

# The role of G.U. infections in male infertility

- Infertility is a challenging health issue that affects approximately 15% of couples who are at their reproductive age desiring to have children.
- This condition is defined by the inability to achieve a pregnancy after at least 1 year of well-timed.
- Approximately 50% of the infertility cases are attributed to the male due to numerous andrological problems
- The role of infection in infertility is often neglected. Worldwide, infection is the most frequent preventable cause of human infertility.
- Male urogenital tract infection (UTI) is one of the most important causes of male infertility, being associated with 8%-35% of male infertility.

- The literature reports that about 15% of the male infertility cases are linked to male genital tract infections (MGTIs).
- Sixty percent of patients treated with assisted reproductive technology (ART) had suffered inflammation or infection.
- There is direct relation between male infertility and genital tract infection, this relation represents important problem in contemporary andrology.

- The bacteria responsible for semen infection may originate from the urinary tract or be sexually transmitted.
- Pathogenic bacteria may interfere with infertility treatment involving the application of in vitro fertilization. Microorganisms might affect the spermatozoa function in different ways:
  - a) By direct contact on sperm cells; by the help of some organelles such as pili; causing agglutination of motile sperm, reducing ability of the acrosome reaction, and also causing alterations in cell morphology.
  - b) Trigger a local inflammatory reaction leading to increase in reactive oxygen species (ROS).
  - c) Induction of sperm autoantibodies.
  - d) Production of cytotoxic factors.
  - e) Infection treatment with antibiotics for long time may lead to defect in the sperm.

# Seminal Leukocytes

- Some authors have claimed that seminal leukocytes may not be just a response to infection, but rather act to scavenge abnormal germ cells and would play some kind of positive role in surveillance and phagocytosing of abnormal and dead spermatozoa.
- Lackner et al. suggest that the effects of leukocytes on sperm motility and normal sperm morphology would be concentration-dependent as at concentrations lower than  $0.5 \times 10^6$  leukocytes/ml a higher leukocyte concentration would be beneficial for motility and morphology.
- According to the WHO guidelines (WHO, 2010), the seminal leukocyte concentration should not exceed  $1 \times 10^6$ /ml. Leukocyte concentrations higher than this cut-off value are regarded as leukocytospermia.
- Leukocytospermia induced sperm damage is a likely result of the high levels of leukocyte-derived ROS and inflammatory mediators.

- The incidence of pathological leukocytospermia in infertile men ranges from 2% to 35% with usually reported averages between 10% and 20%.
- In addition to genital tract infections, smoking, consumption of alcohol and marijuana may also increase seminal leukocyte concentrations.
- Leukocytospermia is associated with significantly lower progressive motility.
- This deterioration of sperm motility is a likely result of the high levels of ROS and inflammatory
- mediators that are produced by leukocyte

- Elevated levels of ROS can trigger apoptosis in mature human spermatozoa and thereby reduce sperm fertilizing capacity.
- Yet, SDF was significantly higher in semen samples with oxidative stress.
- It is generally believed that an increase in seminal leukocytes in ejaculated semen may indicate MGTI.

# Origin of seminal leukocytes

- These leukocytes play a major role in immunosurveillance and phagocytic clearance of abnormal spermatozoa.
- It is thought that a major amount of leukocytes is originating from the rete testis or epididymis.
- While these white blood cells are rather the macrophages, and T- and B-lymphocytes, the prostate and the seminal vesicles are rather the origin of granulocytes.



## Types of leukocytes, origin and numbers in fertile and infertile men

Leukocyte type	Percentage in the ejaculate	Main origin
Total		
Granulocytes	50%–60%	Prostate Seminal vesicle
Macrophages	20%–30%	Epididymis Rete testis
T-lymphocytes	2%–5%	Epididymis Rete testis
B-lymphocytes	Very small percentage	Epididymis Rete testis

1. Non-inflammatory semen

2. Inflammatory semen

- Several studies reported a strong association between inflammatory semen with the presence of leukocytes and male infertility.
- Leukocytes release not only ROS, but also pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\alpha$ , interleukin-6 or interleukin-8 leading to an inflammatory response.

- Evidence suggests that inflammation of the genital tract affects semen quality and can lead to deterioration of the spermatogenesis, impairment of sperm function and obstruction of the seminal tract.
- Inflammatory conditions considerably influence the secretory function of the male accessory organs. Genital tract inflammations can affect urethra, epididymis, testicles and prostate gland.
- Cytokines, especially  $\text{TNF-}\alpha$  and interleukin-8, either alone or in the presence of leukocytes, can trigger sperm lipid peroxidation. A recent study shows that seminal interleukin-8 levels in patients with asthenozoospermia and oligoasthenozoospermia are more than 60% higher than in the controls.
- Cytokines such as  $\text{TNF-}\alpha$ ,  $\text{IL-1}\beta$  and  $\text{IL-6}$  cause a dose-dependent decline in testosterone synthesis in TM3 Leydig cells suggesting that chronic inflammation may even directly affect steroidogenesis by direct modulation of Leydig cell function, thus compromising male fertility.

# Prostatitis

- Prostatitis is an inflammation of the prostate gland with a prevalence between 4% and 11%. Prostatitis is the most common urological diagnosis in younger and middle-aged men.
- Approximately 5%–10% of the diagnosed prostatitis are of bacterial origin. While the Gram-negative bacterium *Escherichia coli* is the cause of bacterial prostatitis in about 80% of the cases other bacterial infections that have been isolated in prostatitis cases are Gram-positive enterococci. *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Nesisseria gonorrhea*, and *Klebsiella* species.

- Prostatitis recognized as one cause of male infertility with the clinical presentation varying from asymptomatic inflammation to severe urological symptoms.
- prostatitis is linked with decreased prostatic excretory function and has negative impact on male fertility potential affecting sperm morphology as well as sperm motility.

# Epididymitis

- Epididymitis is an inflammatory condition of the epididymis in males presenting with acute unilateral or bilateral swelling of the scrotum.
- In young, sexually active men, the inflammation is caused in most cases by *Chlamydia trachomatis* or *Neisseria gonorrhoeae*, whereas *E. coli* is pre-dominantly found in older men.
- In addition to the loss of sperm function, inflammatory obstruction of the epididymal duct has been considered as an underlying cause of persistent azoospermia or oligozoospermia.

- The inflammation may spread to the corresponding testes as 'epididymo-orchitis' and has consistently been associated with high rates of infertility.
- Collective analysis indicates profound deterioration of semen quality (sperm concentration, motility, morphology) together with pronounced leukocytospermia in the acute phase of the disease.
- Due to the bacterial infection ascending to the testis, a testicular involvement with spermatogenesis being affected may occur in 60% of the cases. In these cases, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are etiologically responsible.

# Orchitis

- Orchitis is an inflammatory lesion of the testes that can be caused by *Chlamydia trachomatis* and *Neisseria gonorrhea*. These pathogens are the particular cause of the disease in men younger than 35 years. In contrast, in older men, *Escherichia coli* has been found to be the predominant trigger.
- Furthermore, the mumps virus affects the testicles as mumps orchitis in 20%–30% of the cases which may lead to infertility in up to 87% of the patients.



- Affected seminiferous tubules show degeneration of the germinal epithelium and thickening of the lamina propria which may result in fibrosis of the tubules.
- As a consequence of this infection, testicular atrophy with spermatogenic arrest can occur.
- This would then result in poor sperm quality with low sperm counts.
- In about 15% of the cases of azoospermia, an orchitis is the cause of an intratesticular obstruction.

# Urethritis

- Urethritis is an inflammation of the urethra caused by sexually transmitted pathogens such as *Chlamydia trachomatis*, *Mycoplasma* or *Neisseria gonorrhoea*. *Neisseria gonorrhoea* is isolated in approximately 20% of men with urethritis.
- Non-sexually transmitted uropathogens such as *Enterobacteriaceae* and *staphylococci* are also triggering urethritis with an incidence between 20% and 31%

- Symptoms of urethritis in men typically include urethral discharge, penile itching or tingling, and dysuria.
- In a prospective study, men with gonococcal urethritis showed extensive seminiferous tubular necrosis and inflammatory cell infiltration. After a 2-year follow-up period, 27% of the patients were found to have persistent azoospermia, and 33% had no significant improvement in sperm density.

# Male accessory gland infections (MAGI)

- It comprises the infection/inflammation of the prostate, seminal vesicles and the Cowper's glands.
- However, more recently, elevated levels of polymorphonuclear granulocyte elastase ( $\geq 230$  ng/ml) and pro-inflammatory cytokines such as IL-6 and IL-8 have emerged as more promising parameters in the diagnosis and management of MAGI.
- as these parameters may significantly compromise sperm functions by directly affecting sperm function and increasing seminal ROS levels.
- Considering that the infection/inflammation is also affecting the secretory functions of the accessory glands, determination of their secretions such as citric acid, fructose,  $\alpha$ -glucosidase, phosphatase or zinc can also be done to provide additional information.

# Pathogens causing urogenital infections

- The most prevalent pathogens in the male reproductive tract *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Neisseria gonorrhea*, *Mycoplasma hominis*, *Mycoplasma genitalium* or *Escherichia coli*.
- Except for *Escherichia coli*, which is particularly responsible for epididymo-orchitis or prostatitis in 65%–80% of the cases, the other listed uropathogens are sexually transmitted.

# Chlamydia trachomatis

- Chlamydial infections are the most common sexually transmitted disease.
- Since this infection is rather asymptomatic in about 50% of men and up to 80% in women, this number is rather underestimated and even newborns are infected during delivery accounting for 25%–50% of conjunctivitis and 10%-20% of pneumonia in babies.
- This pathogen is the most important etiologic cause of non-gonococcal urethritis and acute epididymitis in men younger than 35 years

- There is a significant relationship between infections with *Chlamydia trachomatis* and increased IL-8 levels as well as seminal leukocyte concentrations. The percentage of progressively motile spermatozoa decreased in patients with *Chlamydia trachomatis* infections. Infection with these bacteria has significant detrimental effects, not only on sperm parameters in general, but also specifically on DNA integrity.

# Mycoplasmataceae

- Mycoplasmataceae is a family of bacteria which comprises Mycoplasma and Ureaplasma. Mycoplasmas are sexually transmitted bacteria causing clinical presentation and damages to the acrosomal membrane or can damage the sperm DNA via the secondary effects of the infection.

- ✓ **Ureaplasma urealyticum**

- Apart from causing higher seminal viscosity an infection with Ureaplasma urealyticum has also effects on the male germ cells by negatively affecting sperm morphology

- ✓ **Mycoplasma hominis and Mycoplasma genitalium**

- Both pathogens are reported to affect the onset of pregnancy as they can attach and penetrate the sperm plasma membrane



even an asymptomatic infection with *Mycoplasma hominis* significantly affects semen parameters including sperm count, motility, morphology, seminal ROS production and total antioxidant capacity. In contrast, the ejaculatory volume and pH were not negatively affected. After antibiotic treatment with doxycycline, all seminal parameters improved significantly

# Neisseria gonorrhoeae

- Neisseria gonorrhoeae is a Gram-negative diplococcus bacterium, which equally infects men and women at reproductive age.
- In men, it can also lead to prostatitis, epididymo-orchitis and infertility due to testicular damage or ductal obstruction.
- It can attach to cell membrane and damage it.
- This bacteria attract leukocytes to the infection site and consequently increase seminal ROS levels, thereby damaging the male germ cells.

# Escherichia coli

- Escherichia coli is a Gram-negative bacterium causing most infections in the urogenital tract and male accessory gland
- Several studies have described direct detrimental effects of Escherichia coli on sperm motility, acrosome reaction and fertilization potential.
- The detrimental effects on motility and acrosome reaction might be due to the direct interaction of the pathogen by means of pili with the sperm plasma membrane leading to morphological alterations
- Escherichia coli is even able to induce early apoptotic events by activating several caspases and proteases responsible for mitochondrial changes and SDF.

# Viruses

- Among these viruses are the mumps virus (orchitis), human immunodeficiency virus-1 (orchitis and prostatitis), Coxsackie virus (epididymitis), cytomegalovirus (vesiculitis), human papillomavirus and herpes simplex virus (prostatitis).
- There are two different modes by which viral infections can enter the male urogenital tract system: ascent through the urethra or hematogenously.
- Adverse effects can be exerted either by direct toxic effects on the cells or indirectly via local inflammatory or immunological reactions.

- Since angiotensin-converting enzyme-2 is present on Leydig and Sertoli cells and the fact that SARS-CoV-2, the virus that causes COVID-19, utilises this enzyme as a receptor to enter human cells, it is plausible that COVID-19 could affect testicular function and therefore male fertility

# Treatment of infections

- Considering that male genital tract infections are in their majority caused by bacterial pathogens, such infections can be treated with antibiotics and anti-inflammatories and are therefore potentially correctable causes of male infertility.
- It is to be noticed that many of these pathogens are sexually transmitted. Therefore, both partners have to be treated after proper diagnosis and administration of a suitable antibiotic after semen culture.

- for viral infections such as mumps orchitis, a systemic treatment with 2 $\beta$ -interferon was used to prevent testicular atrophy.
- On the other hand, lesions due to the inflammatory processes can be alleviated with an anti-inflammation therapy, with both corticosteroids and non-steroids, and have shown significant positive effects on various semen parameters.
- Antioxidant therapies to counteract the oxidative stress may be considered, but are still debated.