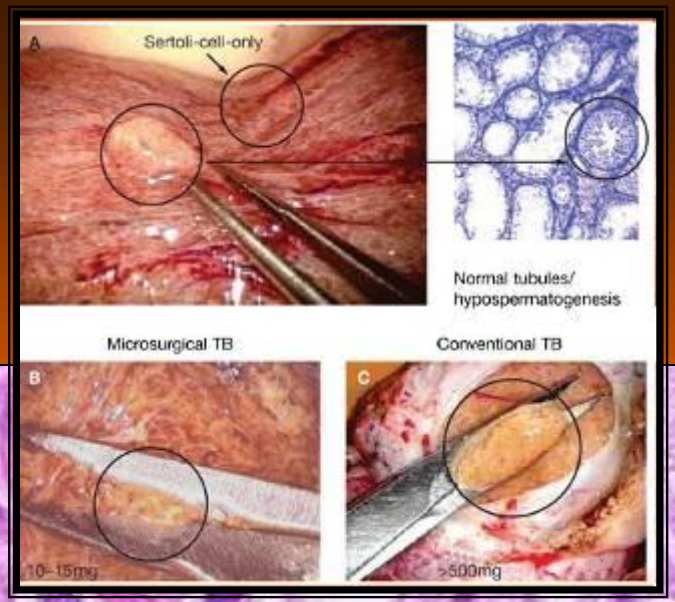


Treatment – NOA

- Testicular biopsy can be part of an ICSI in patients with clinical evidence of NOA.
- Spermatogenesis may be focal
- In about 50-60% of men with NOA spermatozoa can be found that can be used for ICSI
- Taking several testicular is samples recommended

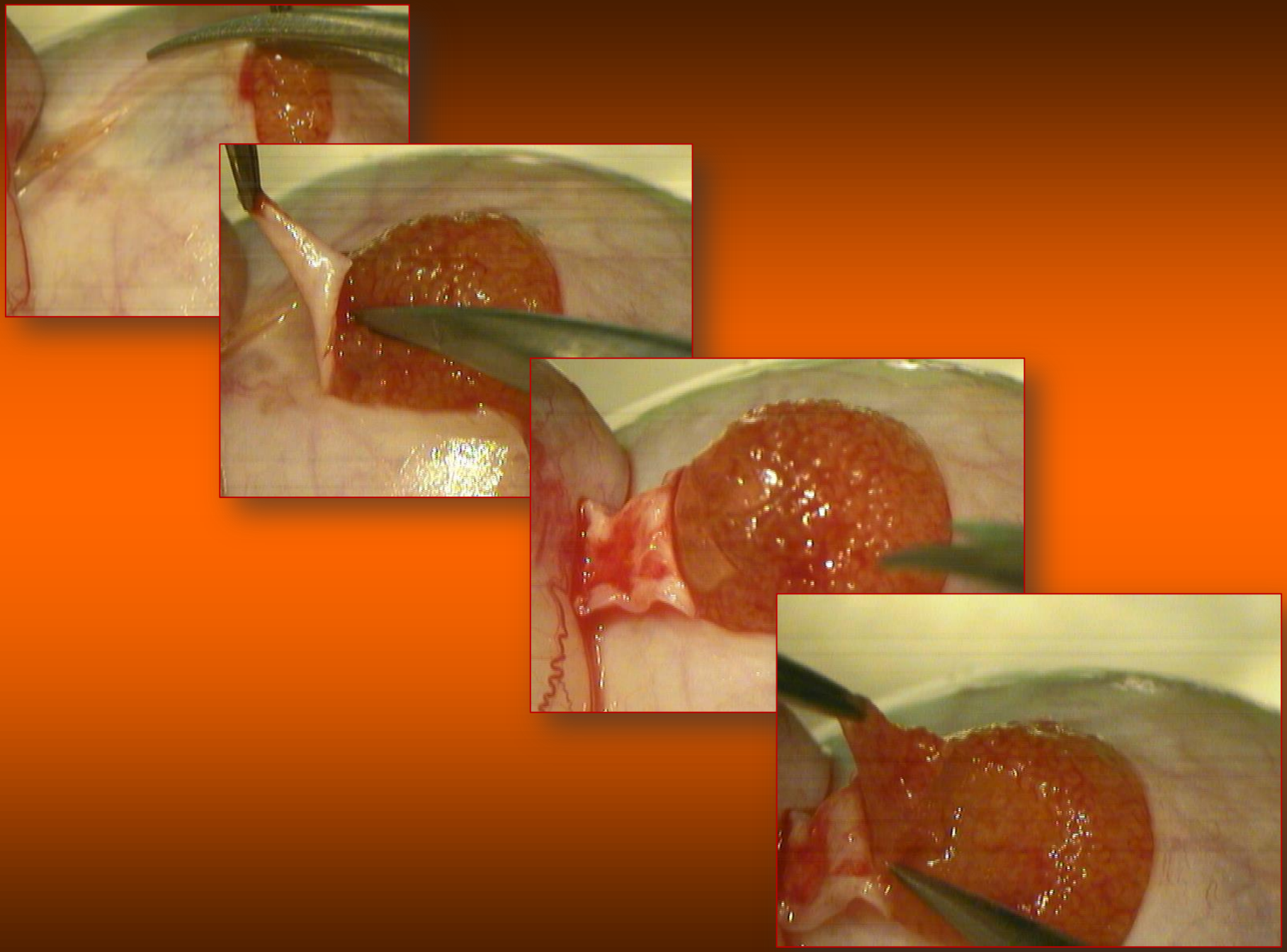
- Testicular fine-needle aspiration (TEFNA) results in lower retrieval rates and does not allow histological examination to detect for instance carcinoma in situ (CIS) and testicular malignancies
- TEFNA may also result in more tubular and vascular damage than TESE

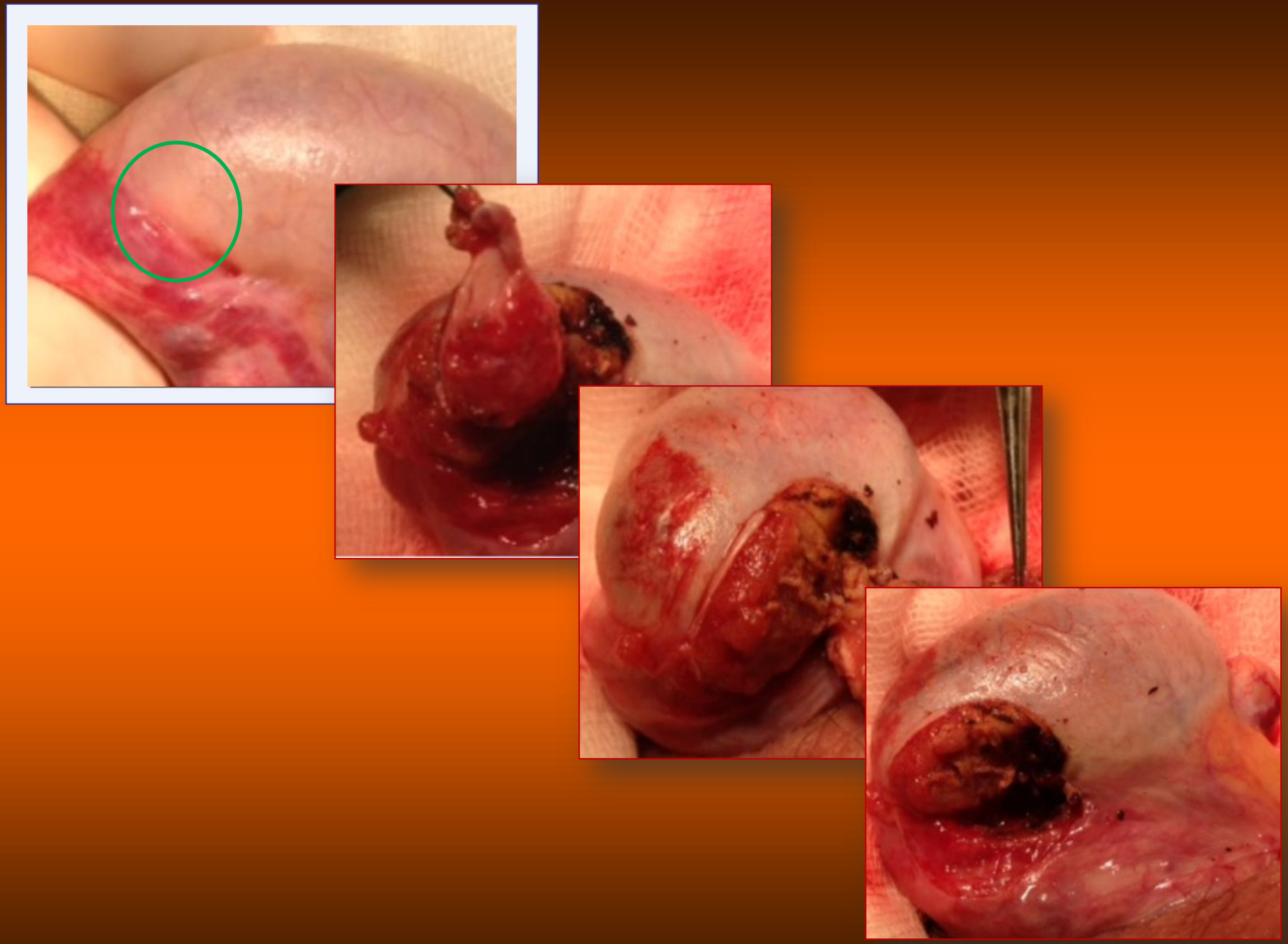
Testicular biopsy



- **Biopsy**
  - Testis
    - **TeSE**
      - **microTeSE**
    - Multiple
  - Epididymis
    - Open surgery
  - Vas deferens
    - Wash out

Cryopreservation !!!





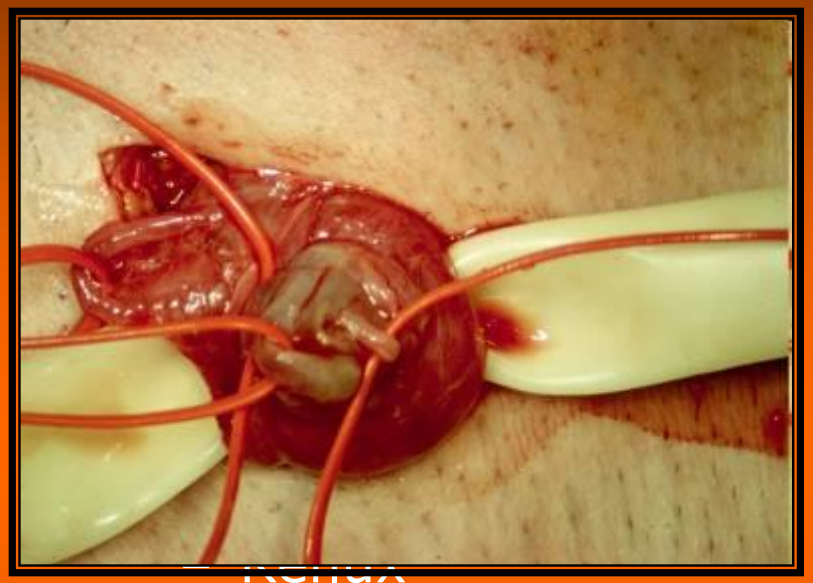
Treatment - Varicocele

- In some men, the presence of varicocele is associated with progressive testicular damage from adolescence onwards and consequent reduction in fertility.
- Although treatment of varicocele in adolescents may be effective, there is a significant risk of over-treatment.
- Varicocele repair may be effective in men with subnormal semen analysis, a clinical varicocele and otherwise unexplained infertility.

Treatment - Varicocele

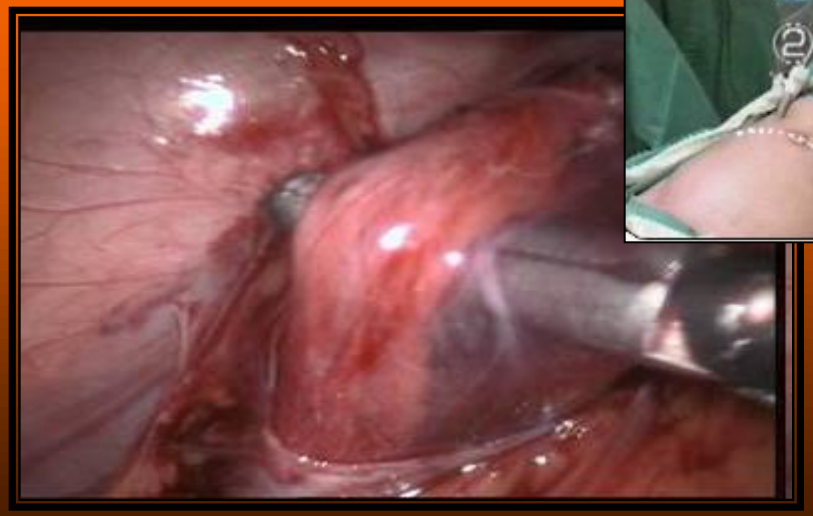
Treatment (reference)	Recurrence/persistence	Complication rates
• Antegrade sclerotherapy (20)	9%	Complication rate 0.3-2.2%; testicular atrophy; scrotal haematoma; epididymitis; left-flank erythema.
• Retrograde sclerotherapy (21)	9.8%	Adverse reaction to contrast medium; flank pain; persistent thrombophlebitis; vascular perforation.
• Retrograde embolisation (22,23)	3.8-10%	Pain due to thrombophlebitis; bleeding haematoma; infection; venous perforation; hydrocele; radiological complication (e.g. reaction to contrast media); misplacement or migration of coils; retroperitoneal haemorrhage; fibrosis; ureteric obstruction.
Open operation		
Scrotal operation	–	Testicular atrophy; arterial damage with risk of devascularisation and gangrene of testicle.
• Inguinal approach (24)	13.3%	Possibility of missing out a branch of testicular vein.
• High ligation (25)	29%	5-10% incidence of hydrocele.
• Microsurgical (26,27)	0.8-4%	Post-operative hydrocele arterial injury; scrotal haematoma.
• Laparoscopy (28,29)	3-7%	Injury to testicular artery and lymph vessels; intestinal, vascular and nerve damage; pulmonary embolism; peritonitis ; bleeding; post-operative pain in right shoulder (due to diaphragmatic stretching during pneumo-peritoneum); pneumo-scrotum; wound infection.

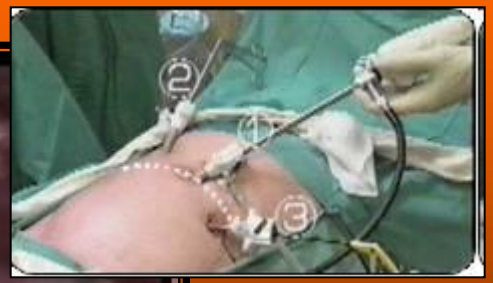
Varicocele

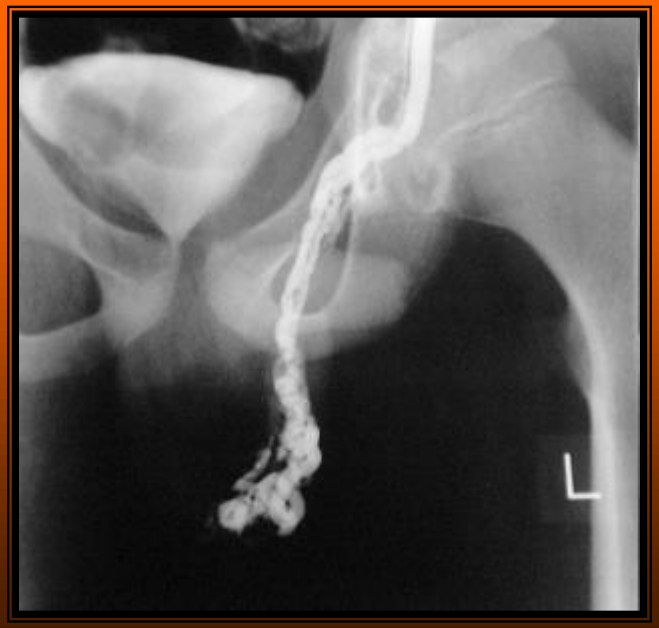


Surgery

- subinguinal aproach
- mikrosurgery
- laparoscopy
- interventiona radiology







„Medicine is art, an art based on science.”

Sir William Osler – 1892

(„father of modern medicine”)