DISSERTATIONES
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UNIVERSITY OF TARTU
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Dissertation has been accepted for the commencement of the degree of Doctor of Medical Sciences on June 21, 2023 by the Council of the Faculty of Medicine, University of Tartu, Estonia

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Commencement:	September 15th, 2023

Publication of this dissertation is granted by the University of Tartu.

This study was supported by Estonian Research Council (Grants No. IUT34-19 and PUT181), Estonian Ministry of Education and Research (Grant No. KOGU-HUMB) and Enterprise Estonia (Grant No. EU48695).

ISSN 1024-395X (print)
ISBN 978-9916-27-267-1 (print)
ISSN 2806-240X (pdf)
ISBN 978-9916-27-268-8 (pdf)

University of Tartu Press
www.tyk.ee

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LIST OF ORIGINAL PUBLICATIONS

I. Tjagur, S., Mändar, R., & Punab, M. (2018). Prevalence of Mycoplasma genitalium and other sexually transmitted infections causing urethritis among high-risk heterosexual male patients in Estonia. *Infectious Diseases, 50*(2), 133–139. https://doi.org/10.1080/23744235.2017.1366044

II. Tjagur, S., Mändar, R., & Punab, M. (2020). Profile of sexually transmitted infections causing urethritis and a related inflammatory reaction in urine among heterosexual males: A flow-cytometry study. *PloS ONE, 15*(12). e0242227. https://doi.org/10.1371/journal.pone.0242227

III. Tjagur, S., Mändar, R., Poolamets, O., Pomm, K., & Punab, M. (2021). *Mycoplasma genitalium* Provokes Seminal Inflammation among Infertile Males. *International Journal of Molecular Sciences*, *22*(24), 13467. https://doi.org/10.3390/ijms222413467

Contribution of Stanislav Tjagur to original publications:

Paper I: conceptualisation, methodology, formal analysis, investigation, writing – original draft preparation, visualisation.

Paper II: conceptualisation, methodology, formal analysis, investigation, writing – original draft preparation, visualisation.

Paper III: conceptualisation, methodology, formal analysis, writing – original draft preparation, visualisation.

ABBREVIATIONS

AR – androgen receptor
BMI – body mass index
BPH – benign prostate hyperplasia
CT – *Chlamydia trachomatis*
CI – confidence interval
DHT – 5α-dihydrotestosterone
EB – elementary body
EBV – Epstein-Barr virus
FSH – follicle stimulated hormone
FVU – first-voided urine
GnRH – gonadotropin-releasing hormone
GSS – Gram-stained urethral smear
GU – gonococcal urethritis
HHV-4 – human herpesvirus 4
HIV – human immunodeficiency virus
HPF – high power field
hsCRP – high-sensitivity C-reactive protein
HSV – herpes simplex virus
HSV-1 – herpes simplex virus type-1
HSV-2 – herpes simplex virus type-2
IL-6 – interleukin-6
IPSS – International Prostate Symptom Score
LH – luteinizing hormone
MG – *Mycoplasma genitalium*
MOMP – major outer membrane protein
MSM – men who have sex with men
MTO – Maailma Tervishoiu Organisatsioon
NAATs – nucleic acid amplification tests
NCNGU – non-gonococcal non-chlamydial non-gonococcal urethritis
NG – *Neisseria gonorrhoeae*
NGU – non-gonococcal urethritis
NIH-CPSI – National Institutes of Health Chronic Prostatitis Symptom Index
NSU – non-specific urethritis
OmcB – outer membrane complex protein B
PCR – polymerase chain reaction
PMNL – polymorphonuclear leukocyte
PmpD – polymorphic membrane protein subtype D
PSA – prostate-specific antigen
RB – reticulate body
ROC – receiver operating curve
SARA – sexually acquired reactive arthritis
STI – sexually transmitted infection

STLI – seksuaalsel teel levivad infektsioon
TD – testicular damage
TRUS – transrectal ultrasound study
TV – *Trichomonas vaginalis*
UI – uncertainty interval
WBCs – white blood cells
WHO – World Health Organization
Zn^{2+} – zinc

INTRODUCTION

Sexually transmitted infections (STIs) affect the health of people worldwide, causing several adverse consequences. According to the World Health Organization, the global prevalence estimates of STIs for men in 2016 were 2.7% for chlamydia, 0.7% for gonorrhoea and 0.6% for trichomoniasis. These numbers have remained generally unchanged in comparison with the global data for 2012 (Rowley, et al., 2019). In women, the prevalence of the above-mentioned diseases has been shown to be 3.8% for chlamydia, 0.9% for gonorrhoea and 5.3% for trichomoniasis (Rowley, et al., 2019). STI prevalence is higher among people with high-risk sexual behaviour (Workowski, et al., 2015).

Although STIs may present without symptoms or with mild and transient symptoms, they may have serious consequences such as male infertility (following to complications of urethritis such as epididymitis, orchitis, prostatitis, male accessory sex gland obstruction, and disseminated infection) but also female infertility and ectopic pregnancy (Gimenes, et al., 2014, b; Pellati, et al., 2008).

During recent years more attention has been paid to *Mycoplasma genitalium* (MG) infection along with well-known classic STIs such as chlamydia, gonorrhoea, trichomoniasis and syphilis. The prevalence of *M. genitalium* infection in the global population is estimated at 1–4% in men and 1–6.4% in women, being even more prevalent in the STI testing centres (4–38%) (Cazanave, et al., 2012). In men, *M. genitalium* is associated with urethritis, balanitis and posthitis but in some cases its carriage can be asymptomatic. It may also contribute to couple infertility and increased human immunodeficiency virus (HIV) infection risk (Taylor-Robinson & Jensen, 2011; Cazanave, et al., 2012; Munoz & Goje, 2016). Testing for *M. genitalium* is currently not routinely performed in most countries, so the disease is usually diagnosed syndromically, for example non-chlamydial non-gonococcal urethritis. Furthermore, macrolide-resistance (Couldwell & Lewis, 2015; Manhart, et al., 2015) and fluoroquinolone-resistance (Manhart, et al., 2015; Deguchi, et al., 2016) is increasing in the case of MG infection.

Infertility is defined as the inability of a couple to become pregnant despite unprotected intercourse for a period of more than twelve months (Zegers-Hochschild, et al., 2009). The prevalence rate of infertility varies from 3.5% to 16.7% in more developed countries and from 6.9% to 9.3% in less developed countries, with an estimated overall median prevalence of 9% (Boivin, et al., & Nygren, 2007). In 50% of involuntarily childless couples, a male infertility-associated factor is found, usually together with abnormal semen parameters (Jungwirth, et al., 2012). The impact of sexually transmitted infections on male reproductive function is a continuously controversial topic. It is found that urogenital infections and inflammation can contribute to male infertility in 6.9% to 16% of men (Weidner, et al., 2013; Punab, et al., 2017). It is also known that inflammation and oxidative stress could serve as core mechanisms linking STI with male infertility (Dutta, et al., 2021). However, depending on the local

prevalence of STIs and the availability of medical care, the impact of STIs on the male urogenital system and fertility (as a consequence of infection) may appear regionally different (Ochsendorf et al., 2008). The inconsistent diagnostic criteria applied to date could also explain the controversy regarding the role of infection and inflammation in the genital tract as a cause of infertility (Schuppe, et al., 2017). Research has continued for many decades but the evidence from scientific studies is still uneven – most of the published works are focused on *C. trachomatis* (Ahmadi, et al., 2016), and to a lesser extent to *N. gonorrhoeae* (Fode, et al., 2016), and *T. vaginalis* (Mielczarek & Blaszkowska, 2016). Information about a relatively new pathogen, *M. genitalium*, is rather limited (Taylor-Robinson & Jensen, 2011; Huang, et al., 2015).

Traditionally, diagnosis of urethritis is based on physical signs/symptoms and laboratory methods. In parallel with classic methods (i.e. microscopy of Gram-stained urethral smear and dipstick test of urine) new techniques became available, including flow-cytometric analyses of the urine. However, this method has largely not been evaluated in diagnostics of urethritis and there is no international consensus on the threshold levels as yet.

The general aim of the study was to reveal the prevalence of urethritis among different populations in Estonia, its impact on the male uro-genital system, and the applicability of the novel diagnostic options for urethritis patients.

The studies described in this thesis were conducted at the Centre of Andrology, Tartu University Hospital, Estonia. The doctoral thesis was completed in collaboration with the Centre of Andrology, Tartu University Hospital, Estonia, and the Department of Microbiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia.

REVIEW OF LITERATURE

1. Anatomy, histology and physiology of the male reproductive tract

The components of the male reproductive system include the central nervous system, pituitary gland, testis, epididymis, prostate, seminal vesicles, testes, and penis.

The components of male reproductive system act together to produce functional spermatozoa, and to deliver these spermatozoa to the female reproductive tract. Spermatozoa are produced in the testes and undergo maturational changes during passage of the epididymis. The *vas deferens* transports the spermatozoa from the epididymis to the ejaculatory duct in the prostate. The spermatozoa and secretions of the seminal vesicles empty together, with secretions from the prostate, into prostatic urethra. Secretions from the bulbourethral glands contribute to the ejaculate as the mixture exits the body through the penile urethra. The entire system is dependent on testosterone, also produced in the testis, and its regulation by the pituitary and hypothalamus (The American Society of Andrology, 2010). A short summary of the components of male reproductive system and their main functions is given in Table 1.

Table 1. Components of male reproductive system and their main functions.

Hypothalamus	Gonadotropin-releasing hormone (GnRH) production and secretion; control of follicle stimulated hormone (FSH) and luteinizing hormone (LH) production and secretion.
Hypophysis (pituitary)	FSH and LH production and secretion; control of testicular function.
Testis	Production of the male gametes and androgens.
Epididymis	Maturation of spermatozoa and their storage prior to ejaculation.
Vas (ductus) deferens	Transportation of spermatozoa from the epididymis to the urethra; secretion of fluid for sperm transport; resorption of spermatozoan remnants from the duct lumen.
Seminal vesicles	Resorption of fluids or dissolved substances; spermatophagy; secretions that are involved during coitus (coagulation of seminal plasma), in the maturation of spermatozoa (motility, freezability, condensation of chromatin, capacitation), in the female genital tract (local immunodepression and stimulation of smooth muscle with prostaglandins), in the male genital tract (antibacterial capacity of seminal vesicles' secretions).
Prostate	Control of the ejaculation process, semen liquefaction, and sperm motility; antibacterial function.
Cowper's (bulbourethral) glands	Urethral lubrication; involvement in immune defence of the genitourinary tract.
Urethra	Transport of urine and semen; neutralisation of residual urine's acidity; lubrication during sexual intercourse; role in innate and acquired immunity.

1.1. Anatomy and histology

1.1.1. Prostate

The prostate is both an accessory gland of the male reproductive system and a muscle-driven mechanical switch between urination and ejaculation. The normal prostate weighs 18 g; measures 3 cm in length, 4 cm in width, and is 2 cm in depth; and is traversed by the prostatic urethra (Wein, et al., 2012).

The prostate is composed of approximately 70% glandular elements and 30% fibromuscular stroma. The stroma is continuous with capsule and is composed of collagen and abundant smooth muscle. It encircles and invests the glands of the prostate and contracts during ejaculation to express prostatic secretions into the urethra (Wein, et al., 2012; Lee, et al., 2011; McNeal, 1988).

The arterial supply to the prostate generally arises from the inferior vesical artery (Tummala, et al., 2020). Lymphatic drainage is primarily to the obturator and internal iliac nodes. A small proportion of drainage may initially pass through the presacral group or, less commonly, the external iliac nodes (Wein, et al., 2012; Boscolo-Berto, et al., 2020). Sympathetic and parasympathetic innervation from the pelvic plexus travels to the prostate through the cavernous nerves. The nerves follow branches of the capsular artery to ramify in the glandular and stromal elements. Parasympathetic nerves end at the acini and promote secretion; sympathetic fibres cause contraction of the smooth muscle of the capsule and stroma (Wein, et al., 2012; Lee, et al., 2011).

In general, the glands of the prostate are tubuloalveolar with relatively simple branching and are lined with simple cuboidal or columnar epithelium. Scattered neuroendocrine cells, of unknown function, are found between the secretory cells. Beneath the epithelial cells, flattened basal cells line each acinus. Each acinus is surrounded by a thin layer of stromal smooth muscle and connective tissue (Wein, et al., 2012; McNeal, 1988).

The glandular elements of the prostate have been divided into discrete zones, distinguished by the location of their ducts in the urethra, by their differing pathologic lesions, and, in some cases, by their embryologic origin. The zones of the prostate gland are the transitional zone, central zone, peripheral zone, and anterior fibromuscular stroma (Pradidarcheep, et al., 2011; Wein, et al., 2012; McNeal, 1988).

1.1.2. Urethra

The urethra connects the urinary bladder to the outside world to pass urine. It also participates in transportation of the seminal fluid. The approximately 20-cm-long male urethra is subdivided into the pelvic urethra, with prostatic and membranous portions, and the penile urethra, which includes the bulbar portion (Pradidarcheep, et al., 2011).

The prostatic portion of the urethra connects to the bladder neck and runs through the core of the prostatic mass and is lined with transitional cell

epithelium. This segment contains the openings of the ejaculatory ducts and the numerous openings of the prostatic ducts (Pradidarcheep, et al., 2011).

In its course from the apex of the prostate to the perineal membrane, the membranous urethra spans on average 2 to 2.5 cm (range 1.2 to 5 cm). It is surrounded by the striated (external) urethral sphincter. Autonomic innervation to the intrinsic smooth muscle of the membranous urethra is likely to be given by the cavernous nerves as they pass nearby, although dividing these nerves does not appear to affect urinary continence significantly (Wein, et al., 2012). The ducts of the bulbourethral glands enter the urethra just distal to the perineal membrane. The membranous urethra is lined with pseudostratified columnar epithelium (Pradidarcheep, et al., 2011).

The anterior urethra lies inside *corpus spongiosum*. The anterior urethra is dilated in its bulbar and glanular segments (*fossa navicularis*) and narrowest at the external meatus. Proximally, it is lined by stratified and pseudostratified columnar epithelium, distally by stratified squamous epithelium. The mucus-secreting glands (of Littre) may be seen as small outpouchings of the mucosa (Wein, et al., 2012).

1.1.3. *Vas deferens*

Vas (ductus) deferens arises from the tail of the epididymis and at the epididymal end it is tortuous for 2 to 3 cm. It runs posterior to the vessels of the cord and through the inguinal canal and emerges in the pelvis. At the internal ring, it diverges from the testicular vessels and passes medial to all structures of the pelvic side wall to reach the base of the prostate posteriorly. The terminal vas is dilated and tortuous (*ampulla*) and is capable of storing spermatozoa (Wein, et al., 2012).

Histologically, the *vas deferens* may be considered a system in which the lumen, bounded by mucosa, is surrounded by a muscular wall. The muscular wall consists of three layers: longitudinally oriented smooth muscle cells surrounding the mucosa; circumferentially oriented smooth muscle cells; and, once again, longitudinally oriented smooth muscle cells. The *vas deferens* is surrounded by the adventitia which contains the veins and the incoming neural axons. Structure and arrangement of mucosa epithelium of the *vas deferens* vary along the length of the *vas deferens*. Near the epididymis, it is similar to the epithelium found in the *cauda epididymidis*. These are large columnar cells with regularly placed pairs of cilia. Going away from the epididymal end, the epithelium is nonciliated, smaller in size, and in the ampulla it appears in more than one layer (strata). The epithelium lies on a layer of connective tissue containing numerous elastic fibres (Batra, 1974).

Blood supply comes from the inferior vesical artery. The *vas deferens* is innervated by autonomic postganglionic nerve fibres primarily originating from neurons in pelvic ganglia, and, to a lesser extent, from neurons in the caudal mesenteric ganglion and sympathetic chain ganglia, and also by sensory nerve fibres arising from dorsal root ganglia (Koslov & Andersson, 2013).

1.1.4. Seminal vesicles

The seminal vesicles were described for the first time in 1521 by the Italian anatomist Berengario a Carpi. They were regarded as mere storage organs for semen, hence the designation seminal vesicles (Aumüller & Riva, 1992).

The seminal vesicle is a lateral outpouching of the *vas deferens*, approximately 5 cm long, with a capacity of 3 to 4 ml. It is a paired organ. Seminal vesicles do not store sperm but contribute the largest portion of fluid to the ejaculate (about 70% of the seminal fluid) (Aumüller & Riva, 1992; Wein, et al., 2012). The seminal vesicle comprises a single coiled tube with several outpouchings that is lined by columnar epithelium with goblet cells (Wein, et al., 2012). The seminal vesicles resemble a skein of coral rather than comprising a single strand (Lee, et al., 2020). The tube is encased in a thin layer of smooth muscle and is held in its coiled configuration by a loose adventitia. The seminal vesicle and ampulla of the vas lie posterior to the bladder. The ureter enters the bladder medial to the tip of the seminal vesicle. As the seminal vesicle and vas join to form the ejaculatory duct, their smooth muscle coats fuse with the prostatic capsule at its base (Wein, et al., 2012).

The human seminal vesicle epithelium is pseudostratified and consists of two cell populations, basal cells and principal cells, the latter being the majority of the cells within the seminal vesicle epithelium. The seminal vesicle is nonfunctional until puberty is reached (Gonzales, 1989).

The mesenchyme of the seminal vesicle is derived from the urogenital septum and surrounds the undifferentiated epithelium. It is suggested that the mesenchyme has an important function in helping to shape the developing seminal vesicle (Gonzales, 1989).

The blood supply for the seminal vesicles and vasa comes from the vesiculodeferential artery, a branch of the superior vesical artery. Additional arterial supply may come from the inferior vesical artery. The pelvic vas and seminal vesicle drain into the pelvic venous plexus. Lymphatic drainage passes to the external and internal iliac nodes. Innervation arises from the pelvic plexus, with major excitatory efferents contributed by the (sympathetic) hypogastric nerves (Wein, et al., 2012).

1.1.5. Bulbourethral glands

Cowper's glands also known as the bulbourethral glands were named after the 17th century English surgeon William Cowper. The two main Cowper's glands are situated within the urogenital diaphragm, with a second pair of accessory glands situated in the bulbospongiosal tissue. The main Cowper's ducts enter the ventral surface of the bulbourethra near the midline by piercing the spongiosum. The accessory ducts can enter the urethra directly or drain into the main duct. The bulbourethra extends from the inferior urogenital diaphragm to the suspensory penile ligament superiorly and penoscrotal junction inferiorly. A Cowper's gland consists of well-demarcated lobules of small, compact tubulo-

alveolar glands radiating from a central excretory duct lined by pseudostratified epithelium, and entrapped within fascicles of muscle. The glands have a thin connective tissue capsule composed of simple columnar epithelium (Chughtai, et al., 2005).

1.1.6. Penis

The root of the penis is fixed to the perineum within the superficial pouch. The *corpora cavernosa* join beneath the pubis (penile hilum) to form the major portion of the body of the penis. They are separated by a septum that becomes pectiniform distally. They are enclosed by the tough *tunica albuginea*, which is predominantly collagenous. Its outer longitudinal and inner circular fibres form an undulating meshwork when the penis is flaccid and appear tightly stretched with erection. Smooth muscle bundles traverse the erectile bodies to form the endothelium-lined cavernous sinuses. Distal to the bulb, the *corpus spongiosum* tapers and runs on the underside (*ventrum*) of the *corpora cavernosa* and then expands to cap them as the *glans penis*. The *corona* separates the base of the *glans* from the shaft of the penis (Wein, et al., 2012).

The spongiosum is traversed throughout its length by the anterior urethra, which begins at the perineal membrane. Buck fascia surrounds both cavernosal bodies dorsally and splits to surround the spongiosum ventrally (Wein, et al., 2012).

The skin of the penile shaft is highly elastic and without appendages (hair or glandular elements), except for the smegma-producing glands at the base of the *corona*. Distally, it folds over the glans as the foreskin and attaches firmly below the *corona*. Its blood supply is independent of the erectile bodies and is derived from the external pudendal branches of the femoral vessels (Wein, et al., 2012).

The blood supply of *corpora cavernosa* and *corpus spongiosum* derives from the internal pudendal artery. At the base of the glans, several venous channels coalesce to form the dorsal vein of the penis, which runs in a groove between the corporal bodies and drains into the preprostatic plexus (Wein, et al., 2012).

The dorsal nerves provide sensory innervation to the penis. Small branches from the perineal nerve supply the *ventrum* of the penis near the urethra as far as the glans distally. The route of the cavernous nerves has been described. After piercing the corporal bodies, they ramify in the erectile tissue to supply sympathetic and parasympathetic innervation from the pelvic plexus. Tonic sympathetic tone inhibits erection. Parasympathetic nerves release acetylcholine, nitric oxide, and vasoactive intestinal polypeptide, which cause the cavernosal smooth muscle and arterial relaxation necessary for erection. It is thought that during erection, the subtunical venules are occluded by being compressed against the nondistensible *tunica albuginea* (Wein, et al., 2012).

1.1.7. Scrotum

The scrotum or scrotal sac is located at the base of the penis and consists of a suspended dual-chambered sac of skin and smooth muscle. The scrotal skin is pigmented, hair bearing, devoid of fat, and rich in sebaceous and sweat glands. It varies from loose and shiny to highly folded with transverse rugae, depending on the tone of the dartos smooth muscle. A midline raphe runs from the urethral meatus to the anus and represents the line of fusion of the genital tubercles. Deep to this raphe, the scrotum is separated into two compartments by a septum. The testes are suspended by their cords in the scrotal compartments. As the testes descend, they acquire coverings from the layers of the abdominal wall, known as the spermatic fascia, that form part of the scrotal wall. The parietal and visceral *tunica vaginalis* surround the testis with a mesothelium-lined pouch and are derived from the peritoneum (Wein, et al., 2012).

The anterior wall of the scrotum is supplied by the external pudendal vessels and the ilioinguinal and genitofemoral nerves. The back of the scrotum is supplied by the posterior scrotal branches of the perineal vessels and nerves. In addition, the posterior femoral cutaneous nerve (S3) gives a perineal branch to supply the scrotum and perineum. In accordance with their origin, the spermatic fasciae have a blood supply (cremasteric, vasal, testicular) separate from that of the scrotal wall (Wein, et al., 2012).

The penis, scrotum, and perineum drain into the inguinal lymph nodes. These nodes may be divided into superficial and deep groups, which are separated by the deep fascia of the thigh (*fascia lata*). The scrotal lymphatics do not cross the median raphe and drain into the ipsilateral superficial inguinal lymph nodes (Wein, et al., 2012).

1.1.8. Testis and epididymis

A testicle or testis is a paired organ. The testes are 4 to 5 cm long, 3 cm wide, and 2.5 cm deep (Wein, Kavoussi, Novick, Partin, & Peters, 2012). The mean Prader orchidometer-based testicular volume reported in the European general population is 20.0 ± 5.0 ml (Lotti & Maggi, 2015). They are enclosed in a tough capsule comprising: (1) the visceral *tunica vaginalis*; (2) *tunica albuginea*; and (3) the *tunica vasculosa*. The epididymis attaches to the posterolateral aspect of the testis. Beneath it, the *tunica albuginea* projects inward to form the *mediastinum testis*, the point at which vessels and ducts traverse the testicular capsule. Septa radiate from the mediastinum to attach to the inner surface of the *tunica albuginea* to form 200 to 300 cone-shaped lobules, each of which contains one or more convoluted seminiferous tubules. Interstitial (Leydig) cells lie in the loose tissue surrounding the tubules and are responsible for testosterone production. Toward the apices of the lobules, the seminiferous tubules become straight (*tubuli recti*) and enter the *mediastinum testis* to form an anastomosing network of tubules. This network, known as the *rete testis*, forms 12 to 20 efferent ductules and passes into the largest portion of the epididymis, the *caput*.

Here, the efferent ductules enlarge, become more convoluted, and form conical lobules. The duct from each lobule drains into a single epididymal duct, which winds approximately 6 m within the fibrous sheath of the epididymis to form its body and tail. As the duct approaches the tail, it thickens and straightens to become the *vas deferens* (Wein, et al., 2012).

In the seminiferous tubules, adjacent Sertoli cells form complex networks of specialised tight junctions (*zonula occludens*) that cause isolation of the tubular contents from the blood vascular compartment. This barrier is called the blood-testis barrier. Sperm are transported in the tubular fluid (Nieschlag, et al., 2010).

The testicular arteries arise from the aorta (Wein, et al., 2012). The testicular veins form the pampiniform plexus. At the level of the inguinal canal, the veins join to form two or three channels and then a single vein that drains into the inferior *vena cava* on the right and the renal vein on the left. Testicular lymphatic vessels drain to the para-aortic and interaortocaval nodes (Wein, et al., 2012).

Visceral innervation to the testis and epididymis travels by two routes. A portion arises in the renal and aortic plexuses and travels with the gonadal vessels. Additional gonadal afferent and efferent nerves course from the pelvic plexus in association with the *vas deferens*. Some afferent and efferent nerves cross over to the contralateral pelvic plexus. The genital branch of the genito-femoral nerve supplies sensation to the parietal and visceral *tunica vaginalis* and the overlying scrotum (Wein, et al., 2012).

1.2. Physiology

1.2.1. Testis

The testes have two main functions – production of the male gametes (by tubular compartment of the testis) and the male sexual hormones – androgens (by interstitial compartment of the testis). Spermatogenesis in men lasts for at 74 days by including time for spermatogonial renewal (Nieschlag, et al., 2010; Ježek, 2013).

The seminiferous epithelium consists of supporting Sertoli cells and spermatogenic cells: spermatogonia, primary and secondary spermatocytes, and early and late spermatids. When early spermatids are released into the tubular lumen, they are considered mature spermatozoa (Ježek, 2013). The process whereby germ cells progress from diploid cells (spermatogonia) to meiotic cells (spermatocytes) to haploid cells (spermatids) is called spermatogenesis (The American Society of Andrology, 2010; Nieschlag, et al., 2010). Sertoli cells are part of the blood-testis barrier that provides the physical isolation of haploid and thereby antigenic germ cells to prevent recognition by the immune system and participates in the preparation of a special milieu for the meiotic process and sperm development (Nieschlag, et al., 2010). Sertoli cells are stimulated by intratesticular testosterone and follicle stimulated hormone (FSH) (The American Society of Andrology, 2010).

The Leydig cells are the site of production and secretion of the hormone testosterone. Male sexual development and function is regulated by testosterone's direct action and that of its metabolites (dihydrotestosterone, oestradiol). The Leydig cells are regulated by luteinizing hormone (LH) (The American Society of Andrology, 2010).

1.2.2. Epididymis

In the epididymis, spermatozoa mature and are stored prior to ejaculation. The time for spermatozoa to pass through the epididymis lies between two and 11 days, depending on testicular sperm output (Nieschlag, et al., 2010).

1.2.3. Prostate

The main functions of the prostate include control of the ejaculation process, semen liquefaction, and sperm motility, as well as antibacterial function.

The main function of the stromal compartment of the prostate gland is to ensure the appropriate microenvironment for the epithelial compartment. The stromal compartment provides many supportive signals to retain or restore gland homeostasis in healthy conditions or during regeneration processes (Verze, et al., 2016).

The prostate epithelial compartment has the main glandular function as it secretes the prostatic fluid that constitutes approximately one-fifth to one-third of the volume of the entire ejaculate. Prostatic fluid contains a number of factors and regulatory proteins, which are necessary for semen liquefaction, the clotting cycle and sperm motility. These factors are as follows: kallikreins, a specific subfamily of 15 serine proteases, which include prostate specific antigen (PSA), encoded by *KLK3*; citrate, an intermediate metabolite of the Krebs cycle; and zinc (Zn^{2+}), a trace element actively stored within the cytoplasm of the prostatic epithelial cells. The normal human prostate accumulates the highest levels of Zn^{2+} of any soft tissue in the human body (about 4% of the overall Zn^{2+} content within the human male body) (Verze, et al., 2016).

Firstly, Zn^{2+} blocks the initial step of the Krebs cycle – the oxidation of citrate in isocitrate, leading to the accumulation of high levels of citrate. Prostatic citrate mainly acts as an energy substrate for sperm, increasing its adenosine triphosphate production. Secondly, Zn^{2+} causes the temporary inactivity of prostatic tissue kallikreins. Under physiological conditions within the prostate and prostatic fluid, kallikreins are inactivated by allosteric reversible binding of Zn^{2+}. Thus, Zn^{2+} represents the major inhibitor of the prostatic kallikreins' proteolytic cascade, the triggering cues of which depend on ejaculatory stimuli driven by the central nervous system (Verze, et al., 2016). Zinc was also found to be a prostatic antibacterial factor (Frick & Aulitzky, 1991).

Intraprostatic accumulation of Zn^{2+} and citrate, inhibition of the Krebs cycle and prostatic fluid release are regulated by male sex steroids via their main intracellular effector, the androgen receptor (AR). The AR is a nuclear receptor

and has a dual function, acting as both an intracellularly located receptor and as a ligand-activated transcription factor. Within the prostate, testosterone is converted by the enzyme 5α-reductase into the more potent (in terms of binding affinity for the AR) androgen, 5α-dihydrotestosterone (DHT) (Verze, et al., 2016).

1.2.4. Seminal vesicles and *vas deferens*

Along with the ampulla of the *vas deferens* and the ejaculatory duct, seminal vesicles form a functional unit (ampullo-vesiculoductal complex). Its functions consist of resorption, spermatophagy, and secretion (Aumüller & Riva, 1992). Secretion by the seminal vesicles is androgen dependent (Marty, et al., 2003).

The function of the *vas (ductus) deferens* is to convey spermatozoa from the epididymis to the urethra. During emission, its coordinated muscular contractions propel the spermatozoa toward the urethra. However, the vas does not only serve as a conduit, but also contributes to the secretion of fluid for sperm transport and possibly to the resorption of spermatozoan remnants from the duct lumen (Koslov & Andersson, 2013).

1.2.5. Urethra

Male urethra functions as an exit for urine as well as semen, it neutralises acidity of residual urine present in the lumen of the urethra during sexual excitement, provides a lubricant during sexual intercourse, and plays an important role in innate and acquired immunity (Pudney & Anderson, 2011).

The urethral mucosa can recognise pathogens through the expression of Toll-like receptors on epithelial cells and intraepithelial immune cells, and is capable of secreting a wide array of antimicrobial factors that may be retained by a viscous mucosal layer secreted by glands of Littre. Little is known about adaptive immune responses at this site, but the urethral mucosal appears to have an entire contingent of humoral and cellular immunological mediators that could participate in a local immune response (Pudney & Anderson, 2011). In the urethra, slender epithelial cells with apical microvilli ('brush cells') serve as a chemosensory sentinel for potential hazardous compounds in the urethral lumen, triggering a protective mechanism (flushing through micturition) against further ascent (Kummer & Deckmann, 2017). There is also a recently investigated new group of cells in the urethra, called telocytes. These cells are located in the subepithelial region of the urethra. Telocytes could act in the tissue organisation of the stroma. These cells are also observed around the blood vessels and nerve endings. The telocytes may participate in the micturition reflex in the bladder urethra (Sanches, et al., 2021). However, the exact role of telocytes in the physiology of the urethra still needs further investigation. Transient receptor potential ion channels are responsive to a broad range of sensory signals such as temperature (hot and cold), osmolarity, pH, stretch, taste, and odorants. Expression of these ion channels in the urethra is implicated

in the regulation of the urethra-to-bladder reflex and detrusor overactivity (Toktanis, et al., 2018).

1.2.6. Bulbourethral glands

The Cowper's or bulbourethral gland is an accessory sexual organ that contributes to urethral lubrication. The bulbourethral gland is a paired organ. Cowper's glands develop from the membranous urethra and are most significantly under the control of dihydrotestosterone (Chughtai, et al., 2005).

During sexual excitement, these glands secrete clear glycoproteins into the bulbous urethra. The male sex accessory tissues require the continued function of the testes for their development, growth and maintenance of secretions that form the major components of the ejaculate. Cowper's glands secrete glycoproteins during sexual stimulation, which functions as a lubricant for the semen. In response to sexual stimulation, the bulbourethral glands secrete an alkaline mucus-like fluid. This fluid neutralises the acidity of the urine residue in the urethra, helps to neutralise the acidity of the vagina, and provides some lubrication for the tip of the penis during intercourse. Cowper's gland secretions contain no sperm. Cowper's glands are involved in the immune defence of the genitourinary tract and secrete many glycoproteins, including PSA, and mucin MG1 (Chughtai, et al., 2005; Piludu, et al., 2009; Kutia, et al., 2021). Mucins represent a first line of defence for mucosal surfaces against bacterial, fungal and viral attacks. Their glycosidic groups bind to specific components of microbial walls, thus mediating the entrapment and clearance of bacteria from the body tracts (Piludu, et al., 2009). Immunohistochemical studies on whole-mount cadaveric Cowper's gland and cystoprostatourethrectomy samples showed that, although PSA and prostate-specific acid phosphatase are mostly produced by prostatic tissue, it was not exclusive. These findings support the hypothesis of extraprostatic sources of PSA and may impact on the specificity and sensitivity of PSA serum levels after radical prostatectomy (Chughtai, et al., 2005).

1.2.7. Neuroendocrine control of the reproductive system

Both LH and FSH are involved in the initiation of spermatogenesis. Pulses of LH elicit increases in androgen concentrations. As age increases, pulsatile gonadotrophin secretion increases in frequency and amplitude in response to pulsatile gonadotropin-releasing hormone (GnRH) secretion. During puberty, inhibin from Sertoli cells is the primary negative feedback agent to control FSH release. Testosterone regulates both FSH and LH at the level of the hypothalamus. From puberty onward, androgen increases libido (Marty, et al., 2003). In primates, the androgen receptor can not only be found in the classical androgen-dependent organs, such as muscles, prostate, seminal vesicles, epididymis and testes, but also in almost every tissue, e.g., hypothalamus, pituitary, kidney, spleen, heart, and salivary glands. Hence, testosterone exerts a variety of actions on many body targets (Nieschlag, et al., 2010).

In clinical practice, measurement of blood testosterone, LH and FSH level in men is used in diagnostic investigations and monitoring of hypogonadism. The analogues of gonadotrophins and testosterone are used in the treatment of male patients with hypogonadism and the treatment of male infertility (Corona, et al., 2020; Bhasin, et al., 2018; Barbonetti, et al., 2018).

1.2.8. Erection and ejaculation

The ejaculatory process consists of two sequential processes under the control of the autonomic nervous system. Under psychological, visual, auditory, olfactory and tactile stimuli, parasympathetic impulses travelling over the *nervi erigentes* liberate acetylcholine that vasodilates the arteries. This increases blood flow into the *corpora cavernosum* and *corpus spongiosum*, which compresses venous outflow, thus increasing the turgidity of the penis and causing an erection. Parasympathetic impulses also lead to secretion by the urethral and bulbourethral glands. The next phase, emission (the passage of spermatozoa into the urethra) is under sympathetic control with nerve impulses travelling over the *rami communicantes* and hypogastric nerves to liberate adrenalin that initiates contraction of the smooth muscles surrounding the *ampulla*, *ductuli deferentia* and terminal *cauda epididymidis*. During ejaculation proper, parasympathetic fibres from the lower lumbar and upper sacral centres, together with somatic inflow via the pudendal nerve, initiate contraction of the bulbocavernous muscles, leading to forcible ejection of the semen from the urethra at the same time as ascending impulses give rise to the sensation of orgasm. Finally, detumescence of the penis is caused by the sympathetic release of noradrenalin causing dilation of the penile vasculature and penile flaccidity (Nieschlag, et al., 2010).

1.2.9. Ejaculate

Semen is composed of spermatozoa, which make up about 2–5% of the volume of the whole ejaculate, and seminal plasma, which mostly consists of various fluids secreted by the seminal vesicles, prostate epithelium and bulbourethral glands (Verze, et al., 2016).

In fertile men the sequence in which the accessory glands contribute their secretions to the ejaculate is fixed: the bulbourethral glands secrete an alkaline solution with glycoproteins that neutralises urinary tract acidity and lubricates the tract before ejaculation; the prostate, epididymis and *vasa deferentia* contract together, discharging spermatozoa and prostatic secretions; finally the seminal vesicles contract and expel the pellet of spermatozoa to the urethra with their secretions before forceful expulsion to the outside (Nieschlag, et al., 2010).

Once the ejaculation cue is triggered, the sperm-enriched epididymal fluid is mixed with both prostatic fluid enriched with Zn^{2+}, citrate and kallikreins and with semenogelin-containing seminal vesicle secretions, which together form the bulk of the semen. After ejaculation, semenogelins 1 and 2 and fibronectin

aggregate to form a gelatinous mass and then within a few minutes the activated kallikreins, including PSA, start the semen liquefaction process that enables sperm to be released and move towards the Fallopian tube (Verze, et al., 2016).

The ejaculate that is collected and analysed in the laboratory differs from that produced at coitus since all the various fractions are pooled in the collection vessel and cannot be analysed until liquefaction has occurred. Of all the accessory glands that contribute to the ejaculate, the seminal vesicles provide the bulk volume but proper functioning of all the organs is necessary to provide sufficient fluid of the optimum composition for a normal ejaculate. Sperm-free seminal plasma is assayed to assess accessory gland function. The major secretions of the sex organs that appear in seminal plasma (fructose from the seminal vesicles; zinc, acid phosphatase, citric acid, prostate-specific antigen from the prostate, l-carnitine, glycerophosphocholine, neutral α-glucosidase from the epididymis) are analysed clinically to diagnose possible causes of infertility; the amount of secretion provides information about the presence, functioning or blockage of the glands (Nieschlag, et al., 2010).

1.2.10. Seminal interleukin-6

Beside measurement of leucocyte concentration in semen or prostate-specific materials, the assessment of male accessory gland inflammation also includes the measurement of granulocyte elastase and proinflammatory cytokines (Schuppe, et al., 2017), including interleukin-6 (IL-6). IL-6 is a pro-inflammatory cytokine, initially discovered and cloned in the Kishimoto laboratory as a B-cell stimulatory factor (Rose-John, 2020). IL-6 is produced by different cell types including monocytes/macrophages, fibroblasts, keratinocytes, endothelial cells, mesangial cells, glial cells, chondrocytes, osteoblasts, smooth muscle cells, T cells, B cells, granulocytes, mast cells, and certain tumour cells (Akira, et al., 1993). In semen, macrophages seem to be the main source of IL-6 (Fathy, et al., 2014; Haidl, et al., 2015). The actual source of these cytokines in the semen is unknown, but apparently the cytokines are restricted to the urogenital system because they are not detected in patient blood samples (Basu, et al., 2004). A predominantly prostatic origin of IL-6 in seminal fluid is suggested (Comhaire, et al., 1994; Matalliotakis, et al., 1998). However, the seminal vesicles could also be implicated (Friebe, et al., 2003). Friebe *et al.* (2003) found the significant circannual variation of seminal IL-6 levels with a maximum in December and a peak-to-trough variation of 33% of the mean. The authors speculate that this may, however, be merely the consequence of a higher frequency of seminal tract inflammations in autumn and winter.

Many studies have controlled the correlation of seminal IL-6 with different parameters. The results of these studies are presented in Table 2.

Table 2. Correlation of seminal interleukin-6 (IL-6) with different parameters.

Parameter	Positive correlation	Negative correlation	No correlation
Seminal white blood cells (WBCs)	Eldamnhoury, et al., 2018; Dehghan Marvast, et al., 2016; Ausmees, et al., 2013; Aghazarian, et al., 2011, 2013, 2015, 2019; Martínez-Prado, et al., 2010; Kokab, et al., 2010; Korrovits, et al., 2006, 2008, 2011; Moretti, et al., 2009; Kullisaar, et al., 2008; Kopa, et al., 2005; Krause, et al., 2003; Friebe, et al., 2003; Eggert-Kruse, et al., 2001; Paradisi, et al., 1997; Depuydt, et al., 1996, 1998; Shimoya, et al., 1995; Zalata, et al., 1995; Comhaire, et al., 1994	NA	NA
Seminal polymorpho-nuclear/granulocyte elastase	Moretti, et al., 2009; Kratzsch, et al., 2008; Kopa, et al., 2005	NA	NA
Total sperm count	NA	Ausmees, et al., 2013; Naz & Kaplan, 1994	Elfassy, et al., 2020; Camejo, et al., 2003
Sperm concentration	Elfassy, et al., 2020	Moretti, et al., 2021; Micheli, et al., 2019; Furuya, et al., 2003; Paradisi, et al., 1997	Ausmees, et al., 2013; Nandipati, et al., 2005; Kopa, et al., 2005; Nallella, et al., 2004; Friebe, et al., 2003; Koçak, et al., 2002; Matalliotakis, et al., 1998; Dousset, et al., 1997; Depuydt, et al., 1996; Huleihel, et al. 1996
Sperm motility	Elfassy, et al., 2020	Micheli, et al., 2019; Moretti, et al., 2014, 2021; Kopa, et al., 2005; Paradisi, et al., 1997; Gruschwitz, et al., 1996; Naz & Kaplan, 1994	Ausmees, et al., 2013; Nandipati, et al., 2005; Kopa, et al., 2005; Nallella, et al., 2004; Friebe, et al., 2003; Camejo, et al., 2003; Koçak, et al., 2002; Matalliotakis, et al., 1998; Dousset, et al., 1997; Depuydt, et al., 1996; Huleihel, et al. 1996
Sperm vitality	Elfassy, et al., 2020	Moretti, et al., 2021; Kopa, et al., 2005	Gruschwitz, et al., 1996

Parameter	Positive correlation	Negative correlation	No correlation
Sperm morphology	NA	Moretti, et al., 2021; Micheli, et al., 2019; Matalliotakis, et al., 1998; Paradisi, et al., 1997	Elfassy, et al., 2020; Friebe, et al., 2003; Camejo, et al., 2003; Koçak, et al., 2002; Dousset, et al., 1997; Gruschwitz, et al., 1996; Huleihel, et al. 1996
Spermatozoa abnormal forms	Attia, et al., 2021	NA	NA
Seminal dead sperm	Micheli, et al., 2019; Moretti, et al., 2014	NA	NA
Seminal fluid viscosity	Castiglione, et al., 2014	NA	NA
Semen volume	NA	Elfassy, et al., 2020; Aghazarian, et al., 2013; Ausmees, et al., 2013; Martínez-Prado, et al., 2010	Moretti, et al., 2021; Friebe, et al., 2003
Semen pH	NA	NA	Friebe, et al., 2003; Gruschwitz, et al., 1996
Seminal fructose	NA	Krause, et al., 2003; Friebe, et al., 2003	NA
Seminal α-glucosidase	NA	NA	Friebe, et al., 2003
Seminal interleukin-8	Dehghan Marvast, et al., 2016; Aghazarian, et al., 2013; Kokab, et al., 2010; Moretti, et al., 2009; Friebe, et al., 2003; Eggert-Kruse, et al., 2001; Depuydt, et al., 1996	NA	Furuya, et al., 2003
Seminal interleukin-1β	Moretti, et al., 2009; Comhaire, et al., 1994	NA	NA
Seminal interleukin-18	Qian, et al., 2014	NA	NA
Seminal tumour necrosis factor α	Micheli, et al., 2019; Moretti, et al., 2009, 2014; Koçak, et al., 2002	Attia, et al., 2021	NA
Seminal monocyte chemotactic and activating factor	Shimoya, et al., 1995	NA	NA
Seminal hepatocyte growth factor	Depuydt, et al., 1996	NA	NA
Seminal immunoglobulin G	Friebe, et al., 2003	NA	NA
Seminal immunoglobulin A	NA	NA	Friebe, et al., 2003

Parameter	Positive correlation	Negative correlation	No correlation
Seminal anti-heat shock protein-60 antibodies	Martínez-Prado, et al., 2010	NA	NA
Semen complement fraction C_3	Eggert-Kruse, et al., 2001	NA	NA
Seminal ghrelin	NA	Micheli, et al., 2019	NA
Seminal obestatin	NA	Micheli, et al., 2019	NA
Seminal adipocytokine resistin	Kratzsch, et al., 2008; Moretti, et al., 2014	NA	NA
Seminal oxidative stress markers/reactive oxygen species	Micheli, et al., 2019; Nandipati, et al., 2005; Nallella, et al., 2004; Camejo, et al., 2001; Depuydt, et al., 1996, 1998	NA	NA
Seminal antioxidant system molecules	NA	Micheli, et al., 2019	NA
Semen lipid peroxidation	Moretti, et al., 2021	NA	NA
Spermatozoa DNA integrity	NA	Haidl, et al., 2015	Kopa, et al., 2005
Sperm DNA fragmentation	NA	NA	Derbel, et al., 2021
Body mass index (BMI)	Ausmees, et al., 2013	NA	NA
Age	Ausmees, et al., 2013	NA	Friebe, et al., 2003
Total prostate volume	Ausmees, et al., 2013	NA	NA
Intraprostatic T lymphocytes	NA	John, et al., 2003	NA
Blood PSA	Ausmees, et al., 2013, 2014	NA	NA
Blood cholesterol	NA	Attia, et al., 2021	NA
Blood FSH, LH, or testosterone	NA	NA	Ausmees, et al., 2013; Furuya, et al., 2003; Gruschwitz et al., 1996; Dousset, et al., 1997

Abbreviations: NA – not available; FSH, follicle stimulated hormone; LH, luteinizing hormone; DNA, deoxyribonucleic acid

Seminal IL-6 is positively correlated with seminal white blood cells (WBCs), seminal polymorphonuclear/granulocyte elastase, different cytokines and miscellaneous factors. There are studies that found positive, negative and no correlation between seminal IL-6 and spermatozoa concentration, motility and morphology (see Table 2). Moretti *et al.* (2021) found a correlation between seminal IL-6 and on lipid peroxidation of the spermatozoa in an *in vivo* study. However, Martínez *et al.* (2007) did not show the effect of IL-6 on lipid peroxidation of the spermatozoa plasma membrane *in vitro*. Lampiao and du Plessis (2009) demonstrated that IL-6 can inhibit both the non-physiological as well as physiologically elicited acrosome reaction by calcium ionophore and progesterone respectively acrosome reaction. Thus, there is a potential impact of this cytokine on fertilisation. Reactive oxygen species can increase the secretion of IL-6 by seminal leucocytes (Li, et al., 2020, b).

Basu *et al.* (2004) demonstrated higher seminal plasma concentration of IL-6 in patients with a spinal cord injury compared with healthy men without such an injury. The *in vitro* study by Yoshida *et al.* (2004) showed that a combination of IL-6 and soluble IL-6 receptor may be associated with glycoprotein 130 expressed in the sperm and reduce sperm motility. An *in vivo* study by Cohen *et al.* (2004) showed that neutralisation of tumour necrosis factor α, interleukin 1-β, and IL-6 via monoclonal antibodies enhanced sperm motility in men with a spinal cord injury. In the next study by the same group it was shown that these cytokines act at the level of the sperm receptor to inhibit sperm motility (Brackett, et al., 2007).

Although the measurement of IL-6 in the ejaculate is still considered facultative/experimental by the World Health Organization (WHO) and is not used for routine semen analysis (Schuppe, et al., 2017; World Health Organization, 2021), the determination of cytokines in the case of inflammation/infections of the seminal ducts or chronic prostatitis/chronic pelvic pain syndrome could be useful for diagnosis and monitoring of the therapy (Pilatz, et al., 2013).

A relationship between seminal IL-6 and STI is inconclusive. Some *in vivo* studies found elevated seminal IL-6 in *C. trachomatis*-positive men (Kokab, et al., 2010; Dehghan Marvast, et al., 2016). There are no studies analysing seminal IL-6 in case the of MG, TV or NG infection.

2. Urethritis

2.1. Definition, clinical features and epidemiology

Urethritis, or inflammation of the urethra, is a multifactorial condition, which is sexually acquired in the majority of cases. It is characterised by symptoms such as urethral discharge, dysuria and/or urethral discomfort/itch, and penile tip irritation, but may be also asymptomatic. The signs of urethritis include urethral discharge and penile tip erythema. However, physical examination can also be

normal. Complications of STI-associated urethritis can include epididymo-orchitis, prostatitis, and sexually acquired reactive arthritis (acute or chronic).

Urethritis is described as either gonococcal (GU), when *Neisseria gonorrhoeae* (NG) is detected, or non-gonococcal (NGU) when it is not. The term non-specific urethritis (NSU) applies to non-gonococcal non-chlamydial urethritis and to prevent confusion it should be avoided (Horner, et al., 2016). Urethritis that occurs in the absence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (CT) is called non-gonococcal non-chlamydial non-gonococcal urethritis (NCNGU) (Wada, et al., 2021). The main causes of NCNGU include *Mycoplasma genitalium* and *Trichomonas vaginalis* (TV), rarely other bacteria or viruses.

The epidemiology of urethritis is diverse and depends on the population of interest. For example, Rossignol *et al.* (2019) estimated incidence rates for adult male urethritis (men older than 15 years) reported by general practitioners in France between 2007 and 2017. The overall trend in estimated incidence rates for urethritis remained stable during this period, from 226 (95% confidence interval, CI [172–280]) in 2007 to 196 (95% CI [165–227]) per 100,000 men in 2017 (p-value = 0.9). In this study the authors also evaluated the age-specific burden of illness by using the relative illness ratio. The latter was highest for the age groups 20–29 years and 30–39 years (Rossignol, et al., 2019). The prevalence of urethritis is also dependent on laboratory methods and the definition of urethritis *per se* used in diagnostics (Rietmeijer & Mettenbrink, 2017; Wasef, et al., 2005). For example, the prevalence of urethritis among urologic patients was estimated at 26% detecting leukocytes by the Gram-stained urethral smear and 18% using first-void urine (Krieger, et al., 2000). The performance of light microscopy evaluating urethral smear slides can have considerable intraobserver variation in diagnosing urethritis (Smith, et al., 2003). In addition, the sensitivity of the urethral Gram stain is highly dependent upon collection technique (Bachmann, et al., 2015). Even in the STI clinics, the prevalence of urethritis is highly variable, comprising 27–94% (Gottesman, et al., 2017; Schwebke & Hook, 2003; Gaydos, et al., 2009; Högdahl & Kihlström, 2007; Bowden, 1998).

2.2. Etiopathogenetic aspects

2.2.1. *Neisseria gonorrhoeae*

Neisseria gonorrhoeae was described for the first time by Albert Neisser in Gram-stained microscopy of urethral discharge in 1879. *N. gonorrhoeae* is a diplococcal (composed of two joined bacterial cells), Gram-negative micro-organism; it belongs to the bacterial class *Betaproteobacteria* and the family *Neisseriaceae*. The pathogenesis and pathophysiology of *N. gonorrhoeae* have been studied for decades; however, detailed knowledge regarding many fundamental properties is lacking.

A characteristic cell envelope of bacteria consists of a cytoplasmic membrane (the inner membrane), a periplasmic space containing the peptidoglycan cell wall and the outer membrane containing lipo-oligosaccharide, which is an important virulence factor responsible for most clinical symptoms (Unemo, et al., 2019; Morse, 1978).

N. gonorrhoeae is a fastidious organism that is sensitive to many environmental factors such as oxygen, nonphysiological temperatures, desiccation and the presence of toxic substances (such as many fatty acids), among others; thus, the bacterium does not survive for long outside the human host, and is difficult to culture. Many strains have incomplete biosynthetic capabilities for amino acids, presumably because amino acids and other important nutrients are readily obtained from the human host. Iron (which is essential for bacterial growth) is acquired from the host by binding iron-containing host proteins (Unemo, et al., 2019; Morse, 1979; Rohde & Dyer, 2003).

Many gonococcal strains contain a plasmid encoding a penicillinase (mostly TEM-1 or TEM-135 β-lactamase), which results in high-level penicillin resistance, and conjugative plasmids, which sometimes carry *tetM* causing high-level tetracycline resistance (Unemo, et al., 2019; Unemo & Shafer, 2011, 2014).

The colonisation determinants of *N. gonorrhoeae* include: the type IV pilus, the opacity protein family (Opa proteins), the porin PorB, efflux pumps and metal transport systems (Unemo, et al., 2019).

N. gonorrhoeae infects the mucosal epithelium of the male and female urogenital tracts, the rectum, pharynx or conjunctiva. The main forms of transmission are unprotected vaginal, anal or oral intercourse. *N. gonorrhoeae* infection amplifies the risk for acquisition and transmission of HIV and several other STIs. Women with *N. gonorrhoeae* infection can effectively transmit the infection to their children during birth, which may result in *ophthalmia neonatorum* (Unemo, et al., 2019, 2020; Cornelisse, et al., 2019; Cohen, et al., 1997).

N. gonorrhoeae can survive the various antimicrobial functions of polymorphonuclear leukocytes (PMNLs) including phagocytosis, the release of reactive oxygen species, cationic peptides and antimicrobial enzymes, metal sequestration and PMNL extracellular traps (Criss & Seifert, 2012; Unemo, et al., 2019). *N. gonorrhoeae* can also modulate the apoptosis of epithelial cells, macrophages, T cells and PMNLs. *N. gonorrhoeae* uses several mechanisms to limit complement-mediated killing by blocking deposition or activity of several complement factors (Massari, et al., 2003). People with complement deficiencies are at an increased risk of disseminated gonococcal infection (Densen, 1989). Antigenically variable surface antigens (type IV pilus, Opa proteins and lipo-oligosaccharide) ensure immune avoidance, which enables reinfection. *N. gonorrhoeae* does not produce any exotoxins that can destroy host cells, but has peptidoglycan fragments, outer membrane vesicles and lipo-oligosaccharide that are toxic to mammalian cells and can specifically inhibit the ciliated cells on fallopian tube tissues (Melly, et al., 1984). Moreover, PMNL antimicrobial products are released, which can also damage human tissue, particularly during disseminated gonococcal infections in which, in addition to fever, dermatitis,

infectious arthritis and (less frequently) septicaemia, endocarditis and meningitis can occur (Unemo, et al., 2019).

In men, acute urethritis is predominant with symptoms of urethral discharge (>80%) and dysuria (>50%), usually starting within 2–8 days of exposure. Mucopurulent urethral discharge is the most common symptom, which may be accompanied by erythema of the urethral meatus. Asymptomatic urethral infection in men is rare (<10% of infections). Rectal and oropharyngeal infections are usually asymptomatic. Rare symptoms include anal discharge and perianal/anal pain or discomfort and sore throat (Unemo, et al., 2020).

The epidemiological diversity of gonorrhoea manifests itself in the variability of the geographical distribution and the prevalence among certain populations; determinants of such variability include sexuality and sexual orientation, socioeconomics, demographics, geographical and cultural ramifications (including stigma and taboos), and access to and quality of sex education, prevention, testing and diagnostics, as well as political commitment in the provision of health services (Unemo, et al., 2019). In the European Union/European Economic Area, the number of reported gonorrhoea cases has increased from 29,434 cases in 2008 (with an incidence of 7.85 per 100,000 population) to 89,239 cases in 2017, with the highest numbers of cases in the UK, France, the Netherlands and Spain. The higher prevalence in these countries might be accounted for in part by the availability of comprehensive sexual health systems, frequent testing and/or surveillance. The highest incidence of gonorrhoea in the European Union/European Economic Area is in young adults (15–24 years of age) (Unemo, et al., 2019). The 2016 global prevalence estimates of *N. gonorrhoeae* in men aged 15–49 years was 0.7% (95% uncertainty interval, UI: 0.5–1.1) (Rowley, et al., 2019).

2.2.2. *Chlamydia trachomatis*

Chlamydia trachomatis is a small obligate intracellular Gram-negative bacterium surrounded by a rigid cell wall. The bacterium alternates between two developmental forms, the elementary body (EB) and the reticulate body (RB). EBs are small (300 nm), able to survive in the extracellular environment, and display little or no metabolic activity. It is the EB form of the organism that is infectious, and, upon encounter with host cells, EBs induce their own uptake into a vacuolar compartment termed an inclusion. Within 2 h, EBs differentiate into the metabolically active but non-infectious RB form. The RBs then replicate by binary fission within the expanding inclusion. After about 18 h, the RBs begin to differentiate back into EBs (Roan & Starnbach, 2008). This unique lifecycle takes part in living cells because of its inability to synthesise essential nutrients, thus strictly depending on host biosynthesis pathways (Mackern-Oberti, et al., 2013; Wyrick, 2000).

The *C. trachomatis* species is divided into serovars. Serovars A–C infect the conjunctival epithelium and can give rise to trachoma, the leading cause of preventable blindness. Serovars D–K are associated with urogenital tract infec-

tions (including male urethritis), termed chlamydial infection or simply chlamydia. Serovars L1–L3 also establish infection in the urogenital tract but can additionally spread into the draining lymph nodes to cause a relatively rare systemic disease called *lymphogranuloma venereum* (Roan & Starnbach, 2008).

Although the different *C. trachomatis* serovars exhibit different tissue tropisms, they all primarily infect epithelial cells, in which the organisms undergo a similar intracellular developmental cycle. The bacteria can persist in host cells in a viable, but culture-negative state. It has recently been revealed that CT has the capacity for rapid evolution by exchanging deoxyribonucleic acid (DNA) between strains at a much higher rate than predicted, which allows this pathogen to evade the immune response and chronically persist in a host (Mackern-Oberti, et al., 2013; Harris, et al., 2012).

Several bacterial virulence factors have been linked to EBs' attachment to epithelial cells, such as major outer membrane protein (MOMP), outer membrane complex protein B (OmcB) and polymorphic membrane protein subtype D (PmpD). Chlamydia can manipulate host trafficking to avoid degradation and to acquire sphingolipids and cholesterol (Mackern-Oberti, et al., 2017).

EBs are released after host cell lysis and can survive in the extracellular environment as infectious agents. During lysis, chlamydial components trigger the host immune response. Cells of the immune system migrate into this necrotic and ulcerous epithelium. Local inflammation can subsequently lead to fibrosis followed by scarring (Mackern-Oberti, et al., 2013). Factors that may influence whether the immune response to *C. trachomatis* is protective or pathological include the nature of the antigens presented during infection, the effector functions deployed by immune effectors, as well as immune evasion strategies implemented by the *Chlamydia* organisms (Roan & Starnbach, 2008).

The first isolation of CT from the genital tract was performed in the 1950s (Paavonen, 2012). Although this finding was reported several decades ago, until the 1990s most CT genital infections in both genders were underestimated because of their asymptomatic or mild clinical course (Mackern-Oberti, et al., 2013).

Transmission of *C. trachomatis* usually takes place by direct mucosal contact between two individuals during sexual intercourse (vaginal, anal or oral sex) or at birth through an infected cervical canal (Lanjouw, et al., 2016).

In men, CT is responsible for urethritis, epididymitis, epididymo-orchitis, and it is becoming increasingly accepted as a causative agent of prostatitis. In addition, CT infection has been reported to be a major cause of reactive arthritis (Mackern-Oberti, et al., 2013; Carter & Hudson, 2010). *C. trachomatis* infection of the rectum is typically asymptomatic; however, the infection may cause anal discharge and anorectal discomfort and also progress to proctocolitis (Boisvert, et al., 1999; Quinn, et al., 1981). Pharyngeal chlamydial infection is also usually asymptomatic, but symptoms of a mild sore throat can occur (van Rooijen, et al., 2015). Ocular infection can result in conjunctivitis in neonates and adults, and can lead to chronic conjunctivitis and persist for several months if left untreated (Hu, et al., 2010). Infants born to mothers through an infected

birth canal may become colonised and develop conjunctivitis and/or pneumonia (Lanjouw, et al., 2016; Darville, 2005).

At the same time, up to 75% of CT infections in women and up to 50% of those in men can be asymptomatic (Detels, et al., 2011; Coble, et al., 2006; Carne, et al., 2013). Serious difficulties are involved in determining the duration of infection in humans, because the onset of infection is generally unknown, re-exposure is common, and clearance is rarely followed up (Cunningham & Beagley, 2008; Mackern-Oberti, et al., 2013). The factors that determine whether infection develops as symptomatic or asymptomatic are unknown. However, a high prevalence of serotype E and its lack of associated clinical symptoms may suggest that this serotype might be more successful in maintaining a sub-clinical infection than other, less prevalent serotypes (Mackern-Oberti, et al., 2013). No study has consistently followed up infection in men for greater than 4 weeks; thus, the frequency of prolonged infections is unknown. For comparison, most evidence in women suggests that infection persists for more than 60 days and even up to years in the upper female reproductive tract, and it has been suggested that a CT infection can maintain itself for up to 4 years within a couple. This may have implications for fertility (Cunningham & Beagley, 2008).

The current high incidence of the *Chlamydia trachomatis* genital infection is the result of its chronic nature and the absent or mild symptoms, which lead to an undiagnosed disease. Although the true prevalence of genital CT infection is unknown, it has been shown to vary between 1% and 40%, depending on the population (Mackern-Oberti, et al., 2013). The 2016 global prevalence estimates in men aged 15–49 years for chlamydia were 2.7% (95% uncertainty interval, UI: 1.9–3.7) (Rowley, et al., 2019). Although the prevalence of chlamydial infection is similar in men and women, current research and screening are still focused on women, who develop the most severe complications, leaving the study of male genital tract infection underrated (Chen & Donovan, 2003).

2.2.3. *Mycoplasma genitalium*

Mycoplasma genitalium is a small slowly growing facultatively intracellular bacterium lacking a cell wall around its cell membrane. It has the smallest genome (580 kb) for a self-replicating organism. It is frequently bottle shaped with a terminal rod-like structure (the terminal organelle). *M. genitalium* was first isolated and described in 1980 from urethral specimens from 2 of 13 men with non-gonococcal urethritis (Tully, et al., 1981; Taylor-Robinson & Jensen, 2011).

M. genitalium causes inflammation in the urogenital tract by adhesion to host epithelial cells eliciting acute inflammatory signals via highly expressed innate immune sensors. It results in activation of pro-inflammatory signals including potent chemokines, ultimately resulting in leucocyte recruitment to the site of infection. Multiple factors to aid pathogenesis include the ability for adhesion, gliding motility and cell invasion. It has been postulated that antigenic

variation may occur. Factors leading to immune evasion are unclear and the role of antibodies and cell-mediated immunity also requires further study (Gnanadurai & Fifer, 2020).

The terminal organelle in MG is a polar structure that has a pivotal role in the virulence of mycoplasmas. It assists the mycoplasma in adhesion and further invasion of the host cells, plays a relevant function in cell division and contains the molecular motor that propels and directs the cell movement when gliding on solid surfaces (García-Morales, et al., 2016). This gliding motility could be critical for host invasion (García-Morales, et al., 2016). MG can adhere by terminal organelle to glass and plastic surfaces and to various human and animal cells (epithelial cells, spermatozoa and erythrocytes), the proteins responsible for adherence are MgPa and P110 in collaboration with accessory proteins (Taylor-Robinson & Jensen, 2011).

In electron microscopic observations, *M. genitalium* becomes intracellular in about 10% of Vero cells infected *in vitro*. The intracellular location could protect the mycoplasmas from the effects of the host immune system and antibiotics (Taylor-Robinson & Jensen, 2011; Sethi, et al., 2012).

M. genitalium has been detected in human urogenital, respiratory and rectal specimens. The urogenital tract is the preferred site of colonisation of MG in humans (Taylor-Robinson & Jensen, 2011). Transmission of MG occurs by direct genital-genital mucosal contact, by genital-anorectal contact and less often by oral-genital contact. MG infection can facilitate HIV transmission (Jensen, et al., 2016).

M. genitalium is associated with acute and chronic urethritis in men (Taylor-Robinson & Jensen, 2011; Manhart, et al., 2011). At the same time, nearly 70% of MG infections in men are symptomatic in STI clinic settings. In women, 40–75% of *M. genitalium* infections are asymptomatic among STI clinic attendees, while in the general population less than 5% of those infected report symptoms (Jensen, et al., 2022).

There are no estimates of the global burden of *M. genitalium* infection. In sexually transmitted infection clinic patients, the prevalence of MG is usually lower than that of chlamydia (ranges from 75% to 90% of that of CT), but in some settings, it is even higher than chlamydia. In the general population, *M. genitalium* is detected in 1% to 3.3% of men and women (Jensen, et al., 2022). *M. genitalium* can be responsible for 15–25% of acute NGU cases (Cazanave, et al., 2012; Taylor-Robinson & Jensen, 2011; Manhart, et al., 2011). In a study by Sonnenberg *et al.*, there were no positive *M. genitalium* tests in British men aged 16–19, and prevalence peaked at 2.1% in men aged 25–34 years (Sonnenberg, et al., 2015).

2.2.4. *Trichomonas vaginalis*

Trichomonas vaginalis is a protozoa with size 10–20 μm long and 2–14 μm wide. Four flagella project from the anterior portion of the cell and one flagellum extends backwards to the middle of the organism, forming an undulating

membrane. An axostyle extends from the posterior aspect of the organism (Kissinger, 2015). Trichomonadida thrive in anaerobic habitats (Kusdian & Gould, 2014). *T. vaginalis* was first described by a European physician, M. A. Donné, in 1836 (Kampmeier, 1978; Fichorova, 2009).

Trichomonas vaginalis exists as a trophozoite without the true cystic stages (Mielczarek & Blaszkowska, 2016). Free-swimming *Trichomonas* cells are pyriform. During infection the virulent strains go through morphogenesis and become amoeboid within minutes when exposed to host tissue (Kusdian & Gould, 2014). Binary fission is the preferred mode of division in TV, but the parasite is also observed to form multinucleated forms that can actively migrate on host cells, and from which individual cells can bud off (Kusdian & Gould, 2014). TV reproduces every 8–12 h (Mielczarek & Blaszkowska, 2016).

Outside the host, the parasite can survive for 6–24 h in urine, semen and swimming pool water (Pereira-Neves & Benchimol, 2008), but only up to 30 min when exposed to air. TV can grow over a wide range of pH values with an optimum level between pH 6 and 6.3 (Petrin, et al., 1998; Mielczarek & Blaszkowska, 2016).

TV mainly affects the urogenital tract of both men and women, where the parasite invades the squamous epithelium (Kusdian & Gould, 2014). The cytopathogenic action of TV may be divided into four stages: adhesion, cytolysis following contact, phagocytosis and intracellular digestion. The parasite is able to efficiently phagocytize and degrade lactobacilli, yeast cells, vaginal and cervical epithelial cells, leukocytes, erythrocytes, prostatic cells and spermatozoids (Mielczarek & Blaszkowska, 2016; Midlej & Benchimol, 2010; Benchimol, et al., 2008; Pereira-Neves & Benchimol, 2007). TV phagocytosis is thought to be both an efficient means of obtaining nutrients for the parasite and an important factor in the pathogenesis of trichomonal infections (Mielczarek & Blaszkowska, 2016). In addition, TV is apparently able to internalise viable viruses such as HIV or herpes simplex virus (HSV), which is why it has been suggested that the parasite itself might act as a vector for these pathogens. Moreover, viruses are known to influence the gene expression of the parasite and could have an impact on TV's virulence (Kusdian & Gould, 2014; Mielczarek & Blaszkowska, 2016). Studies show an association between TV and vaginitis, cervicitis, urethritis, bacterial vaginosis, candidiasis, herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2), CT infection, gonorrhoea, and syphilis (Kissinger, 2015). TV can evade the immune system by molecular mimicry, evasion of complement-mediated destruction, and the ability to coat itself with host plasma proteins (Mielczarek & Blaszkowska, 2016; Ryan et al., 2011; Figueroa-Angulo et al., 2012; Ibáñez-Escribano et al., 2015).

T. vaginalis is commonly spread through sexual contact with the vaginal or urethral discharges of infected persons (Petrin, et al., 1998). Transmission of protozoa is also possible via artificial insemination of infected cryobanked semen (Sherman, et al., 1991; Habib, et al., 2004). The occurrence of this protozoan in the respiratory tract has been linked to orogenital sexual activity (Rebhun, 1964). There are documented cases of vaginal, urinary, nasal and

respiratory tract TV infections in neonates who acquired the infection vertically from the maternal genitourinary tract. Non-sexual transmission is rare, but has been observed in cases involving freshly contaminated showers, moist towels, toilet seats, or specula (Mielczarek & Blaszkowska, 2016; Charles, 1991; Adu-Sarkodie, 1991; Peterson & Drame, 2010; Crucitti, et al., 2011).

The majority of women (85%) and men (77%) with TV are asymptomatic (Kissinger, 2015). Symptomatic men experience symptoms of urethritis. An incubation period for men with TV urethritis is 3–9 days (Poole & McClelland, 2013). Infection may occur in other areas of the urogenital system causing epididymitis, prostatitis, and impaired sperm function (Kissinger, 2015; Van Gerwen, et al., 2021; Mielczarek & Blaszkowska, 2016). Although TV has been identified in urethral discharge, urine, semen and prostatic fluid, its presence in these fluids does not confirm infection of either the prostate or seminal vesicles, since fluid can be contaminated when it passes through the urethra (Mielczarek & Blaszkowska, 2016).

Symptomatic TV infection in men is typically cleared spontaneously within 10 days. By contrast, symptomatic TV infection in women can persist for years (Poole & McClelland, 2013). Gender differences in TV persistence could be explained by the following factors: 1) the zinc-rich environment of the prostate inhibits persistent infection; 2) it is possible that urination helps to clear TV parasites from the male genital tract; 3) the absence of oestrogen and the iron-depleted environment of the male genital tract (Poole & McClelland, 2013). All these circumstances may make men poor long-term TV reservoirs.

TV infection is not a reportable infection. Recent estimates of *T. vaginalis* prevalence for men range from 6% of asymptomatic men to 20% of men with urethritis in Africa and from 4% to 17% of men attending US STI clinics. The higher prevalence among symptomatic men may be related to lower detection among asymptomatic men due to lack of screening recommendations (Van Gerwen, et al., 2021). The 2016 global prevalence estimates in men aged 15–49 years for trichomoniasis were 0.6% (95% uncertainty interval, UI: 0.4–0.9) (Rowley, et al., 2019). In sharp contrast with other curable STIs including *C. trachomatis* and *N. gonorrhoeae*, significantly higher rates of TV are found in older men and women compared with adolescents and younger adults (Poole & McClelland, 2013).

2.2.5. Other causative agents of urethritis

Ureaplasma

Ureaplasmas were first isolated from male NGU patients in 1954, and due to the tiny colony size upon agar plates, these bacteria were originally referred to as “T-strain” or “tiny” mycoplasmas. In 2002 a single species of human-associated ureaplasmas, *Ureaplasma urealyticum*, was subdivided into two separate human-associated species – *Ureaplasma parvum* (serovars 1, 3, 6 and 14) and *Ureaplasma urealyticum* (serovars 2, 4, 5, and 7 to 13) (Beeton, et al., 2019).

Similarly to mycoplasmas, ureaplasmas lack a rigid cell wall, and are small enough to pass through bacterial-retaining filters. As mycoplasmas evolved from Gram-positive bacteria that underwent significant genome reduction, members of this genus can exhibit host and tissue specificities and have limited metabolic options for replication and survival, forcing them to adapt to procure metabolic precursors from the host (Yiwen, et al., 2021).

There is some evidence that ureaplasmas exist as quasi-species rather than as stable serovars in their native environment. Therefore, differential pathogenicity and clinical outcome of a ureaplasmal infection is most likely not on the serovar level, but may rather be due to the presence or absence of potential pathogenicity factors in an individual ureaplasma clinical isolate and/or patient-to-patient differences in terms of autoimmunity and microbiome (Paralanov, et al., 2012).

The controversy about the association of ureaplasmas with male urethritis is still unresolved. Some studies find an association between *U. parvum* and/or *U. urealyticum* and urethritis (Deguchi, et al., 2004; Yoshida, et al., 2005; Couldwell, et al., 2010; Ondondo, et al., 2010; Kawaguchi, et al., 2012; Seike, et al., 2013; Shimada, et al., 2014; Deguchi, et al., 2015; Cox, et al., 2016; Frølund, et al., 2016), while others do not (Bradshaw, et al., 2006; McKechnie, et al., 2009; Carne, et al., 2013; Ito, et al., 2014, b; Khosropour, et al., 2015; Moi, et al., 2017, b; Strauss, et al., 2018; Frølund, et al., 2019; Jordan, et al., 2020). A meta-analysis conducted by Zhang *et al.* (2014) found that the *U. urealyticum* positive rate was significantly higher in NGU patients compared to controls; and also the *U. parvum* positive rate was significantly higher in controls compared to NGU patients.

It has been suggested that bacterial load may be important in the development of symptoms. This helps to explain why *U. urealyticum* in high bacterial loads might cause a small proportion of male NGU, but the majority of men infected/colonised with *U. urealyticum* do not develop the disease (Yoshida, et al., 2007; Shimada, et al., 2014; Frølund, et al., 2016; Horner, et al., 2018). At the same time, some other studies did not find any differences in *U. urealyticum* bacterial load between controls and cases (Cox, et al., 2016; Frølund, et al., 2019). Implication of bacterial load in case of NGU was also confirmed for *U. parvum* in one study (Deguchi, et al., 2015), while not in others (Frølund, et al., 2016; Cox, et al., 2016; Frølund, et al., 2019).

The population prevalence of *U. parvum* and *U. urealyticum* in men is largely unknown (Horner, et al., 2018). *U. urealyticum* can be detected in 7–33% of urine specimens from symptomatic urethritis patients using nucleic acid amplification tests (NAATs) (Wada, et al., 2021). The prevalence of *U. parvum* in STI clinic settings varies between 6% and 14% (Shigehara, et al., 2011; Cox, et al., 2016; Moi, et al., 2017, b; de Souza, et al., 2021).

Adenovirus, Herpes simplex virus and Epstein-Barr virus are sometimes implicated in the aetiology of urethritis (Bachmann, et al., 2015; Perkins & Decker, 2016; Sarier & Kukul, 2019).

Herpes simplex virus

Herpetic genital lesions were already described in the third millennium BC. HSV-1 and HSV-2 belong to the *Herpesviridae* family, *Alphaherpesvirinae* subfamily, genus *Simplexvirus*. HSV is an enveloped virus with a linear double-stranded DNA genome (Rechenchoski, et al., 2017). HSV can be transmitted through contact with herpetic lesions, mucosal surfaces, genital secretions, or oral secretions. The virus can be shed in the absence of lesions (Cole, 2020). Generally, HSV-1 infections are mostly related to the oropharynx and the virus is transmitted by respiratory droplets or saliva, and most often by kissing. The main transmission of HSV-2 is through sexual activity (Rechenchoski, et al., 2017).

Genital herpes in men can manifest as ulcerative vesicles in the penis or perineum, or urethritis (Bachmann, et al., 2015; Rechenchoski, et al., 2017). Other manifestations include infections in the oropharyngeal region, keratoconjunctivitis, paronychia/whitlow (fingers), glossitis, *herpes gladiatorum* (body), and multiform erythema. Primary infection can be complicated by urinary retention, and encephalitis which is associated with a high mortality rate and neurologic sequelae among survivors (Haanpää & Paavonen, 2004; Patel, et al., 2017; Rechenchoski, et al., 2017). Patients compromised by malnutrition or immunosuppression are at an increased risk of severe HSV infections involving the respiratory tract, oesophagus, and intestinal mucosa (Rechenchoski, et al., 2017).

Asymptomatic HSV shedding can occur in the male urethra (Deardourff, et al., 1974; Strand, et al., 1986). However, while symptomatic, HSV infection can be atypical and difficult to recognise (Uusküla & Raukas, 2004). Urethritis is commonly seen (15–30%) in patients with primary HSV and less common in recurrent HSV (Bachmann, et al., 2015). HSV-1 is responsible for approximately two-thirds of HSV urethritis while HSV-2 is only responsible for one-third of cases (Bradshaw, et al., 2006; Ito, et al., 2016; Ong, et al., 2017). In male patients with HSV-positive acute urethritis the herpetic lesions are not found in up to 73.7% of patients. Urethra should spontaneously cease in most cases of HSV-positive NGU without antiviral therapy, and urethritis symptoms are alleviated. The development of meatitis and the mononuclear cell response in the urethral smear could be helpful to diagnose HSV-induced NGU (Bradshaw, et al., 2006; Ito, et al., 2016, 2017). Severe dysuria, genital ulceration (penile shaft, meatus, *glans penis* or foreskin), inguinal lymphadenopathy, and constitutional symptoms (fever, myalgia, and/or fatigue) can accompany HSV urethritis (Lautenschlager & Eichmann, 2002; Bradshaw, et al., 2006; Ito, et al., 2016; Ong, et al., 2017).

In STI clinics HSV prevalence varies between 4% and 21.6% of men with acute NGU (Madeb, et al., 2000; Malathi, et al., 2002; Srugo, et al., 2003; Bradshaw, et al., 2006; Frølund, et al., 2016; Ito, et al., 2016; Kim, et al., 2017; de Souza, et al., 2021). In a study by Sturm *et al.* (2004) evaluating the aetiology of urethritis in the area with a high HIV prevalence, HSV was found in 6% of males. Cases with HSV in men are associated with insertive oral sex and male partners (Bradshaw, et al., 2006; Ito, et al., 2016).

Adenovirus

Adenoviruses belong to the genus *Mastadenovirus* of the family *Adenoviridae* (Hiroi, et al., 2020). Adenovirus is a nonenveloped double-stranded DNA virus first isolated by Rowe *et al.* in 1953 from surgical specimens of adenoid tissue. More than 100 serotypes in seven subgroups, A to G, have been identified and these can cause different clinical diseases (respiratory tract infection, kerato-conjunctivitis, cystitis, hepatitis, gastroenteritis) (Liddle, et al., 2015; Hanaoka, et al., 2020). Adenovirus subgroup D types 8, 9, 19, 37, 56, 64 are the most common in the genital tract; however, type 4, 35, 49, 85, subgroup B2 and B7d adenovirus infections are also implicated in urethritis cases (Tabrizi, et al., 2007; Liddle, et al., 2015; Hanaoka, et al., 2019, 2020; Hiroi, et al., 2020).

Uncertainty exists over how adenovirus is transmitted to the urethra. The transmission to the urethra could result from direct inoculation via oral, vaginal or anal sex from a sexual partner. Alternatively, urethral adenovirus might reflect inoculation elsewhere: as a local manifestation of systemic adenoviral infection or via autoinoculation from a remote site such as the conjunctiva (Liddle, et al., 2015; Samaraweera, et al., 2016). A recent study showed that adenovirus infections can be transferred from the urethra to the eyes (Hanaoka, et al., 2019).

The mean incubation period for adenoviral urethritis is 10 days (range 1–21 days) (Hanaoka, et al., 2019). The persistence of adenovirus in the genital tract is short-lived, a mean time of symptoms to resolve is *ca* 14 days (Harnett & Newnham, 1981; Liddle, et al., 2015), but in some cases symptoms may persist for more days (Bradshaw, et al., 2002; Liddle, et al., 2015; Hanaoka, et al., 2019, 2020). The infection is self-limiting (Liddle, et al., 2015). The virus can be isolated for up to 12 days after urethritis symptoms have disappeared (Hanaoka, et al., 2019). However, in another study viral DNA could be detected even after one month after the first sampling (Hiroi, et al., 2020).

Cases with adenovirus urethritis are significantly more likely to be present with meatitis and/or balanitis and moderate to severe dysuria (Bradshaw, et al., 2006; Ito, et al., 2016). In addition, some cases can present with conjunctivitis, constitutional symptoms (fatigue and malaise), pharyngitis, and inguinal adenopathy (Harnett & Newnham, 1981; Azariah & Reid, 2000; Bradshaw, et al., 2002; Bradshaw, et al., 2006; O'Mahony, 2006; Hiroi, et al., 2012; Tønsberg & Hartgill, 2014; Avolio, et al., 2014; Liddle, et al., 2015; Frølund, et al., 2016; Ito, et al., 2016; Hanaoka, et al., 2019). Adenovirus urethritis in men can be associated with insertive oral sex, insertive vaginal sex, and anal sex. Males

with adenovirus urethritis are more likely to have had male sex partners during the past month (Bradshaw, et al., 2002, 2006). However, Ito *et al.* (2016) did not find an association between adenovirus and insertive oral sex. The clinical manifestations of adenoviral urethritis between heterosexual men and men who have sex with men (MSM) does not differ (Samaraweera, et al., 2016). Some researchers have found that adenovirus infections are seasonally clustered (autumn, winter) (Bradshaw, et al., 2002, 2006; Liddle, et al., 2015; Samaraweera, et al., 2016), while others have not (Avolio, et al., 2014; Hanaoka, et al., 2019; Hiroi, et al., 2020).

In routine urethral smear microscopy, the elevated numbers of polymorphonuclear cells are not always present in adenovirus-positive men (Azariah & Reid, 2000; Bradshaw, et al., 2006; Samaraweera, et al., 2016). However, in some cases large numbers of mononuclear cells are noted (Azariah & Reid, 2000; Tønsberg & Hartgill, 2014; Hiroi, et al., 2020).

Harnett and Newnham reported isolation of adenovirus from 13 males with NGU in 1981 (Harnett & Newnham, 1981). Today, in STI clinics the adenovirus prevalence varies between 3% and 5.6% of men with acute NGU (Bradshaw, et al., 2006; Tabrizi, et al., 2007; Frølund, et al., 2016; Ito, et al., 2016; Hanaoka, et al., 2019).

Epstein-Barr virus

Epstein-Barr virus (EBV), also known as human herpesvirus 4 (HHV-4), is a member of the genus *Lymphocryptovirus* that belongs to the *Herpesviridae* family. EBV was first discovered in 1964 from Burkitt lymphoma. This virus is associated with different cancers such as Hodgkin lymphoma, NK/T cell lymphoma, Burkitt lymphoma, diffused large B cell lymphoma, HIV-associated lymphomas, epithelial cancers including nasopharyngeal carcinoma and a subset of gastric cancers. In addition, EBV is linked to non-malignant diseases such as infectious mononucleosis, oral hairy leukoplakia, systemic lupus erythematosus, and multiple sclerosis (Damania, et al., 2022).

Primary EBV infection is often asymptomatic and can occur at a young or later age. EBV transmission primarily occurs through saliva. However, breast milk, body fluids, and transplantation of EBV-positive organs can also spread the virus. EBV can persist life-long in the human host by infecting B cells and residing in memory B cells in healthy people where it is asymptomatic and does not cause disease. EBV-associated cancers can develop upon the influence of both intrinsic factors (e.g., genetic mutations and deficiencies) and extrinsic factors (e.g., immunosuppression, HIV infection, salted or preserved fish diet) (Damania, et al., 2022).

Before NAAT possibilities, attempts to demonstrate EBV in the male urethra were precluded by the insufficiency of urethral secretions and by toxicity of both semen and inflammatory penile discharge for cultured primary B lymphocytes used for the isolation of EBV. Israele *et al.* (1991) reported isolation of EBV DNA from urethral discharge in males with gonorrhoea in 1991. Later, Näher *et al.* (1992) isolated EBV in men without STI and signs of inflammation

from coronal sulcus and showed that EBV can shed subclinically in the male genital tract. Thomas *et al.* (2006) support the possibility that EBV could on occasion be transmitted sexually, however, the low levels detected in genital secretions compared to saliva suggest that this is not a major transmission route. The finding of small quantities of cell-associated virus from cervical and urethral samples suggests a latent infection; thus, EBV is probably in the B lymphocyte rather than in the epithelial cell component of the cervical and urethral secretions (Thomas, et al., 2006). A study by Berntsson *et al.* (2010) found an independent association between male urethritis and EBV. In addition, there is a case report on a primary non-Hodgkin's B-cell lymphoma of the urethra in a 78-year-old female where EBV genome was found in the tumour cell nuclei (Ohsawa, et al., 1994).

The epidemiological estimates for EBV urethritis are scarce. In one study, the prevalence of EBV among males with urethritis attending STI clinics was 21% (Berntsson, et al., 2010).

Miscellaneous pathogens

Haemophilus influenzae, *Haemophilus parainfluenzae*, and *Neisseria meningitidis* are, among other bacterial species, responsible for acute urethritis. Orogenital contact is considered to be the most important form of transmission. Data about *Candida* species, *Streptococcus* species, *Moraxella catarrhalis*, *Gardnerella vaginalis*, and *Mycoplasma hominis* are insufficient to draw a clear conclusion about their implication in male urethritis (Sarier & Kukul, 2019). There is some evidence that uncultured or fastidious organisms commonly found in bacterial vaginosis can also play a role in urethritis (Bachmann, et al., 2015).

Noninfectious causes of urethritis

Mechanical urethritis can occur after insertion of foreign bodies into the urethra. Foreign bodies are apparently inserted into the urethra as a form of attention seeking, curiosity, attempt to cure oneself of urinary symptoms, sexual stimulation, erotic games or to maintain/enhance erections (Péc, et al., 1992; Weber & Lamb, 2005; Boscolo-Berto, et al., 2010; Rahman, et al., 2010; Ratkal, et al., 2015; Palmer, et al., 2016). Self-insertion of foreign bodies into the urethra can also be as a result of psychiatric disturbance or alcohol intoxication (Forde, et al., 2009; Simms, et al., 2020). The patients can be reluctant to confess to such practices, which can underlie sexual or psychological problems in the patient or their sexual partners. Some individuals practise this kind of sexual stimulation repeatedly. Originally inserted into the urethra, during erections and subsequently slipped into the bladder, foreign bodies cannot be manually removed. Consequently, cystitis, cystopyelitis, cystopyelonephritis, injury of the urogenital tract and surrounding tissues (urethral false passage, urethral stricture, urethral laceration, rectal or periurethral abscess and/or fistula, calcification of

foreign bodies, and stone formation) can develop. The severe consequences of such a practice include septicaemia, Fournier's gangrene, and death (Péc, et al., 1992; Forde, et al., 2009; Brooks, et al., 2013; Prasad Ray, et al., 2015; Palmer, et al., 2016).

2.2.6. Complications of urethritis

N. gonorrhoeae

Ascending gonorrhoea can impair different parts of the male uro-genital tract, including the prostate and epididymo-testicular region. There is the risk of urethral strictures that are generally located in the bulbar urethra, probably because of the localisation of gonococci in the numerous urethral glands (synonyms: paraurethral glands, periurethral glands or Littré's glands[1]) (Singh & Blandy, 1976; Greenberg, 1979). A loss of urethral distensibility and a narrowing of the channel may occur as long as 10 to 20 years after the initial infection. Frequently, there is urinary retention. Ejaculatory duct stenosis may also result from infection or inflammation, causing obstructive azoospermia (Greenberg, 1979). Further ascent of infection to the upper genital tract can cause epididymo-orchitis and infertility.

A rare local complication of gonorrhoea, gonococcal tysonitis[2] can occur (Burgess, 1971; Fiumara, 1977; Subramanian, 1981; Abdul Gaffoor, 1986). In one study, its prevalence was *ca* 0.3% among men with gonococcal urethritis (GU) (Fan, et al., 2022). Gonococcal tysonitis lesions manifest as abscesses, nodules, and sinus-like lesions (Fan, et al., 2022).

Infection of the paraurethral duct by *N. gonorrhoeae* with consequent paraurethral duct dilatation is another rare localised complication in male patients with gonorrhoea (Fan, et al., 2014, 2016, 2019). Paraurethral infection by NG can be complicated by periurethral abscess formation which in turn can

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[1] Urethral glands (synonyms: paraurethral glands, periurethral glands, Littré's glands) are the male developmental equivalent of Skene's glands in women. Littré's glands are male accessory sexual tissue (Wein, et al., 2012). The ducts inside the paraurethral glands are small blind channels that run parallel to the terminal part of the urethra for varying distances. The paraurethral ducts in men are composed of squamous epithelium and surrounded by mucous glands, and appear to be rare embryological remnants (Hirsch, 1927; Singh & Blandy, 1976; Fan, et al., 2014; Puppo & Puppo, 2016). The proximal bulbar urethra has a rich distribution of paraurethral glands that extend deeply into the *corpus spongiosum* and are distributed circumferentially around the urethra. At the penoscrotal junction these glands became sparse and smaller in size. In the penile urethra glands are almost absent except for the short segment behind the meatus, where there is a small collection of mucous glands (Singh & Blandy, 1976). Littré's glands secret mucus that together with Cowper's gland secretions comprise 1–5% (0.1 to 0.2 ml) of total semen volume (Puppo & Puppo, 2016; Wein, et al., 2012).

[2] Gonococcal tysonitis – an inflammation of Tyson's glands. These glands are a pair of sebaceous hairless glands with columnar epithelium-lined ducts located on both sides of the preputial frenulum. Tyson's glands serve to produce smegma (Fan, et al., 2022).

result in urethral stricture, urethrocutaneous fistula, or urethral diverticulum (Campbell, 1931; Kenfak-Foguena, et al., 2010).

Gonococcal bacteraemia is rare, but can be more common in high-prevalent gonorrhoea areas and may be expected to increase when the gonorrhoea incidence increases. This is usually manifested as skin lesions, fever, arthralgia, acute arthritis, and tenosynovitis (disseminated gonococcal infection) (Unemo, et al., 2020). Endocarditis and meningitis are very rare nowadays (Sherrard, 2014).

C. trachomatis

Complications of the CT-caused urethritis in men include epididymitis, epididymo-orchitis, and sexually acquired reactive arthritis (SARA) (<1%) (Lanjouw, et al., 2016). The development of CT epididymitis is most predominant in younger men, with a number of studies demonstrating significantly higher rates in men under the age of 35 years (Cunningham & Beagley, 2008).

Chlamydial antigen has been detected in urethral or urine samples from 11% to 35% of men presenting with epididymo-orchitis (Cunningham & Beagley, 2008), and a causative link between CT infection and epididymo-orchitis in men is now accepted (Holmes, et al., 1979; Berger, et al., 1978, 1979, 1980, 1987; Scheibel, et al., 1983; Kiviat, et al., 1987; Melekos & Asbach, 1987, 1988; Ito Y., 1989; Doble, et al., 1989; Villegas, et al., 1991; Deguchi, et al., 1992; Hori & Tsutsumi, 1995; Molijn & Bogdanowicz, 1997). Decreased sperm counts and decreased motility are often demonstrated in cases of acute epididymo-orchitis of nonspecific aetiology, and this pathology is also consistently associated with high rates of infertility. Ascending urethral infection to the sites of spermatogenesis provides a plausible means by which CT can interact with and impair sperm function and, thus, affect fertility (Cunningham & Beagley, 2008).

A rare local complication of CT-urethritis is infection of the paraurethral glands (synonyms: urethral glands, periurethral glands or Littré's glands). There are several studies which discuss this issue (Fan, et al., 2013, 2014, 2016). Infection of the paraurethral glands may lead to periurethral abscess formation. If the abscess penetrates Buck's fascia, a necrotising fasciitis may occur, with extensive tissue destruction (Sanders & Mulder, 1998).

M. genitalium

In the case of *M. genitalium*-caused urethritis, the main possible complications are epididymitis and SARA (Jensen, et al., 2022). However, the clinical evidence for this is weak, although biologically plausible (Horner & Martin, 2017). Ocular infections can result in conjunctivitis in adults but it has not been systematically studied (Jensen, et al., 2022).

Evidence about the implication of *M. genitalium* in the pathogenesis of epididymitis is limited (Hamasuna, 2012). In some studies, MG is detected in

1.9–8.9% patients with acute epididymitis (Ito, et al., 2012; Eickhoff, et al., 1999). There is little evidence to suggest an association between MG and chronic prostatitis (Taylor-Robinson & Jensen, 2011; Horner & Martin, 2017) or prostate cancer (Tantengco, et al., 2021).

The first case reports about the isolation of MG from patients with arthritis were published in the 1990s (Tully & Baseman, 1991; Taylor-Robinson, et al., 1994; Tully, et al., 1995; Taylor-Robinson & Schaeverbeke, 1996). Later, in the study by Henry *et al.* MG was detected by polymerase chain reaction (PCR) in 35% of patients undergoing surgery for internal derangement of the temporomandibular joint (Henry, et al., 2000). However, in the last decade there were only single-case reports about this topic (Chrisment, et al., 2013; Simos & Stewart, 2022).

In a recent meta-analysis by Huang *et al.* an association of *M. genitalium* with male infertility was not supported (Huang, et al., 2015). However, they included only three original studies regarding this bacterium and, therefore, the analysis gave borderline significance value, despite a good odds ratio [3.27 (95% CI: 0.80–13.29)]. In addition, the following meta-analysis by Farahani *et al.* (2021) found that *M. genitalium* significantly decreases concentration of spermatozoa.

The natural history of *M. genitalium* infection in males is scarce and further studies in this field are required.

T. vaginalis

If left untreated in men, *T. vaginalis* can ascend and affect the prostate, leading to prostatitis (Van Gerwen, et al., 2021). An association between persistent TV infection and the development of prostate cancer is reported. It is unclear, however, whether this infection is related to a higher risk of advanced or fatal disease in patients who develop prostate cancer given the multifactorial nature of these studies. Other reported sequelae of persistent TV include balanoposthitis, epididymitis, and infertility (Van Gerwen, et al., 2021). In one study by Sivaraj *et al.* (2021) TV was implicated in 0.8% of epididymitis cases in sexual and reproductive health clinics (Sivaraj, et al., 2021).

2.3. Impact of urethritis on organs of male genital tract and reproductive parameters

2.3.1. Caveats applying in research of impact of urethritis on reproductive parameters

From the perspective of etiopathogenesis, the notion that there is a connection between male infertility and epididymitis/epididymo-orchitis resulting from STI-related urethritis seems plausible (Schuppe, et al., 2017). Fode *et al.* (2016) speculated that there may be an association between STIs and male infertility of unknown genesis and possibly with different pathogenic mechanisms for diffe-

rent pathogens. Alternatively, some STIs may cause male infertility, whereas others may not; however, there is hardly a strong correlation. Unfortunately, at present, for all relevant urethritis-associated STI pathogens, the studies are contradictory and generally of limited quality. High-quality studies on the subject are therefore needed (Fode, et al., 2016). It is also important to keep in mind that the impact of STIs on male fertility is strongly dependent on the local geographical prevalence of the STIs. Therefore, the practical relevance of these agents in the aetiology of male infertility will differ (Ochsendorf, 2008).

There is also a problem with the diverse contribution of the different accessory glands to the ejaculate as a whole in terms of volume and content. A changing biological factor is also the different time spans during which germ cells or sperm can interact with microorganisms in the seminal pathways. Second, cellular and humoral inflammatory components in the various parts of the seminal pathways need to be taken into account, and third, although the majority of inflammatory disorders within the male genital tract are of infectious origin, non-infectious causes of inflammation also have to be considered (Weidner, et al., 2013). Inconsistent diagnostic criteria have been applied to date, and this may explain the controversial debate regarding the role of infection and inflammation in the genital tract as a cause of infertility (Schuppe, et al., 2017).

There are also important epidemiological issues in studying STI and infertility. Some studies make comparisons within an infertile population (i.e. STI positives *vs.* STI negatives) that may yield results that are biased toward the null hypothesis because there may be many reasons for sperm aberrations among infertile men. Absence of an association between STI and sperm characteristics does not exclude a link between infection and infertility. Instead, it excludes the tested parameters as intermediate markers of an effect. The retrospective nature of the evidence associating STI with infertility does not allow for any assessment of temporality (Ness, et al., 1997).

There are also methodological considerations. For example, the male infertility definition as it exists in the context of couple infertility. Second, there is the absence of specific intermediate markers of infection-associated male infertility (with the exception of urethral inflammation with ongoing infection). Third, definitions of infertility must incorporate time (i.e. the amount of time spent trying to conceive). Finally, male factor infertility has a variety of aetiologies that sometimes overlap, which can dilute any relation with STI (Ness, et al., 1997).

Issues regarding measuring exposure include sensitivity and specificity of diagnostic tests for STIs. Improved detection of asymptomatic *C. trachomatis* with the more sensitive PCR and ligase chain reaction assays significantly increased the reported prevalence of chlamydial infections among males (Ness, et al., 1997). Increased sensitivity of the detection methods may have an impact on the association between asymptomatic infection and infertility (Ness, et al., 1997). Another problem is that different diagnostic tests capture different windows of time. The effects of acute infection (as measured by culture, PCR/

ligase chain reaction, or immunoassay) on acute fertility potential may differ from the effects of past exposure (as measured by serologies) on chronic fertility potential (Ness, et al., 1997). The issue of technology modifications can also distort the study of male infertility. For example, earlier studies dealing with *C. trachomatis* antibodies did not differentiate between species-specific antibodies, which also include antibodies against *C. pneumoniae* and genus-specific antibodies (Krause & Bohring, 2003). Another issue is refining the classification of pathogens. In 2002 a single species of human-associated *Ureaplasma*, the *Ureaplasma urealyticum*, was subdivided into two separate human-associated species – *U. parvum* and *U. urealyticum*. Studies prior to 2002 solely reported results as *U. urealyticum* and therefore may have over-represented this species among clinical samples from both cases and control groups (Beeton, et al., 2019).

Confounding can also seriously complicate exploration of STI and male infertility. A confounder is a factor related to both the exposure and the outcome of interest. A confounder results in a distortion of the effect of the exposure because the exposure is mixed with the effect of the confounding factor. In the context of STIs and infertility, examples of confounders include race, socioeconomic status, and age at initiation of conception attempts (Ness, et al., 1997).

2.3.2. Impact on epididymis and testis

The recent systematic review on the long-term effects of STIs on male reproductive functions concluded that only limited new knowledge on the long-term effects on male reproductive functions has been added. The existing knowledge that ascending infections can cause epididymo-orchitis or prostatitis was confirmed. Due to epithelial inflammatory responses these infections can result in scarring with resulting infertility due to obstruction (Henkel, 2021). Obstructions of the *ductus epididymidis* or *ductus deferens* are more frequent than obstructions of the ejaculatory ducts (Schuppe, et al., 2017). Although acute epididymitis is typically unilateral, disturbed spermatogenesis and a decreased number of germ cells can be observed bilaterally in testicular biopsies. The pathomechanisms of the contralateral testicular impairment are unclear, but the implication of a T cell-mediated autoimmune response is possible (Rusz, et al., 2012). The involvement of *Chlamydia trachomatis,* and *Neisseria gonorrhoeae* in the aetiology of epididymitis/orchitis is described (Henkel, 2021; Chen, et al., 2017; Pilatz, et al., 2015; Rusz, et al., 2012; De Jong, et al., 1988; Mulcahy, et al., 1987; Hawkins, et al., 1986; Kristensen & Scheibel, 1984; Greenberg, 1979; Harnisch, et al., 1977; King Wade, 1927; Blahd, 1918), with proof of the detection of chlamydia (Holmes, et al., 1979; Berger, et al., 1978, 1979, 1980, 1987; Scheibel, et al., 1983; Kiviat, et al., 1987; Melekos & Asbach, 1987, 1988; Ito Y. , 1989; Doble, et al., 1989; Villegas, et al., 1991; Deguchi, et al., 1992; Hori & Tsutsumi, 1995; Molijn & Bogdanowicz, 1997) and gonorrhoea (Furness, et al., 1971; Campbell, 1927; Ingram, 1926) from epididymis.

The mechanisms by which STI organisms cause testicular inflammation and impair spermatogenesis are limited, and many assumptions are based on the responses in the female genital tract (Bryan, et al., 2020). There is an observation derived from animal models that chlamydia can infect the macrophages at the site of initial infection (penile urethra) and when entering the bloodstream infected cells can reach and enter the testes. In the testicular tissue chlamydia is able to infect the testicular macrophages, Sertoli cells, Leydig cells and spermatogonial stem cells, having downstream effects on the testicular environment and fertility at different points (Bryan, et al., 2020). However, the data from human studies are rather tentative. In the study by Bryan *et al.* chlamydia was found in 16.7% of fresh testicular biopsies and 45.3% of fixed testicular biopsies taken from a selection of infertile men (Bryan, et al., 2019). Villegas *et al.* (1991) also detected chlamydia in epididymal and testicular tissue by using electron microscopy. However, Sripada *et al.* (2010) did not detect the presence of *Chlamydia trachomatis*-specific DNA by PCR in the epididymis or testis of 14 asymptomatic men with obstructive azoospermia and 22 azoospermic men seeking vasectomy reversal. The authors' hypothesis that unrecognised, asymptomatic chlamydial infection will lead to complete bilateral obstruction of the male genital tract remained unproven. In the prospective study of 71 patients with azoospermia by Pilatz *et al.* (2019) there was only one patient with *C. trachomatis* detected in a two-glass test and semen, whereas the pathogen was not detected in testicular swab and testicular tissue. There were no cases with *M. genitalium* or *N. gonorrhoeae* detected either in a two-glass test, semen, testicular swab or testicular tissue in this study (Pilatz, et al., 2019).

The evidence of the implication of *M. genitalium* (Chirwa, et al., 2021; Hamasuna, 2012; Ito, et al., 2012; Eickhoff, et al., 1999) and *T. vaginalis* (Amar, 1967; Fisher & Morton, 1969; Weidner, et al., 1987; Sivaraj, et al., 2021; Banyra, et al., 2019; Bonner, et al., 2021) in epididymitis/orchitis is limited (Jírovec & Petrů, 1968). There are few case reports that directly assessed the presence of *T. vaginalis* in testicular (Lloyd, et al., 2003; Gong, et al., 2018) or epididymal (Janssenswillen, et al., 1997; Morgentaler, 1998) tissues. To the best of our knowledge, there are no studies directly indicating the presence of *M. genitalium* in epididymo-testicular tissues.

Evidence supporting the role of *U. urealyticum* in epididymo-orchitis is somewhat scarce (Chirwa, et al., 2021; Pilatz, et al., 2019; Ito, et al., 2012). Information about the role of *U. parvum* is even scarcer (Ito, et al., 2012). One case report implicated *U. parvum* in orchitis in a man with lymphoma (Korytny, et al., 2017). Studies conducted before the distinction of *U. parvum* and *U. urealyticum* species also did not support their role in epididymitis as no ureaplasmas could be isolated from epididymal tissues (Ito Y. , 1989; Weidner, et al., 1987; Melekos & Asbach, 1987; Berger, et al., 1978, 1979; Holmes, et al., 1979), while some other authors succeeded in the isolation of ureaplasmas from epididymal tissue. However, false-positive results are possible in the case of broth cultures that were used in the past (Jalil, et al., 1988; Doble, et al., 1989).

2.3.3. Impact on seminal vesicles

There is also some proof of retrograde canalicular ascension of STI from ultrasound studies. In a study by Ghaly *et al.* (1994) 61.5% of patients with urethritis had abnormal prostate findings during transrectal ultrasound study (TRUS) scan, whereas this was only 31.3% in the control group. Almost all the patients with chlamydial or non-specific urethritis showed abnormality on a TRUS. No abnormalities were seen in the patients presenting with gonococcal urethritis (Ghaly, et al., 1994). A study by Krishnan and Heal (1991) has shown that the ipsilateral seminal vesicle was significantly larger than its mate in 72% to 80% of cases of unilateral acute epididymitis using TRUS. A total of 92% of these enlarged vesicles returned to normal size in 12 weeks. The authors postulated that this swelling in seminal vesicles is due to an inflammatory condition similar to that in the ipsilateral epididymis and that this condition is present early in the onset of epididymitis (Krishnan & Heal, 1991).

Furuya *et al.* (2004) also found cytologically inflammatory responses in the seminal vesicles of patients with acute epididymitis by TRUS-guided puncture of seminal vesicles. *Chlamydia trachomatis* was the causative pathogen most frequently detected in seminal vesicle fluid with results positive for the microorganism on first-voided urine. In addition, the frequency of inflammatory findings was significantly higher in fluid from the ipsilateral seminal vesicle than from the contralateral one (100% *vs.* 40%) when the puncture was successfully accomplished. The authors also found dilatation of the seminal vesicle on the side ipsilateral to epididymitis in 92.3% of patients on TRUS examination. After treatment seminal vesicle size on the ipsilateral side was markedly reduced. The authors speculated that the microorganism induced urethral infection that caused seminal vesiculitis to develop, followed by epididymitis in some patients (Furuya, et al., 2004). As with the study by Krishnan and Heal (1991), the authors could not determine whether seminal vesiculitis is the cause or the result of epididymitis. Later, Furuya *et al.* reported a case where seminal vesiculitis preceded acute epididymitis caused by *C. trachomatis*, supporting the idea of the retrograde route of microbial transmission (Furuya, et al., 2005). The further description by Furuya *et al.* of two cases with bacteriologically and cytologically proven chlamydial seminal vesiculitis (transperineal puncture of the seminal vesicles under TRUS) who had asymptomatic urethritis but not epididymitis supports the principle of the retrograde route of microbial transmission (Furuya, et al., 2006). The authors suggested that seminal vesiculitis caused by chlamydia may serve as a source of its latent infection (Furuya, et al., 2006). In a large-scale study by the same author patients with urethritis were likely to have accompanying seminal vesiculitis using TRUS imaging (Furuya, et al., 2009). This suggests a close interrelationship between urethritis, seminal vesiculitis and epididymitis. The authors speculated that there are two opposite roles of seminal vesicles in patients with urethritis. On the one hand, they act as a reservoir of microorganisms, on the other, seminal vesicles protect against spreading microorganisms (Furuya, et al., 2009).

There is still no explanation as to why not all patients with STI urethritis have seminal vesiculitis and epididymitis. Despite some cases with urethritis causing bilateral seminal vesiculitis, there is no indication as to why acute epididymitis only occurred on the unilateral side (Furuya, et al., 2004). Perhaps this not only depends on properties of pathogens, but also on the host organism's immune system status (Korytny, et al., 2017), urogenital tract anatomy and its functionality (Muro, et al., 2021; Park, et al., 2020; Lee, et al., 2020; Nistal, et al., 1992).

2.3.4. Impact on prostate gland

Prostate inflammation: impact of particular STI agents

In the pre-antibiotic era (the first half of the 20th century), prostatitis complicated with prostatic abscess was frequently caused by *Neisseria gonorrhoeae*. The postulated mechanisms of infection were either metastases from a distant focus of infection (an ascending urethral infection) or previous urethral instrumentation. Not infrequently, the clinical presentation of the disease was rather dramatic because of the spread of the infection to the periprostatic space and/or spontaneous rupture into the urethra, perineum, rectum, and even the bladder and peritoneum. Surgical drainage was the only effective therapeutic option in the cases diagnosed before rupture. Morbidity and mortality were relatively high, with the latter reaching a rate of 30%. Prostatic abscess was widely accepted as a serious disease, and the prognosis was poor without surgery. Although rare, prostatic abscess in children was also reported, and the gonococcus was implicated in several of these cases. The clinical distinction between prostatic abscess and prostatitis is rather difficult. Moreover, prostatic abscess may not be differentiated from other urinary tract and prostatic diseases on the basis of history and physical examination alone (Weinberger, et al., 1988).

Studies from the 1970s and 1980s claimed that the prostate in patients with non-acute prostatitis may be a reservoir for gonococci and a source of infection (Mårdh & Colleen, 1975; Molin & Danielsson, 1970; Vandenbroucke-Grauls, et al., 1982). However, the later studies on this issue rather deny any role of *N. gonorrhoeae* in chronic prostatitis (Weidner, et al., 1991; Xiao, et al., 2013; Campos, et al., 2021).

According to the recent systematic review by Whelan *et al.* (2021), there were no any observational studies with a comparator group regarding the prostatitis issue in *N. gonorrhoeae* infection.

The role of *C. trachomatis* infection in the development of prostatitis remains controversial (Cunningham & Beagley, 2008). Some studies support the role of *C. trachomatis* in chronic prostatitis (Weidner, et al., 1991; Krieger, et al., 1996; Skerk, et al., 2002, 2004, 2009; Badalyan, et al., 2003; Bielecki, et al., 2020), while others do not (Papeš, et al., 2017).

The recovery rate of *T. vaginalis* in the patients with chronic prostatitis is low (Mårdh & Colleen, 1975; Weidner, et al., 1991; Krieger, et al., 1996; Skerk,

et al., 2009; Campos, et al., 2021; Papeš, et al., 2017), although some studies reported higher prevalence (Skerk, et al., 2002, 2004).

The prevalence of *M. genitalium* among patients with chronic prostatitis is low (Krieger, et al., 1996; Papeš, et al., 2017).

Prostate inflammation: methodological dilemmas of the pathogen detection

Semen and prostatic secretion samples may be contaminated via passage through an infected urethra, making diagnosis of upper male reproductive tract infection, including prostatitis, difficult. It has been suggested that care must also be taken in the interpretation of prostatic biopsy findings when determining the presence of infection, because some biopsy samples may contain prostatic-urethral material (Cunningham & Beagley, 2008). In addition, the significance of positive results with PCR is uncertain. PCR results are dependent on the primers used. Also, PCR tests for fragments of bacteria, and it does not confirm the presence of live bacteria. Lysed bacteria would also be positive with PCR (Lee, 2000). On the other hand, previously used culture diagnosis of urogenital pathogens in men with chronic prostatitis represented an especially challenging clinical microbiology problem because of inhibitory substances secreted by prostate and multiple previous courses of antimicrobial treatment (Bielecki, et al., 2020; Krieger, et al., 1996; Molin & Danielsson, 1970). This could be an explanation for the negative yield by culture, albeit this does not pertain to causative agents of chronic bacterial prostatitis (NIH II category) (Skerk, et al., 2002). There is also the possibility that some of the study subjects initially had negative urinary tract samples, were correctly diagnosed with chronic prostatitis, and later acquired the infection. All the above-mentioned factors virtually pertain to all the STI agents whose role is studied in the aetiology of chronic prostatitis.

Prostate inflammation: detection of PSA, hsCRP and Ig in STI patients

Another approach to investigate the relationships of STI with prostatitis is the utilisation of PSA assay. Sutcliffe *et al.* (2006) found that patients with laboratory-confirmed STIs including gonorrhoea, chlamydia and trichomoniasis, were more likely to have a 40% or greater increase in PSA than patients with no STI diagnoses (32% *vs* 2%, p-value <0.01). They hypothesised that significant PSA increases were likely to represent prostate infection and consequent intraprostatic inflammation, although a generalised response to infection at other genitourinary sites, such as the urethra, could not be ruled out. Interestingly, in some patients convalescent PSA concentrations remained elevated for at least several months after diagnosis and effective antibiotic therapy, raising the possibility of a longer-term influence of STIs on the prostate. This observation may reflect chronic inflammation against residual STI antigens, delayed clearance of inflammation, continued healing or broken tolerance to self-prostatic antigens (Sutcliffe, et al., 2006).

In the following study with a larger number of participants, Sutcliffe *et al.* showed that PSA rise was observed for chlamydia and gonorrhoea, but not for NCNGU (Sutcliffe, et al., 2011). Chlamydia cases were most likely to have a large PSA rise (≥40%), followed by gonorrhoea cases. The authors of the study argued that a possible reason for these differences may be the likelihood of symptoms as a possible marker of duration of infection. The authors hypothesised that men with asymptomatic infections/non-specific symptoms might be more likely to have a prostate infection because of their potentially lower awareness of their STI and consequent delay in seeking treatment. This delay might provide pathogens with a greater opportunity to ascend to and infect the prostate, which is a likely necessary first step for prostate carcinogenesis. This hypothesis is consistent with differences in the likelihood of symptoms for each STI. The absence of PSA elevation in the NCNGU group could be due to the smaller proportion of symptomatic NCNGU infections compared to gonorrhoea. An alternative explanation is that some NCNGU cases might not have an infectious aetiology (Sutcliffe, et al., 2011).

The same authors also investigated the role of *T. vaginalis* in prostate diseases by measuring *T. vaginalis* serum IgG antibodies and serum total PSA concentration in a random sample of young, male US active duty military members (Langston, et al., 2019). Overall, the findings did not provide strong support for prostate involvement during *T. vaginalis* infection. However, a non-significant association between high *T. vaginalis* serostatus score and greater PSA concentrations (≥0.70 ng/mL) suggested the possibility of an influence of *T. vaginalis* infection on the prostate (Langston, et al., 2019).

In another study by the same authors, the high-sensitivity C-reactive protein (hsCRP), a marker of systemic inflammation, was measured during the infection (chlamydia, gonorrhoea or NCNGU), and its levels were compared with those of previously taken samples (9 days to 4 months earlier) (Milbrandt, et al., 2017). Only gonorrhoea cases were significantly more likely to have a large hsCRP rise (≥1.40 mg/L or ≥239%) during infection than the controls. The authors speculated that in the case of gonococcal infections which resulted in a large hsCRP but not PSA rise, these reflect symptomatic urethral infections that contributed to both a systemic immune response and prompt antibiotic therapy, thereby preventing prostate infection. In contrast, for gonococcal and chlamydial infections which resulted in a large PSA but not hsCRP rise, this is more likely to reflect asymptomatic urethral infections that were not treated promptly, thereby allowing the establishment of a chronic, low-grade prostatic infection, or damage to the prostate epithelium from persistent local urethral inflammation (Milbrandt, et al., 2017).

In the later study, the same authors examined the longer-term influence of urethritis-associated STI infections, both individually and cumulatively, on PSA over a mean of 10 years of follow-up (Langston, et al., 2018). The authors found that young men with histories of both genitourinary (CT, gonorrhoea, and NCNGU) and non-genitourinary infections were more likely to have an increase in PSA of any magnitude over an average of 10 years of follow-up than the

controls. However, no large PSA rise (≥40%) was found for chlamydia, gonorrhoea or NCNGU compared to controls. The authors speculate that this small increase in PSA may be important because higher than average PSA concentrations in young- to mid-adulthood have been found to predict future prostate cancer risk and aggressiveness. It is conceivable that this small difference in PSA could translate into a large difference in prostate cancer detection or risk, consistent with the hypothesis that persistent or repeated prostate epithelial cell damage and regeneration (the "injury and regeneration" hypothesis) increases the risk of cellular transformation and prostate carcinogenesis. As a further more troubling possibility, it is also conceivable that infections could contribute to an increase in the trajectory of PSA (i.e., a higher slope or rate of change), particularly if these insults occur during adolescence and young adulthood when the prostate is still growing and developing, and thus may be more susceptible to carcinogenic exposures. This type of increase might lead men to reach higher PSA concentrations than they would otherwise reach without infections, or to reach these concentrations at younger ages (Langston, et al., 2018). However, this study had some important limitations that could impact the results: 1) undiagnosed infections among asymptomatic men; 2) undocumented infections among those seeking care outside the study setting; 3) infections near the time of blood draw; and 4) absence of assessment of sexual behaviour.

Irrespective of the mechanism by which infections raise PSA, a key question that remains to be addressed is the meaning of this elevation for prostate cancer risk.

Prostate cancer

The potential role of genitourinary infection in the aetiology of prostate cancer has been extensively investigated for more than 30 years. Three basic approaches have been used: a) case-control studies with a retrospective design that were based on the self-reported history of STIs and the surrogates of prior risky sexual behaviour and lifestyle practices, b) tissue-based methods (polymerase chain reaction, immunohistochemistry, and *in situ* hybridisation), and c) serologic assays (enzyme-linked immunosorbent assay, immunofluorescence). After performing a critical appraisal of the published evidence by Hrbacek *et al.* (2013), the authors concluded that the association between prostate cancer and infections (including STIs) that has been studied to date is rather unsupported. At the same time, the authors have pointed to several potential limitations that complicate understanding of the association between infection and cancer. In the case of laboratory studies that are based on the direct detection of pathogens in prostatic tissue, the limitations are the following: (1) they are restricted to hypotheses that involve persistent prostatic infections; therefore, if a pathogen interacts with the tissue using a hit-and-run mechanism, it may not be detected at the time of analysis; (2) they are usually small in size; (3) they are prone to selection bias; (4) they do not allow for the assessment of temporal relationships between infection and cancer; (5) the results vary depending on the specific

genetic sequences being investigated; (6) the results may vary depending on the amount and location of the tissue sampled, including the possibility of missing a focal infection; and (7) specimens may be contaminated during collection and processing. Serologic testing has the following limitations: (1) antibody cross-reactivity; (2) waning antibody levels over time and poorly documented "antibody lifetime" for certain pathogens; and (3) difficulties in establishing a temporal relationship between infection and cancer. One specific drawback of large epidemiologic studies of prostate cancer is (4) the definition of the control group (Hrbacek, et al., 2013). The latent existence of clinically unapparent lesions is an inherent characteristic of prostate cancer. If controls are only defined as "cancer-free" or "prostate cancer-free", men with subclinical undiagnosed lesions may be included in the control group, which would shift the statistics towards the null. The difficulties that are inherent to the studies relying on self-reporting of medical history investigating STI issues include poor recall; an unwillingness to report a stigmatised condition; a lack of awareness of subclinical infections; and a lack of awareness of specific diagnoses, e.g., NGU (Hrbacek, et al., 2013).

Caini *et al.* (2014) published a meta-analysis exploring the association between infection caused by *N. gonorrhoeae, T. pallidum, C. trachomatis, T. vaginalis, U. urealyticum, M. hominis*, Herpes Simplex Virus types 1 and 2, Human Herpes Virus 8 and Cytomegalovirus, and prostate cancer. The authors found a significantly increased prostate cancer risk in men who have had gonorrhoea (OR 1.20, 95% CI 1.05–1.37). No other single STI was significantly associated with prostate cancer (Caini, et al., 2014).

A positive association between prostate cancer and *N. gonorrhoeae* infection (OR 1.31, 95% CI 1.14–1.52) was also confirmed by another meta-analysis (Lian, et al., 2015). This association was stronger in African American males (OR 1.32, 95% CI 1.06–1.65) than in whites (OR 1.05, 95% CI 0.90–1.21) (Lian, et al., 2015). Several important limitations mentioned by Hrbacek *et al.* (2013) are still present in meta-analyses by Caini *et al.* (2014) and Lian *et al.* (2015) and preclude making definitive decisions about the implication of NG in prostate cancer.

The implication of *T. vaginalis* in prostate cancer was assessed in the meta-analysis by Najafi *et al.* (2019). The authors found a non-significant increase of prostate cancer observed among individuals with a previous exposure to TV. However, several limitations were pointed to again, including different testing and diagnosing methods and different cohorts in the involved studies (Najafi, et al., 2019).

A recent meta-analysis by Tantengco *et al.* (2021) showed that prostate cancer patients had 2.24 times higher odds (p-value = 0.0005) of being colonised with any species of *Mycoplasma spp*. However, the authors speculated that there is the possibility that the presence of *Mycoplasma* may be a surrogate marker for other STIs that can also increase the risk of prostate cancer, such as *Chlamydia spp.* and *N. gonorrhoeae*. Additionally, the majority of the included studies had control groups with pathologic conditions (e.g.,

benign prostatic hyperplasia, prostatic inflammation), which may have confounded the results (Tantengco, et al., 2021).

Another recent review by Lawson and Glenn (2022) suggested that *N. gonorrhoeae* and *Mycoplasma spp.* may have roles in prostate cancer but the evidence is limited. At the same time, it is unlikely that *T. vaginalis* and *C. trachomatis* have causal roles in prostate cancer (Lawson & Glenn, 2022).

Benign prostate hyperplasia

There are also some studies investigating the STI issue in the context of benign prostatic hyperplasia (BPH). The detection of STIs in the case of BPH using biopsy material varies by study. There are studies that found a high prevalence of TV (Mitteregger, et al., 2012; Iqbal, et al., 2016), of CT (Toth, et al., 2000; Corradi, et al., 1996), and of MG (Miyake, et al., 2019). However, a low prevalence of NG, CT and TV is reported in the study by Ala-Almohadesin *et al.* (2019). Some studies did not observe urethritis-associated STI in BPH biopsy material at all: Kamarkhani *et al.* (2021) did not find any *T. vaginalis* cases among BPH specimens, no cases of CT and NG were found in the study by Iqbal *et al.* (2016). A study from Poland did not find any cases of *M. genitalium* among BPH patients from first-voided urine (FVU) (Smolec, et al., 2021).

Some serological studies were also performed on the STI and BPH topic. In the study by Breyer *et al*. (2016), null results were observed for associations of a self-reported history of STIs (NG, syphilis) and positive STI serologies (CT, TV, human papillomavirus type 16 and 18, HSV-2, human herpesvirus type 8 and CMV) with prevalent and incident BPH/LUTS-related outcomes. However, TV was positively associated with prevalent nocturia, prevalent large prostate volume, and any prevalent BPH/LUTS (Breyer, et al., 2016). In the study by Kim *et al.* (2019), seropositivity to *T. vaginalis* in patients with BPH was 18.7% and it was significantly higher than the 1.7% of the comparing healthy group. In another study, the seroprevalence among BPH patients was 11.4% for *C. trachomatis* and 5.7% for *N. gonorrhoeae* (Hrbacek, et al., 2011).

Ultimately, the same limitations are true for studies evaluating the STI role on the BPH issue as for prostate cancer (see above).

2.3.5. Impact of particular urethritis-associated STI pathogens on male fertility

C. trachomatis has been indirectly associated with male sub-fertility or infertility as a result of a direct effect on sperm production, maturation, motility and viability (Lanjouw, et al., 2016). According to a recent meta-analysis by Ahmadi *et al.* (2016), the prevalence of urogenital *C. trachomatis* was significantly higher in infertile men compared with fertile men (OR = 2.2, 95% CI 1.3–3.7, p-value = 0.003). However, the number of included studies was fewer

than 10, which is insufficient for a good meta-analysis and an accurate conclusion (Ahmadi, et al., 2016).

The scarcity of studies on the *N. gonorrhoeae* is likely to be due to the rarity of infection in developed countries. In a Jordanian study involving 93 infertile men and 70 fertile controls, *N. gonorrhoeae* was detected in semen from 6.5% of infertile men and in none of the fertile men (p-value < 0.05) (Abusarah, et al., 2013). On the contrary, in a large-scale study in Canada, the prevalence of *N. gonorrhoeae* among 5588 infertile males was 0.05%, being lower than the age-adjusted general population prevalence (Domes, et al., 2012).

There is also limited evidence regarding the role of *T. vaginalis* in male infertility (Fode, et al., 2016; Shiadeh, et al., 2016; Crespillo-Andujar, et al., 2018), again, due to the rarity of this infection (Ozdemir, et al., 2011). The results of the recent meta-analysis by Zhang *et al.* (2022) indicated that rate of *T. vaginalis* infection in the infertile male group was higher than that in the fertile group (OR and 95% CI being 1.91 and 1.02–3.58, p-value = 0.04). However, this meta-analysis only included data from five original studies (Zhang, et al., 2022).

A systematic review and meta-analysis by Huang *et al.* could not confirm the association between *M. genitalium* and male infertility (Huang, et al., 2015). However, there were only three original studies included with one study from Europe (Croatia) and two studies from Western Asia (Jordan and Kuwait). All the studies used the in-house PCR methods for the detection of *M. genitalium*.

Ureaplasmas have been investigated in the case of male infertility to some extent. A meta-analysis by Huang *et al.* demonstrated no association with *U. parvum* but suggested an association between *U. urealyticum* and male infertility (Huang, et al., 2015).

2.3.6. Impact of urethritis on semen parameters

The negative impact of *C. trachomatis* on sperm cells has been confirmed in *in vitro* studies while the results of *in vivo* studies are controversial (Redgrove & McLaughlin, 2014). There are some clinical studies supporting an impact of *C. trachomatis* on sperm parameters such as concentration, motility, and morphology (Witkin et al., 1993; Veznik, et al., 2004; Kokab, et al., 2010; Rybar, et al., 2012; Al-Sweih, et al., 2012; Liu, et al., 2014; Sellami, et al., 2014). On the contrary, there are other studies that do not support an impact of *C. trachomatis* on sperm parameters (Dieterle, et al., 1995; Kjaergaard, et al., 1997; Jungwirth, et al., 2003; Hosseinzadeh, et al., 2004; Motrich, et al., 2006; de Barbeyrac, et al., 2006; Gdoura, et al., 2008, a; Gimenes, et al., 2014, a; Dehghan Marvast et al., 2016; Qing, et al., 2017; López-Hurtado, et al., 2018). The recent meta-analysis by Farahani *et al.* (2021) concluded that *C. trachomatis* had no significant impact on mean sperm concentration, progressive motility or morphology.

The impact of *M. genitalium* on semen parameters was assessed in previous studies and the results again tend to be controversial. Some studies have

revealed no impact of *M. genitalium* on semen parameters (Kjaergaard, et al., 1997; Gimenes, et al., 2014, a). On the contrary, Yan *et al.* (2018) reported the negative impact of *M. genitalium* on progressive motility of spermatozoa, Gdoura *et al.* found lower sperm concentration in *M. genitalium*-positive patients (Gdoura, et al., 2007) and higher prevalence of *M. genitalium* in semen samples of azoospermic patients compared with non-azoospermic patients (Gdoura, et al., 2008, a). Qing *et al.* (2017) found that *M. genitalium*-positive men had a higher sperm DNA fragmentation index while the comparisons of other semen parameters (seminal volume, sperm concentration, progressive motility, normal morphology) yielded non-significant results. The meta-analysis by Farahani *et al.* (2021) revealed that *M. genitalium* significantly decreased mean concentration of spermatozoa.

Several researchers have demonstrated *in vitro* the capability of *N. gonorrhoeae* to attach to sperm cells (James-Holmquest, et al., 1974; James, et al., 1976; Gomez, et al., 1979; Gubish, et al., 1979). Liu *et al.* (2002) did not find an impact of *N. gonorrhoeae* on sperm motility and viability in the *in vitro* study. There are a few *in* vivo studies concerning the impact of *N. gonorrhoeae* on semen parameters. No significant impact was seen in spermiogram (semen volume, sperm count, motility, velocity and normal morphology) of asymptomatic men with gonorrhoea in the study by Pérez-Plaza *et al.* (1982) and in the study by Qing *et al.* (2017). One contradictory study has also been published – Rivera *et al.* (2022) found that the samples positive for *N. gonorrhoeae* had a significantly greater percentage of spermatozoa with progressive motility.

Tuttle *et al.* (1977) showed that *in vitro* incubation of human spermatozoa with live *T. vaginalis* resulted in a decrease of sperm motility. Another *in vitro* study also proved the decrease of sperm motility and the spermicidal effect of *T. vaginalis* (Jarecki-Black, et al., 1988). Benchimol *et al.* (2008) observed a decrease in spermatozoa motility and viability, intense semen agglutination, adhesion between trichomonads to the sperm cells, and phagocytosis of spermatozoa by trichomonads after provoked interaction of live *T. vaginalis* with human sperm cells. Zhang *et al.* (2023) demonstrated that incubation of human spermatozoa with excretory secretory proteins of *T. vaginalis* decreased the motility of the sperms, induced sperm apoptosis, and promoted sperm mortality in a dose-dependent and time-dependent manner. In addition, excretory secretory proteins of *T. vaginalis* destroyed sperm acrosome integrity (Zhang, et al., 2023). Wiwanitkit (2008) showed that the *T. vaginalis* whirling movement could disrupt normal sperm movement within the vagina. There is also one study that denies the impact of *T. vaginalis* on semen parameters. Namely, Daly *et al.* (1989) found that *T. vaginalis* did not alter motility or numbers of spermatozoa.

There are scarce data about the impact of *T. vaginalis* on semen parameters in *in vivo* studies. In the study by Gopalkrishnan *et al.* (1990), the authors showed that the seminal fluid viscosity and percentage particulate debris were increased significantly in the *T. vaginalis* group. Spermatozoa motility and morphologically normal forms were also decreased significantly, spermatozoa

viability was altered, and there was a significant change in membrane integrity as shown by the hypoosmotic swelling test (Gopalkrishnan, et al., 1990). Gimenes *et al.* (2014, a) found a statistically insignificant tendency between *T. vaginalis* and necrospermia.

The recent meta-analysis confirmed an increased prevalence of *U. urealyticum* in infertile men (OR: 2.25, 95% CI: 1.47–3.46) and found a negative impact of this bacteria on sperm concentration and morphology (Farahani, et al., 2021).

2.4. Diagnostic options in case of the urethritis

Symptomatic patients and those with a visible discharge should be suspected for the presence of urethritis. However, as other diseases can mimic urethritis and some patients with urethritis can be asymptomatic, the laboratory confirmation of urethritis is required.

The following laboratory methods can be applied to diagnose urethritis: microscopic evaluation of Gram-stained urethral smear (GSS), microscopic evaluation of first-voided urine, urine dipstick test and flow-cytometry.

Urethritis can be confirmed by demonstrating PMNLs from the anterior urethra using a Gram-stained or methylene blue-stained urethral smear, which should contain ≥5 PMNL per high power (×1000) microscopic field (HPF) (averaged over five fields with greatest concentration of PMNLs). Either a 5-mm plastic loop or cotton-tipped swab can be used for specimen collection, which should be introduced about 1 cm into the urethra. Other specimen-collecting methods include a sterile blunt curette or spatula. Alternatively, a FVU specimen can be examined for threads and, if present, these can be stained and interpreted as for a spun deposit (≥10 PMNL/HPF indicates urethritis) (Horner, et al., 2016).

When microscopy is not available, the dipstick test can be used to make a diagnosis of urethritis: the presence of a mucopurulent or purulent urethral discharge on examination; ≥ 1+ on a granulocyte esterase dipstick on an FVU specimen (Horner, et al., 2016). The dipstick test gives only limited quantitative information about an inflammatory reaction in urine, dividing results into discrete categories (Leighton & Little, 1985; Tyndall, et al., 1994; Fraser, et al., 1995). Urine should be considered inflammatory if the dipstick test gives a positive result.

The flow-cytometry of the first-voided urine offers the possibility for the continuous quantitative analysis of bacteria, yeast-like cells and leucocytes in human urine, in addition to the analysis of other particles classified out of urine, e.g. erythrocytes and epithelial cells. There are only a few studies examining the performance of flow cytometry in diagnosis of urethritis, and there is no international consensus on universal technique and threshold levels as yet (Grosso, et al., 2012; Ito, et al., 2014, a; Pond, et al., 2015).

After the urethritis is confirmed by laboratory methods, the etiological diagnosis of urethritis should be made. The diagnostic tests for particular etiological agents are presented below.

2.4.1. *N. gonorrhoeae*

The sensitivity and specificity of the Gram stain, which tests for the presence of characteristic Gram-negative diplococci within PMNLs, can vary substantially between studies and depends upon the specimen; the highest sensitivity and specificity were reported with urethral swab samples from symptomatic males (89% to >98% and >95%, respectively), whereas the sensitivity was as low as 40–50% in urethral specimens from asymptomatic males (Unemo, et al., 2019). A methylene blue staining method is an alternative to the Gram stain, and similar high sensitivity and specificity were reported for diagnosing gonococcal urethritis in men.

Complete antimicrobial resistance testing can only be accomplished if *N. gonorrhoeae* is cultured. Culture of urogenital specimens usually has a sensitivity ranging from 72% to 95%, but can have a sensitivity of 95–100% in settings with extensive experience in appropriate specimen handling and culture (Unemo, et al., 2019). *N. gonorrhoeae* is a fastidious microorganism. Therefore, it needs rapid warm transport to the lab in a special transport medium as well as selective culture media and microaerobic culture conditions.

NAATs are now the preferred and widespread diagnostic tests, being rapid and objective, and having high sensitivity. A major disadvantage of commercial NAATs is the inability to perform antimicrobial resistance testing (Unemo, et al., 2019).

2.4.2. *C. trachomatis*

Laboratory testing of *C. trachomatis* has traditionally consisted of cell culture of inocula prepared from urogenital specimens. The low sensitivity (at most 70–85%), high cost, need for special cell culture lab and high level of technical expertise necessary and the time required to obtain results, are significant disadvantages of the culture method (Wagenlehner, et al., 2006). Therefore, it is no longer used for diagnostic purposes. Later, antigen and nucleic acid detection technologies were developed. The NAATs for *C. trachomatis* have currently been accepted as the standard (Wagenlehner, et al., 2006).

2.4.3. *M. genitalium*

M. genitalium does not possess a cell wall and so cannot be seen in a Gram stain of genital secretions. It is a fastidious organism to culture, requiring weeks to months to grow. Serology testing of antibodies is unfortunately affected by cross reactivity to other mycoplasmas, including *Mycoplasma pneumoniae* (Gnanadurai & Fifer, 2020). Nucleic acid amplification tests (NAATs) identifying *M.*

genitalium-specific nucleic acid in clinical specimens are the only useful methods for diagnosis (Jensen, et al., 2022).

2.4.4. *T. vaginalis*

Wet-mount microscopy has very low sensitivity for male genital specimens (e.g., urethra swab, urine sediment, semen; <51%) due to the lower organism burden and therefore is not commonly used in men (Van Gerwen, et al., 2021; Hobbs & Seña, 2013). *T. vaginalis* culture in liquid medium can be performed on urethral, urine, or semen specimens. However, it is time consuming (needs at least 7 days), and the sensitivity of culture ranges from 40% to 56% for the detection of *T. vaginalis*, although, based on visualisation of viable, motile trichomonads, culture is 100% specific for detection of *T. vaginalis* (Van Gerwen, et al., 2021; Hobbs & Seña, 2013). NAATs are now the gold standard for *T. vaginalis* diagnosis in both men and women (Van Gerwen, et al., 2021).

2.5. Treatment of the urethritis

Empirical treatments are no longer recommended in the latest Centers for Disease Control and Prevention and European Guidelines, although a broad spectrum of antibiotic treatments (single-dose ceftriaxone 1 g intramuscularly plus single-dose azithromycin 1.5 g orally) can be initiated while waiting for the results of the microbiological characterisation of urethral discharge (Bartoletti, et al., 2019). This treatment regimen covers *N. gonorrhoeae, C. trachomatis* and a proportion of *M. genitalium* infections (Unemo, et al., 2020). The use of a Gram stain of urethral discharge or urethral smear to preliminarily diagnose gonococcal urethritis is a useful point-of-care diagnostic. Microbiological characterisation by NAATs to determinate the most appropriate and efficient medical treatment is always indicated and recommended. The risk of developing infections by multiple microorganisms decreases the chance of obtaining successful empirical treatments (Bartoletti, et al., 2019). To avoid reinfection, abstinence of at least seven days should be observed from the start of therapy. All sexual partners of men with urethritis should be referred for evaluation, testing, and treatment. Period to trace contacts varies depending on particular STI agent and can last up to six months depending on STI agent (Tiplica, et al., 2015).

2.5.1. *N. gonorrhoeae*

Ceftriaxone plus azithromycin dual therapy aims to provide a cure for all gonorrhoea cases and, accordingly, to delay the emergence and/or spread of multi-drug resistance and particularly ceftriaxone resistance. This dual therapy also effectively eradicates concomitant *C. trachomatis* infections and a proportion of *M. genitalium* infections, and adherence appears high (Unemo, et al., 2020). A test of cure in asymptomatic patients can be performed with a NAAT

two weeks after completion of treatment (Unemo, et al., 2020). If uncured, a culture study with antibiotic susceptibility testing is suggested.

2.5.2. *C. trachomatis*

According to the 2015 European guideline on the management of *Chlamydia trachomatis* infections, the recommended treatment for uncomplicated urogenital *C. trachomatis* infections is: doxycycline 100 mg twice a day for seven days (oral) or azithromycin 1 g stat (oral) (Lanjouw, et al., 2016). The second-line treatments include erythromycin, levofloxacin, or ofloxacin. The third-line treatments include josamycin (Lanjouw, et al., 2016). A test of cure is not recommended to be routinely performed in patients treated with recommended first-line regimens. Otherwise, the test of cure should be performed four weeks after completion of therapy (Lanjouw, et al., 2016).

2.5.3. *M. genitalium*

Only a few antimicrobial classes have activity against mycoplasmas including tetracyclines, macrolides, fluoroquinolones and streptogramins (Jensen, et al., 2022). It is important to note that doxycycline only has a cure rate of 30–40% (Jensen, et al., 2022), and macrolide-resistance (Couldwell & Lewis, 2015; Manhart, et al., 2015), along with fluoroquinolone-resistance (Manhart, et al., 2015; Deguchi, et al., 2016) are emerging. A test of cure should be considered in all patients and should be collected no earlier than three weeks after completion of treatment (Jensen, et al., 2022).

2.5.4. *T. vaginalis*

The 5-nitroimidazoles (metronidazole, tinidazole, seconidazole) are the only class of antimicrobials effective against *T. vaginalis* (Van Gerwen, et al., 2021). Metronidazole resistance is a well-documented explanation for *T. vaginalis* treatment failure among women; however, no data exist in men. The optimal treatment of metronidazole-resistant *T. vaginalis* infections in men is unknown (Van Gerwen, et al., 2021). The optimal time for retesting for *T. vaginalis* using NAATs is three weeks after completion of treatment (Kissinger, et al., 2022).

2.6. Summary of the literature review

The urethra participates in the transportation of the seminal fluid and urine. At the same time, the urethra can be the entrance point for some urethritis-associated STIs, including *N. gonorrhoeae, C. trachomatis, M. genitalium,* and *T. vaginalis*. STIs passing through urethra provoke urethritis, or inflammation of the urethra. If left untreated, STI-associated urethritis can result in several complications such as epididymo-orchitis, prostatitis, and sexually acquired reactive arthritis. In addition, urethritis-associated STIs can disturb the male reproductive function. Impact of urethritis-associated STIs on men's reproduc-

tive health has been researched for many decades but the evidence from scientific studies is still uneven – most of the published works are focused on *C. trachomatis,* and to a lesser extent to *N. gonorrhoeae,* and *T. vaginalis*. Information about a relatively new pathogen, *M. genitalium*, is rather limited. Moreover, in everyday clinical practice, little attention is paid to *M. genitalium* and its role in urethritis is frequently ignored.

The epidemiology of urethritis-associated STIs varies and depends on different factors (for example, geographic region, study population, diagnostic methods and criteria). In Estonia, there is no clear etiological picture of male urethritis. In addition, there is no general overview about the distribution of urethritis-associated STIs among Estonian males of infertile couples and other populations.

Traditionally, diagnosis of urethritis is based on physical signs and symptoms and laboratory methods. During the last few decades important advances have occurred in diagnostic armamentarium of the urethritis. In parallel with classical methods (i.e. Gram-stained urethral smear, microscopy and dipstick test of urine) new techniques have become available, including flow-cytometric analyses of the urine. However, this method has largely not been evaluated in diagnostics of urethritis and there is no international consensus on the threshold levels as yet.

AIMS OF THE RESEARCH

The general aim of the research was to reveal the prevalence of urethritis among different populations in Estonia, its impact on male uro-genital system, and the applicability of the novel diagnostic options for urethritis patients.

The specific aims of the research were as follows:

1) To describe the prevalence of urethritis-associated STIs (*N. gonorrhoeae, C. trachomatis, M. genitalium,* and *T. vaginalis*) among different populations in Estonia, including heterosexual men with high-risk sexual behaviour, male partners of infertile couples, and male partners of pregnant women.
2) To compare the clinical picture in male patients with urethritis having different STIs.
3) To describe and compare the inflammatory reaction in the male reproductive tract caused by particular urethritis-associated STIs.
4) To evaluate the diagnostic options and criteria for prediction of urethritis-associated STIs in males.
5) To reveal the impact of urethritis-associated STIs on semen quality and blood PSA level.

MATERIAL AND METHODS

1. Subjects and study design

Table 3 provides an overview of the participants of the different studies described in Papers I–III and the thesis.

Table 3. Summary of study subjects.

Studies, study type	Subjects	Type of investigations
High-risk heterosexual males of the STI prevalence study (Paper I). A prevalence study.	825 heterosexual men.	STI questionnaire; concentration of leucocytes from the first-voided urine by dipstick test; detection of NG, CT, MG and TV by PCR from first-voided urine.
Participants of the flow cytometry evaluation study (Paper II). A case-control study.	Cases (N = 306): patients with infectious urethritis caused by CT, NG, MG and/or TV. Control group (N = 192): patients without urogenital complaints, negative tests for CT, NG, MG and TV from first-voided urine and no inflammation in first-voided urine, mid-stream urine and urine after prostate massage.	Detection of NG, CT, MG and TV by PCR from the first-voided urine; first-voided urine and fractionated was analysed using urine particle analyser Sysmex UF-500i.
STI prevalence study among male partners of infertile couples (Paper III). A case-control study.	Study group (N = 2000): males with fertility problems or desire for fertility check. Control group (N = 248): male partners of pregnant women.	Detection of NG, CT, MG and TV by PCR from the first-voided urine; seminal IL-6, semen and fractionated urine, blood analyses (PSA, reproductive hormones, genetic analyses).

Abbreviations: CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; MG, *Mycoplasma genitalium*; TV, *Trichomonas vaginalis*; PSA, prostate-specific antigen; PCR, polymerase chain reaction; IL-6, interleukin-6

1.1. High-risk heterosexual males of the STI prevalence study

The study group included 825 heterosexual men aged 18.0–49.5 years (mean age 30.8±7.3) consulting the andrologist at three departments of Centre of Andrology, Tartu University Hospital, Estonia (departments in Tartu, Tallinn and Pärnu) during the period from 1 January 2013 to 31 August 2015. The reason for STI testing was the patient's self-suspicion of STI risk (Table 3).

1.2. Participants of the flow cytometry evaluation study

The study was organised prospectively. Patients from the Centre of Andrology at Tartu University Hospital in Estonia were examined during the period from March 2015 to January 2018. A group of cases and a control group were recruited for the study. The age of the participants ranged from 18 to 50 years in both groups (Table 3).

The group of cases (n = 306) consisted of patients who referred or self-referred to the Centre of Andrology either for a STI control after a case of high-risk sexual behaviour, for fertility check, or for prophylactic health control. The recruitment criteria for the cases were the following: 1) The patients had done all the four STI PCR tests from first-voided urine for CT, NG, TV and MG; 2) Only heterosexual patients were included; 3) Patients admitted for STI control after STI treatment were excluded.

For the control group (n = 192), we defined uniquely strict inclusion criteria and they were recruited from subjects who visited the Centre of Andrology either for prophylactic purposes or for a fertility check. The recruitment criteria for the control group were as follows: 1) Subjects did not present any symptoms suspicious of urogenital infections or inflammations; 2) Subjects had performed first-voided urine, mid-stream urine and urine after prostate massage with no inflammation found in any of these samples; 3) The timeframe between providing of first-voided and midstream urine/urine after the prostate massage was ≤ 14 days (median 7 days); 4) Patients had done all four STI PCR tests from the first-voided urine for CT, NG, TV and MG; 5) Only heterosexual patients were included.

The patients with inflammation in first-voided and/or mid-stream urine were eliminated from the control group for the reason of including the patients with possible risk of chronic bacterial prostatitis (NIH II group) (European Association of Urology, 2020; Bishop, 2006) or other etiological factors of urethritis (i.e. other bacterial pathogens, viral pathogens, mechanical factors) (Bachmann, et al., 2015; Péc, et al., 1992) which could bias the flow-cytometric counts of bacteria and leucocytes in first-voided urine.

1.3. Male partners of infertile couples

During the period of January-December 2012, there were 3095 referrals to the Centre of Andrology (Tartu University Hospital, Tartu, Estonia) due to family fertility problems (Table 3). After eliminations due to different reasons (Fig. 2 in Paper III), the study group consisted of 2000 men who fulfilled the inclusion criteria (age 18–49 years; correctly delivered first-voided morning urine to the laboratory). In the second stage of the study, we excluded 625 patients with known factors influencing semen quality and reproductive hormone levels to eliminate possible confounding (Supplementary Tables S2 and S3 in Paper III). The selected final study group comprised 1375 men. The formation of the study group is shown in Figure 1.

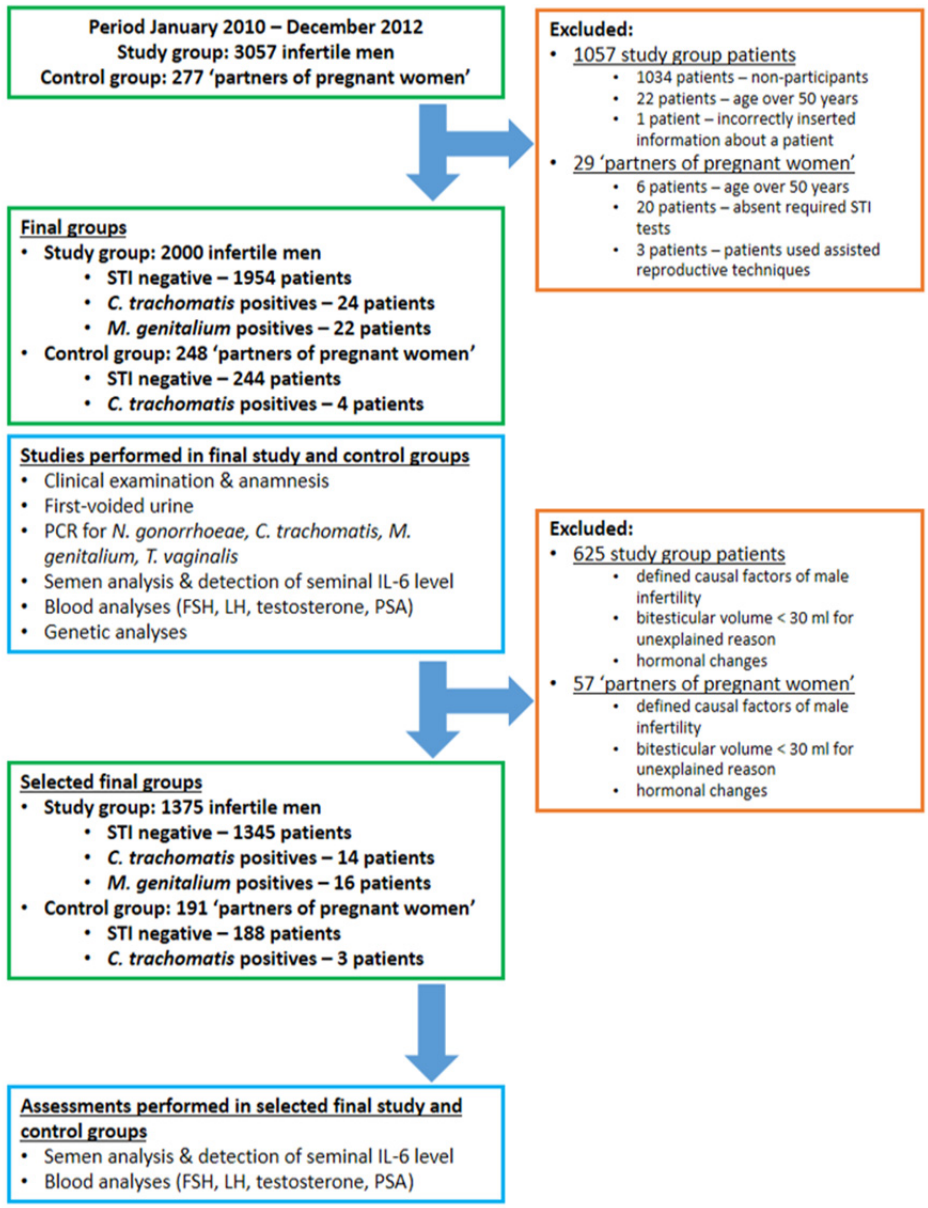

Figure 1. Description of the study III protocol. Abbreviations: STI, sexually transmitted infection; PSA, prostate-specific antigen; FSH, follicle stimulated hormone; LH, luteinizing hormone.

1.4. Male partners of pregnant women

Partners of pregnant women represented a reference sample of fertile control men for the male partners of infertile couples (Figure 2 in Paper III). In 2010–2012, male partners of informed pregnant women at the Women's Clinics of Tartu University Hospital and West-Tallinn Central Hospital were invited to participate in the study and approximately 30% of eligible men agreed. The participants had a choice to complete solely a structured medical questionnaire or additionally to pass a standard andrological physical examination along with blood hormone, semen analysis and first-voided urine sample for urethral inflammation detection and STI tests. Among the 277 participants who comprised the control group, 29 patients were excluded for the following reasons: 20 patients had not performed the required STI tests, six patients were older than 50 years, and three patients used assisted reproductive techniques. Thus, the full dataset including completed questionnaire and physical examination. STI tests, semen analysis (identical to that of the infertility patients) was collected for 248 men who comprised the final control group. To reveal the association of a particular STI agent with the quality of semen parameters and blood PSA level in the second stage of the study, we additionally excluded 57 men from the final control group. The selected final control group consisted of 191 men. The formation of the control group is shown in Figure 1.

1.5. Ethical considerations

Participation in the studies was voluntary. The studies were approved by the Ethics Review Committee on Human Research of the University of Tartu, Estonia (224/T-18 [25.03.2013], 228/M-32 [26.08.2013], 254/M-17 [21.12.2015], 152/4 [18.09.2006], 288/M-13 [17.12.2018], and 188/M-16 [14.12.2009]). The studies were conducted according to the Declaration of Helsinki principles. All participants provided written informed consent.

2. Methods

2.1. Clinical examination

Patients were examined by clinicians, who had received the respective training in clinical assessment and standardised andrological workup, locally and in collaboration with the other centres accredited by the European Academy of Andrology. An assisting nurse recorded the subject's height and weight. Height was measured by roll-up metal length measuring tape for wall mounting rounded to the nearest 0.1 cm and expressed in cm respectively. Body composition was determined using Tanita Body Composition Analyzer (TBF-300MA, Tanita Corporation. 14-2, 1-chome, maeno-cho, Itabashi-ku Tokyo, Japan). Beside weight in kg and body mass index (BMI), actual fat mass in kg and body fat percentage were defined by body analyser. As recommended by

Tanita, a standardised BIA protocol was used in order to obtain the most accurate results (Heyward & Stolarczyk, 1996). Body Composition Analysers are regularly calibrated by the Estonian Accreditation Centre, Metrosert. Physical examination for the assessment of genital pathology and testicular size was performed with the man in a standing position. If necessary, pathologies were further clarified with the man in a supine position. The orchidometer (made of birch wood, Pharmacia & Upjohn, Denmark) was used for the assessment of testicular size. The total testes volume was the sum of right and left testicles. The position of the testicles in the scrotum, pathologies of the genital ducts (*epididymis* and *ductus deferens*) and the penis, presence and grade of varicocele were registered for each study participant. Varicocele was graded according to a traditional system as follows: Grade 1 – palpated only on the Valsalva manoeuvre; Grade 2 – venous distension easily palpable but not visible; Grade 3 – venous plexus bulges through the scrotal skin, visible and palpable (Dubin & Amelar, 1970). Varicoceles were classified according to the highest assigned grade, independent from the affected side. Digital rectal examination was performed if necessary.

2.2. Questionnaires

High-risk heterosexual males of the STI prevalence study (Paper I) received paper-and-pencil STI questionnaire during their visit to andrologist. It comprised 20 items about sexual behaviour that were divided into four groups: seven items about urethral complaints, seven items about sexual behaviour risk factors, two items about external reasons for STI control and four items about unprotected anal sex. The questionnaire was offered to patients in Estonian or Russian (Table 4).

In the case of micturition problems or pelvic pain, the International Prostate Symptom Score (IPSS) and National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) were used.

Table 4. English version of STI risk behaviour questionnaire.

<table>
<tr><td colspan="4">It is suggested to do STI tests at least 3 days after sexual contact.
You will be informed about STI results via phone or e-mail.</td></tr>
<tr><td colspan="4">I wish to receive the results of the analyses:</td></tr>
<tr><td>A. via e-mail</td><td colspan="3">Your e-mail:</td></tr>
<tr><td>B. by phone</td><td>Your phone number:</td><td colspan="2">Signature</td></tr>
<tr><td colspan="4">If it is required to visit a doctor, the fastest way to do this is to send the message to the following e-mail: meestekliinik@kliinikum.ee</td></tr>
<tr><td colspan="4">The upper part of this questionnaire will be detached and destroyed at the end of the data management. Your confidential data will be encoded in your medical history.</td></tr>
<tr><td colspan="4">Sexually transmitted infections' risk behaviour questionnaire.
A. Complaints
1. Persistent cloudy discharge from the urethra
2. Intermittent cloudy discharge from the urethra
3. Persistent clear discharge from the urethra
4. Intermittent clear discharge from the urethra
5. A urethral opening is reddish
6. Persistent discomfort in the urethra
7. Discomfort in the urethra during micturition
B. Sexual behaviour risk factors
8. Sex with a prostitute without protection
9. Sex with a prostitute with protection
10. Oral sex with a prostitute without protection
11. One-night stand sex without protection
12. Oral sex without protection with a random partner
13. One-night stand sex with protection
14. During last 6 months there was more than one regular partner
C. Additional reasons for testing
15. A partner has cystitis or inflammation of genitalia
16. Persistent or frequently recurring inflammation of the prepuce
D. Anal sex (sex via anus) without protection
17. In active position in one-night stand
18. In passive position in one-night stand
19. Frequently in active position
20. Frequently in passive position</td></tr>
<tr><td colspan="4">The following data completes a worker of the Centre of Andrology</td></tr>
<tr><td>Date of analysis:</td><td colspan="2">Concentration of leukocytes in urine per µl:</td><td>Age of patient:</td></tr>
<tr><td colspan="4">Results of analyses:</td></tr>
<tr><td colspan="4">Recommendations:</td></tr>
<tr><td>Date of the feedback to the patient:</td><td>Name:</td><td colspan="2">Signature</td></tr>
</table>

2.3. Sample collection

2.3.1. First-voided urine

First-voided urine (FVU) was defined as the first morning urine or urine provided at least 2–3 hours after the last urination. The first-voided urine was performed at the Centre of Andrology or brought by the patient from home. The first-voided urine was collected into a pre-weighted aseptic 150 ml urine container (Kartell S.p.A., Italy) for polymerase chain reaction studies and for urine flow cytometry and dipstick test. In cases when the patient decided to bring first-voided urine from home a urine container was given to the patient by the clinic staff and the patient was instructed to bring the sample to the Centre of Andrology within 1 hour after collection if at room temperature (+ 24˚C), or within four hours if the sample was stored at + 4˚C. Each patient was asked to provide 15–20 ml of first-voided urine. However, we could observe that the median volume of first-voided urine was higher than recommended (despite the desired volume of 15–20 ml, the actual volume was 54–58 ml), but this reflects the fact that collecting a smaller volume of urine is difficult for patients. To mitigate the effects of this difficulty, we analysed both concentrations and total counts of white blood cells (WBCs) and bacteria and obtained similar results.

2.3.2. Fractionated urine

The fractionated urine samples consisted of the midstream and post-massage urine. The midstream and post-massage urine was always collected at the Centre of Andrology on the same day. The midstream urine was collected either right after first-voided urine collection or after providing 15–20 ml of urine if the test was planned on a separate day. The post-massage urine was collected after a prostate massage that lasted for 2–3 minutes – the prostate secretion and the post-massage urine were collected into the same urine container with the purpose of saving the patient's time and avoiding mixing up different collection tubes by the patient. The obligatory prerequisite before the massage of the prostate was sexual abstinence for 3–7 days before the procedure. The flow-cytometric analysis of urine samples was performed within one hour after sample's arrival at the Centre of Andrology.

2.3.3. Blood

Venous blood of the patient was drawn from the cubital vein in the morning from 08:00 a.m. to 10:30 a.m. after overnight fasting or light meal, and serum was separated immediately.

2.3.4. Semen

Semen samples were obtained by patient masturbation in a private room near the laboratory and all semen values were determined in accordance with the

World Health Organization (WHO) recommendations at the time of recruitment (World Health Organization, 2010). In brief, subjects were told to abstain from ejaculation for three to four days. Semen samples were collected by patients following the washing of the *glans penis* with soap and water and urinating. After ejaculation, semen was incubated at 37˚C for 30–40 min for liquefaction. The semen volume was estimated by weighing the collection tube with the semen sample and subsequently subtracting the predetermined weight of the empty tube and assuming 1 g = 1 ml.

2.4. Semen quality evaluation

For the assessment of the spermatozoa concentration, the samples were diluted in a solution of 0.6 mol/l $NaHCO_3$ and 0.4% (*v/v*) formaldehyde in distilled water. The spermatozoa concentration was assessed using the improved Neubauer hemocytometer. Motility was assessed in order to report the number of progressively motile spermatozoa. Smears for the morphology assessment were made. Following the fixation and Papanicolaou staining, the morphology was assessed according to strict criteria (World Health Organization, 2010).

2.5. Detection of inflammation in reproductive tract

2.5.1. Dipstick test of urine

The analysis of urine specimens was performed by an experienced lab technician. The dipstick tests for urine were performed using Combur10Test UX test strips (Roche Diagnostics Ltd., Rotkreuz, Switzerland) and Urisys 1100 machine (Roche Diagnostics GmbH, Mannheim, Germany) according to producer's instruction.

The Combur10 Test UX are test strips for the *in vitro* qualitative or semi-quantitative determination of leukocytes in urine with the Urisys 1100 urine analyser and by visual reading. The Combur10Test UX test reveals the presence of granulocyte esterases produced by leukocytes. These esterases cleave an indoxyl ester, and the indoxyl once liberated reacts with a diazonium salt to produce a violet dye (Roche, 2021).

The urine samples were analysed at room temperature within two hours of collection. The analysis was performed as follows. The Combur10 Test UX test strip was dipped in urine for about one second. After that, the edge of the dipped test strip was wiped against the rim of the vessel to remove excess urine when withdrawing the strip, and placed into a Urisys 1100 machine. After 60 seconds, the changed colour of the detection pad of the strip was automatically analysed by the Urisys 1100 machine. The possible result values for leucocyte concentration in urine using instrumental reading with Urisys 1100 are the following: negative, 25, 100, 500 WBC/µl (Roche, 2021). Urine was considered inflammatory if ≥25 WBC/µl.

2.5.2. Flow cytometry of urine

The concentration and total count of white blood cells and bacteria in urine were analysed using urine flow cytometry. The analyses were performed using fully automated urine particle analyser Sysmex UF-500i (manufactured by Sysmex Corporation, 1-5-1 Wakinohama-Kaigandori, Chuo-ku, Kobe 651–0073, Japan) according to the producer's instructions at the Centre of Andrology, Tartu University Hospital.

UF-1000i and UF-500i are fluorescence flow cytometers for the quantitative analysis of bacteria, yeast-like cells and leucocytes in human urine, in addition to the analysis of other particles classified out of urine, e.g. erythrocytes and epithelial cells. Two specific fluorescence dyes are used which stain the cells' nucleic acids and other distinctive cellular parts, thus making it possible to detect bacteria, yeasts and leucocytes with high analytical sensitivity and specificity (Sysmex, 2011).

The weight of the first-voided urine specimen was calculated extracting the weight of the pre-weighted urine container. Each urine container was weighted separately using electronic scales. The weight of 1 gram of first-voided urine was approximated with the volume of 1 millilitre of first-voided urine.

Using the measured concentrations of leucocytes and bacteria in the urine sample by flow cytometry, the total counts of white blood cells and bacteria were also calculated using the following formulas:

$$\begin{aligned} &total\ count\ of\ leucocytes \\ &\quad = [volume\ of\ first-voided\ urine\ sample, in\ grams] \\ &\quad \times\ [concentration\ of\ leucocytes\ in\ first \\ &\quad - voided\ urine\ sample, \mu l^{-1}]\ \times 1000 \end{aligned}$$

$$\begin{aligned} &total\ count\ of\ bacteria \\ &\quad = [volume\ of\ first-voided\ urine\ sample, in\ grams] \\ &\quad \times\ [concentration\ of\ bacteria\ in\ first \\ &\quad -\ voided\ urine\ sample, \mu l^{-1}]\ \times 1000 \end{aligned}$$

The cut-off values for the bacteria and leucocyte count in first-voided urine, midstream urine and urine after prostate massage were derived from the patients' cohort of the Centre of Andrology, Tartu University Hospital.

Our empirical cut-off values for inflammation using flow-cytometry were as follows: for first-voided urine and midstream urine – leucocytes ≥ 15/μl, bacteria ≥ 20/μl; for urine after prostate massage – leucocytes ≥ 40/μl, bacteria ≥ 100/μl.

2.5.3. Detection of WBCs and IL-6 in semen

Semen smears were made for the detection of WBCs. The smears were air-dried, Bryan–Leishman stained, and examined with the use of oil immersion microscopy (magnification: 1000×) by an experienced microscopist. Polymorphonuclear leukocytes were differentiated from spermatids by the presence of segmented nuclei, bridges between lobes of a nucleus, and the specific granulation of the cytoplasm. The WBC concentration in the semen was calculated by using the known spermatozoa concentration. In cases of low semen volume (<1.5 ml) and in clinical cases experiencing an orgasm with missing antegrade ejaculation, the retrograde ejaculation was confirmed by examining a sample of post-ejaculatory urine for the presence of spermatozoa.

IL-6 levels in the seminal plasma were measured using the Immulite automated chemiluminescence immunoassay analyser (Immulite DPC, Los Angeles, CA, USA) according to the manufacturer's instructions at the United Laboratories of Tartu University Hospital. If the seminal secretion volume was <20 µl, a known volume of plasma was diluted according to the manufacturer's instructions. Any dilution factor was recorded and results were corrected accordingly.

2.6. Detection of causative agents of STIs

STIs were detected from first-voided urine using the polymerase chain reaction (PCR) method (*C. trachomatis* and *N. gonorrhoeae* DNA by Roche/Cobas® 4800 CT/NG Test [Roche Diagnostics Ltd., Switzerland]; *M. genitalium* DNA by Sacace Biotechnologies/Mycoplasma genitalium Real-TM [Sacace Biotechnologies Srl., Como, Italy]; *T. vaginalis* DNA by Sacace Biotechnologies/ Trichomonas vaginalis Real-TM [Sacace Biotechnologies Srl., Como, Italy]) according to the manufacturer's instructions, at the United Laboratory of Tartu University Hospital.

All STI-positive patients were contacted for the appropriate treatment and follow-up. They were told to inform and motivate sexual contacts to complete the appropriate analyses and treatment.

2.7. Other laboratory analyses

Follicle stimulated hormone (FSH), luteinizing hormone (LH), total testosterone, and total prostate-specific antigen (PSA) levels of blood serum were measured using the Immulite automated chemiluminescence immunoassay analyser (Immulite; Diagnostic Products Corp., Los Angeles, CA, USA) according to the manufacturer's instructions, at the United Laboratories of Tartu University Hospital. For IMMULITE 1000 system analytical sensitivity was 0.1 IU/l for both LH and FSH. The intra- and inter-assay coefficients of variation were 4.2 and 8% for FSH, 4.0 and 7.1% for LH, 6.3 and 9.4% for testosterone.

In the frame of this research, measurement of blood testosterone, LH and FSH level in men were used in diagnostic investigations of hypogonadism and

for differentiation of primary from secondary hypogonadism (Corona, et al., 2020; Bhasin, et al., 2018).

Tests for the known genetic causes of male infertility (karyotyping, Y-chromosome microdeletions [*AZFa, AZFb, AZFc*], *CFTR* mutations [p.F508del, 394delTT, IVS8 5T/7T/9T]) were performed on indication at the United Laboratories of Tartu University Hospital. A detailed description of genetic analyses is provided elsewhere (Punab, et al., 2017).

2.8. Statistical analysis

Data were recorded using Microsoft Office Word 2010 and Excel 2010 software (Microsoft Corporation, Washington, USA).

In the STI prevalence study of high-risk heterosexual males (Paper I) the distribution of patient complaints and leukocyte count in first-voided urine among different STI groups was evaluated by way of comparing the subgroups applying Fisher's exact test, with p-value < 0.05 being considered significant. The SigmaStat program (Systat Software, Chicago, IL, USA) was used for statistical analyses.

In the flow cytometry evaluation study (Paper II) statistical analyses were performed using RStudio software (R version 3.6.1. (2019-07-05), RStudio Inc., Massachusetts, USA). Except for age, the data about first-voided urine volume, total count and concentration of leucocytes and bacteria were through non-parametric statistical distribution. For comparison of the basic parameters of the control and cases' groups a non-parametric Wilcoxon test was used. P-value < 0.05 was accepted as a statistically significant difference. Spearman correlation was used to assess the correlation between the concentration of leucocytes and bacteria. To assess the difference between the leucocyte and bacteria concentration/total count in different groups of STI-positive patients with monoinfection or combined infection Kruskal-Wallis test was used. Mann-Whitney test with Bonferroni correction (using 10 tests, corrected p-value < 0.005; using three tests, corrected p-value < 0.017) was used to assess the statistical significance of the difference of concentration and the total count of bacteria and leucocytes between different patient groups with STI mono-infection and combined infections.

For the STI prevalence study among infertile male partners of infertile couples (Paper III) four dedicated study nurses (two in each department – Tallinn and Tartu) entered the collected epidemiological, laboratory, and clinical examination data into two separate, but identically structured, databases. Prior to statistical analysis, the two databases were merged and duplicate entries were eliminated. The entered laboratory data were counter-controlled from primary sources (lab databases) and, if needed, edited by a specially trained researcher (MP and KP). Clinical data relevant to defining the cause of male infertility were controlled retrospectively for all study subjects from their medical case histories one by one by one author of the study (MP).

Statistical analyses for the third study (Paper III) were performed using RStudio software (R version 3.6.1. (2019-07-05), RStudio Inc., Massachusetts, USA). For comparison of numerical continuous parameters between different STI groups, the non-parametric Mann-Whitney U-test was used for non-normally distributed data, while the parametric unpaired t-test was used for normally distributed data. For categorical nominal data, Fisher's exact test and chi-squared test were used. A two-sided p-value < 0.05 was accepted as statistically significant. Comparing *C. trachomatis*-positive *vs.* STI-negative and *M. genitalium*-positive *vs.* STI-negative patients, the Bonferroni correction of p-values for two tests was used. To find the best cut-off value for semen neutrophils' concentration and semen interleukin-6 for predicting *C. trachomatis* and/or *M. genitalium* infection, the construction of receiver operating curves (ROC) was performed. Due to the relatively small number of STI-positive patients, the construction of multivariate logistic regression models was avoided.

RESULTS AND DISCUSSION

1. Prevalence of urethritis-causing STIs in Estonia

Table 5 summarises the prevalence of urethritis-causing STIs in Estonia among different populations.

Table 5. Prevalence of urethritis-causing STIs in Estonia among different populations

Study group, N	Prevalence, % (N)							
	CT	NG	MG	TV	CT + NG	CT + MG	NG + MG	STI negative
Males with high-risk sexual behaviour (Paper I), N = 691	14.8% (102) [E. F]	2.5% (17)	4.2% (29) [D]	0.7% (5)	0.9% (6)	0.3% (2)	0.3% (2)	76.4% (528) [A. B]
Male partners of infertile couples (Paper III), N = 2000	1.2% (24) [E. G]	0	1.1% (22) [D]	0	0	0	0	97.7% (1954) [A. C]
Male partners of pregnant women (Paper III), N = 248	1.6% (4) [F. G]	0	0	0	0	0	0	98.4% (244) [B. C]

CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; MG, *Mycoplasma genitalium*; TV, *Trichomonas vaginalis*; STI, sexually transmitted infection

P-values were calculated using Fisher's exact test with Bonferroni correction for seven tests.

A, p-value $< 1.54 \times 10^{-15}$

B, p-value $< 1.54 \times 10^{-15}$

D, p-value $= 1.41 \times 10^{-5}$

E, p-value $< 1.54 \times 10^{-15}$

F, p-value $= 1.14 \times 10^{-9}$

1.1. Prevalence among high-risk heterosexual males

In the STI prevalence study of high-risk heterosexual males (Paper I), out of 825 patients, 83.8% (691 patients) reported sexual risk behaviour. Among this group of men, the prevalence of STIs was the following: *C. trachomatis* 14.8%, *M. genitalium* 4.2%, *N. gonorrhoeae* 2.5%, *T. vaginalis* 0.7%, combined STIs 1.5% (two cases with *C. trachomatis* and *M. genitalium*, two cases with *N. gonorrhoeae* and *M. genitalium*, six cases with *C. trachomatis* and *N. gonorrhoeae*) (Table 5).

This is the first study investigating the prevalence of *M. genitalium* in Estonian men, although the study group did not represent the general population. Despite the estimated role as a pathogen in the genito-urinary tract, its registration in Estonian medical system is currently poor, similarly to many other countries.

Our study revealed a higher prevalence of *M. genitalium* than the study of British 16–44-year-old men (Sonnenberg, et al., 2015), Dutch general population (Jenniskens, et al., 2017) and attendees of youth clinics in St. Petersburg (Shipitsyna, et al., 2013), but in comparison with STI testing centres (4–38%) (Cazanave, et al., 2012) our prevalence was in the lowest pole.

As concerns *N. gonorrhoeae*, its prevalence was similar to the prevalence of studies from Russia (Shipitsyna, et al., 2013) and USA (Getman, et al., 2016), however some other researchers found a higher prevalence (Gaydos, et al., 2009; Gottesman, et al., 2017).

The prevalence of *C. trachomatis* in our study was similar to several other studies (Shipitsyna, et al., 2013; Gottesman, et al., 2017; Getman, et al., 2016; Gaydos, et al., 2009; van der Veer, et al., 2016) where youth clinics' and STI clinics' populations were analysed. This bacterium has been considered to be the most frequent causative agent of STIs among men in western countries (Rowley, et al., 2019).

T. vaginalis had the lowest prevalence in our study. Our finding is also in accordance with some previous studies from the USA (Gaydos, et al., 2009; Getman, et al., 2016), the Netherlands (van der Veer, et al., 2016), Japan (Seike, et al., 2013), and Canada (Gratrix, et al., 2017), while studies from Russia (Shipitsyna, et al., 2013) and Israel (Gottesman, et al., 2017) did not find any cases of *T. vaginalis*. Hence, the current evidence does not support the major role for *T. vaginalis* among STIs.

The low prevalence of combined STI cases in our study was also reported in some other papers (Gottesman, et al., 2017; Shipitsyna, et al., 2013; van der Veer, et al., 2016), while some researchers found a high prevalence of combined STI cases in the USA (Gaydos, et al., 2009) revealing most of the combinations *C. trachomatis* with *M. genitalium* (5.9%) and *C. trachomatis* with *N. gonorrhoeae* (5.9%) among men with urethritis.

To sum up, there is a moderate prevalence of urethritis-associated STIs among Estonian males with risky sexual behaviour that may probably be associated with good sexual education and widespread condom use in Estonia. *C. trachomatis* has the highest prevalence among Estonian high-risk men but *M. genitalium* holds an important second place. It is highly recommended to not only track and register the occurrence of *M. genitalium* in the Estonian medical system, but also beyond, as there is a strong possibility, especially in the context of increasing antimicrobial resistance of this STI agent (Manhart, et al., 2015), and the situation with multidrug-resistant strains of *M. genitalium* will disseminate worldwide in the near future over some decades. We compared our data with the studies conducted in STI clinics, however there was one study from a

youth clinic (Shipitsyna, et al., 2013) and one study from general population (Jenniskens, et al., 2017).

1.2. Prevalence among male partners of infertile couples

The insight into the epidemiological situation among male partners of infertile couples is presented in Paper III. We screened 2000 study subjects for four causative agents of STIs (*N. gonorrhoeae, C. trachomatis, M. genitalium* and *T. vaginalis*). Overall, the prevalence of STIs among the male partners of infertile couples was quite low: *M. genitalium* was found in 1.1% and *C. trachomatis* in 1.2% of men. There were no cases with *N. gonorrhoeae*, *T. vaginalis*, or combined infections. This is (more or less) in accordance with the overall STI pattern in developed countries where *C. trachomatis* and *M. genitalium* tend to be more frequent urethritis-associated pathogens than *T. vaginalis* and *N. gonorrhoeae*. On the other hand, substantial differences exist in published STIs' prevalence among the infertile men.

The prevalence of *C. trachomatis* among infertile men varies depending on the place of the study and the year of the publication. Early studies have shown a higher prevalence of chlamydia, while recent studies have shown a lower one. This could suggest the positive effect of preventive work in the field of sexual medicine in developed countries during the last few decades. For example, research conducted in the 1990s has revealed a very high prevalence of *C. trachomatis* among male partners of infertile couples in the USA (39.3%) (Witkin, et al., 1993), and a remarkable prevalence in Germany (10%) (Dieterle, et al., 1995), and France (10.9%) (Levy, et al., 1999). During the last decade, its prevalence has decreased (Veiga, et al., 2020; Salmeri, et al., 2010; Boeri, et al., 2020), however, a quite high prevalence has been revealed in the Czech Republic (13%) (Rybar, et al., 2012).

While there are plenty of studies analysing the role of *C. trachomatis* on male fertility, to date there are only a limited number of works about *M. genitalium*. Its prevalence among infertile men in our study was similar to the studies from Denmark (0.9%) (Kjaergaard, et al., 1997) and Croatia (1.4%) (Plecko, et al., 2014). There are also some studies from places other than Europe reporting the prevalence of *M. genitalium* among infertile men with the following prevalence estimates: 3.5–5.0% in Tunisia (Gdoura, et al., 2007; Gdoura, et al., 2008, a; Sellami, et al., 2014), 4.7% in Kuwait (Al-Sweih, et al., 2012), 3.2% in Jordan (Abusarah, et al., 2013), 3.9% in Brazil (Gimenes, et al., 2014, a), 0–9.7% in Iran (Dehghan Marvast, et al., 2017; Moridi, et al., 2021; Ahmadi, et al., 2018), 0.8% in Vietnam (Tam Le, et al., 2022) and 2.0–3.6% in China (Qing, et al., 2017; Li, et al., 2020, a; Bai, et al., 2022; Bai, et al., 2021). The recent meta-analysis does not support the etiological role in male infertility for *M. genitalium* (Huang, et al., 2015), however, only three original studies regarding this bacterium were included and, therefore, the analysis gave borderline significance value, despite a good odds ratio [3.27 (95% CI: 0.80–13.29)].

Hence, the prevalence of urethritis-associated STIs among male partners of infertile couples in Estonia is satisfactorily low, below 3%. This finding indicates a beneficial epidemiological situation of STIs in our country. At the same time, men with proven fertility status (i.e. male partners of pregnant women) could serve as acceptable controls for future STI studies.

1.3. Prevalence among male partners of pregnant women

STI prevalence among male partners of pregnant women was also addressed in Paper III. These men were investigated as a group of men with proven fertility. The prevalence of STIs in this group was also low – 1.6% (four patients out of 248). All four patients had *C. trachomatis* monoinfection. We did not find any cases of *M. genitalium, N. gonorrhoeae* or *T. vaginalis* in that group of men.

Very little was found in the literature on the question of prevalence of *M. genitalium* among male partners of fertile couples or fertile men. Although the effect of chlamydia on male fertility is greater, even these studies have rarely used the fertile control group. It is important to mention that an even lower number of the research projects controlled the study subjects for all the four urethritis-associated STIs (*C. trachomatis, M. genitalium, N. gonorrhoeae*, and *T. vaginalis*). Therefore, direct comparisons are complicated.

To the best of our knowledge, no studies from Europe concerning the prevalence of *C. trachomatis* in fertile males using PCR exist. However, other studies outside Europe have reported the prevalence of chlamydia among fertile men. The study from Kuwait found a prevalence of *C. trachomatis* among fertile men of 3.7% (Al-Sweih, et al., 2012). A low prevalence of chlamydia among fertile men of 1.4% was found in Jordan (Abusarah, et al., 2013) and 2.3% in China (Liu, et al., 2014), while the recent study from Egypt did not find any *C. trachomatis*-positive cases among healthy normal fertile males (Nasr El-Din, et al., 2021).

We were able to identify only one case-control study from Europe investigating *M. genitalium* among fertile men (Plecko, et al., 2014). The study reported no cases of *M. genitalium* among the subjects of the control group that was comprised of asymptomatic men attending the clinic as a part of an annual physical examination, and thus, their fertility status was unknown. However, there are some studies outside of Europe reporting the prevalence of *M. genitalium* among fertile men. The prevalence of *M. genitalium* among fertile men was 3.2% in Kuwait (Al-Sweih, et al., 2012), 1.4% in Jordan (Abusarah, et al., 2013), and 1.2% in Iran (Ahmadi, et al., 2018).

In conclusion, the prevalence of STIs among male partners of pregnant women in Estonia was low and comparable with the scarce studies of other countries as well as male partners of Estonian infertile couples. Hence, this finding again supports the desirable epidemiological situation in Estonia. Male partners of pregnant women can serve as an acceptable control group for STI studies. Whenever possible, future studies should include the clearly defined control group while investigating the role of STIs in male reproductive health.

2. Clinical picture in STI-positive patients

The clinical picture of different STIs was investigated in detail in Paper I. Figure 2 summarises the distribution of patients' complaints and macroscopic signs of inflammation in the case of different STI agents.

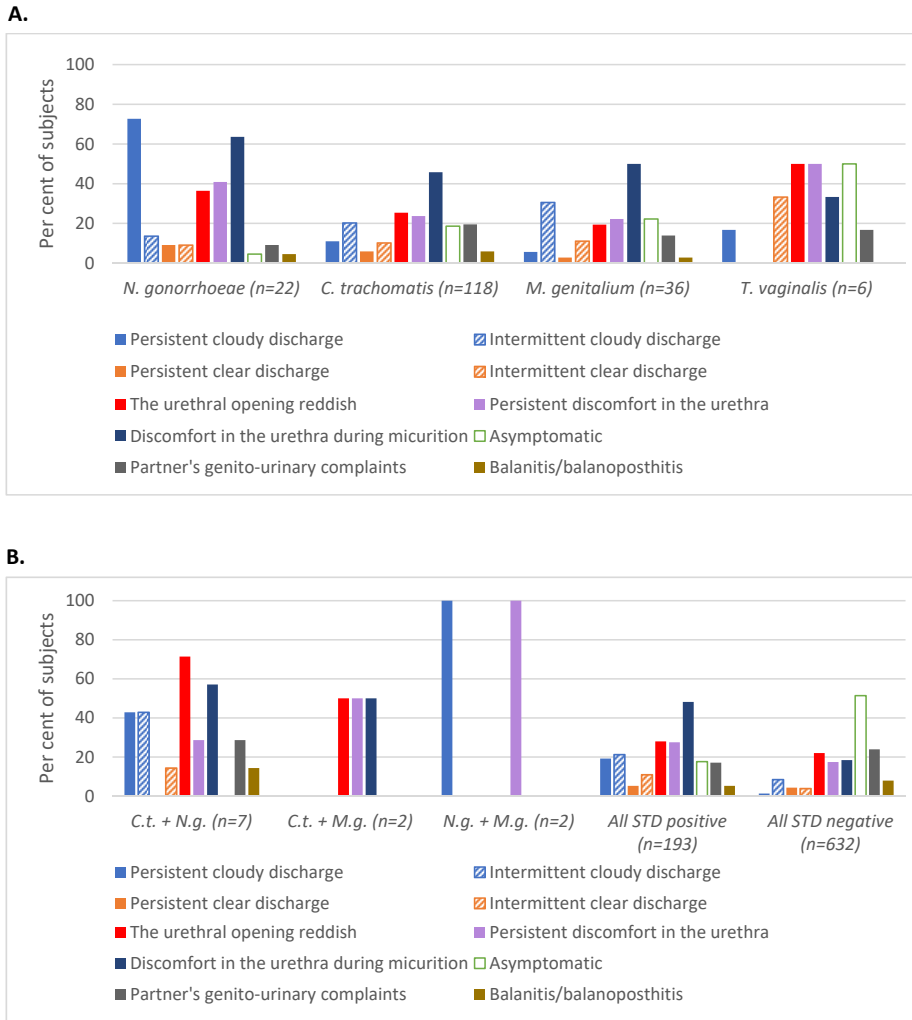

Figure 2. The distribution of patient complaints according to STI monoinfections (A) and their combinations (B).

2.1. Complaints

The complaints profile for different STIs was variable. In the case of mono-infection, *N. gonorrhoeae* and *T. vaginalis* had the highest mean number of complaints per patient (2.5 and 1.8, respectively). Complaints profiles for *M. genitalium* and *C. trachomatis* were similar – mean number of complaints was 1.4 (Table 3 in Paper I). All patients with multiple STIs had complaints, among them *N. gonorrhoeae* + *C. trachomatis* had the highest mean number of complaints per patient (2.6), followed by *N. gonorrhoeae* + *M. genitalium* (2.0).

At the same time, half of the *T. vaginalis* monoinfection cases were asymptomatic while only one out of 22 *N. gonorrhoeae* cases was asymptomatic. Nearly a fifth (18.6–22.2%) of patients were asymptomatic in the case of *C. trachomatis* and *M. genitalium* monoinfection.

T. vaginalis infection can be asymptomatic in as much as 70% of men, because of which a small proportion of men would seek the control in a clinic (Gimenes, et al., 2014, b; Poole & McClelland, 2013; Schwebke & Hook, 2003; Seña, et al., 2007). The small proportion of asymptomatic patients in the case of *C. trachomatis* and *M. genitalium* is in accordance with some previous studies (Taylor-Robinson, et al., 2009; Bowden, 1998; Bradshaw, et al., 2006; Falk, et al., 2004; Gottesman, et al., 2017; Högdahl & Kihlström, 2007), but differs from others (Carne, et al., 2013; Detels, et al., 2011; Coble, et al., 2006; Sonnenberg, et al., 2015; Zheng, et al., 2014). In the case of *N. gonorrhoeae*, a very low prevalence or absence of asymptomatic cases is reported in some studies (Taylor-Robinson, et al., 2009; Gaydos, et al., 2009; Gottesman, et al., 2017), while not in others (Bowden, 1998; Carne, et al., 2013; Detels, et al., 2011).

The most frequent complaint in the case of gonorrhoea was persistent cloudy discharge (72.7%), followed by unpleasant sensations in the urethra during micturition (63.6%). The latter was the most frequent complaint in patients having *C. trachomatis,* and *M. genitalium* monoinfection (45.8% and 50%, respectively). In the case of both *N. gonorrhoeae* and *T. vaginalis*, persistent discomfort in the urethra presented in 40.9% and 50% of cases, respectively (Figure 2). Urethral opening's redness was most frequent in the case of *T. vaginalis* (50%) and *N. gonorrhoeae* (36.4%) monoinfections.

Partner's genito-urinary complaints were the most frequent additional cause for STI testing, especially among *C. trachomatis* + *N. gonorrhoeae* (28.6%) and *C. trachomatis* (19.5%) cases, whereas the presence of balanitis/balanoposthitis was the most frequent cause for STI testing among *C. trachomatis* + *N. gonorrhoeae* (14.3%) cases (Figure 2).

According to a study by Taylor-Robinson *et al.* (2009), urethral discomfort presented in 68% and 48% of *M. genitalium*- and *C. trachomatis*-positive patients, respectively, while all *N. gonorrhoeae*-positive men had symptoms and/or signs of urethritis. In the case of *T. vaginalis* infection, Seña *et al.* (2007) found 76.8% of men were asymptomatic and the most frequent symptoms of trichomoniasis were penile discharge (11.9%), penile tingling (7.3%) and

dysuria or painful urination (7.3%). In the study by Wendel *et al.* (2003), discharge or dysuria was reported in 47% and 22% of men with *T. vaginalis*.

2.2. Macroscopic signs of inflammation

In the case of *N. gonorrhoeae*, the most frequent sign of inflammation was persistent cloudy discharge from the urethra (72.7%), in the case of *C. trachomatis* it was urethral opening's redness (25.4%), in the case of *M. genitalium* it was intermittent cloudy discharge from the urethra (30.6%), and in the case of *T. vaginalis* it was intermittent clear discharge from the urethra (33.3%) (Figure 2A).

Major macroscopic signs such as urethral erythema or balanitis/balanoposthitis were not a specific indication of STI agents and presented quite evenly between STI-positive and STI-negative patients (Figure 2). At the same time, it should be kept in mind that we did not analyse other possible inflammation-causing microorganisms but just four major urethritis-associated causative agents of STIs. It is also important to mention that STI-negative patients with inflammation in first-voided and/or mid-stream urine and/or presence of balanoposthitis or urethral erythema could be at risk of some rare causes such as mechanical factors (Péc, et al., 1992) or other microbiological agents such as *Escherichia coli* (Dan, et al., 2012), *Ureaplasma urealyticum*, herpesvirus, adenovirus, some respiratory tract pathogens (*Haemophilus species*, *Neisseria meningitides*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*) or bacterial vaginosis-associated organisms (Bachmann, et al., 2015; Horner, et al., 2016) that were not analysed in the frame of this study.

Therefore, neither of the macroscopic signs of urethritis is pathognomonic for any particular disease or STI agent. In the case of negative results for urethritis-associated STIs, other less common causes of urethritis should be taken into account.

3. Impact of STIs on inflammation in reproductive tract

3.1. Detection of inflammation in different specimens

Table 6 presents the distribution of inflammation in the case of different STI agents in semen and first-voided urine that were used to test the inflammatory process.

There was a clear difference between the inflammatory parameters between FVU and semen. In the case of urine, the inflammatory reaction was generally present in the case of *C. trachomatis, N. gonorrhoeae*, *M. genitalium,* or in the case of their combination compared with STI negative patients. However, when seminal fluid's parameters are concerned, the difference between *C. trachomatis*-positive and STI-negative patients was still present, albeit with a smaller magnitude and without statistical significance – either in the case of seminal leucocytes' concentration or in the case of seminal plasma IL-6 level

(Table 6). Some other studies also do not support the association of *C. trachomatis* with leucocytospermia (Kjaergaard, et al., 1997; de Barbeyrac, et al., 2006). However, other researchers do support an association between leucocytospermia and *C. trachomatis* infection (Hosseinzadeh, et al., 2004).

As for *T. vaginalis*, the inflammatory reaction in FVU was rather weak and occurred rarely. We could not analyse the magnitude of inflammation in semen in the case of *T. vaginalis* or *N. gonorrhoeae*, as there were no cases with these STI agents in our study with available semen analyses.

3.2. Detection of inflammation in urine with different methods

The distribution of STI-positive cases according to the leukocyte count in first-voided urine analysed by dipstick test was studied in Paper I (Table 2 in Paper I). The flow-cytometric analysis of inflammatory reaction in FVU depending on STI pathogen was performed in Paper II (Figure 1 and Table 3 in Paper II). The comparison of dipstick test and flow-cytometry for FVU assessment are presented in Table 7.

Table 6. Comparison of inflammatory reaction among different STI agents in semen and first-voided urine using different methods.

Parameter (study)	Monoinfections				Combined infections				STI negative
	CT	NG	MG	TV	CT + NG	CT + MG	NG + MG	CT + NG + MG	
Dipstick test of FVU									
Concentration of leucocytes per one µl of FVU ** analysis by dipstick test. Paper I).	100	500	62.5	0	500	50	500	NA	0
Number (N) of study subjects	N = 118	N = 22	N = 36	N = 6	N = 7	N = 2	N = 2		N = 632
Flow cytometry of FVU									
Concentration of leucocytes per one µl of FVU * (analysis by flow cytometer. Paper II).	222.3; (0.9–2400.2); 67.7; 460.9 [B, D]	2067.2; (12.8–7168.1); 1146.0; 4929.7 [A, D, E, F]	108.9; (1.4–2034.1); 36.4; 343.2 [C, E]	9.7; (1.3–666.5); 1.6; 18.7 [F]	3418.9; (3.6–13484.8); 1905.6; 6209.9	475.6; (82.7–821.4); 342.6; 596.8	3176.2	11.9	4.6; (0.0–14.9); 2.6; 7.2 [A, B, C]
Number (N) of study subjects	N = 196	N = 24	N = 64	N = 5	N = 11	N = 4	N = 1	N = 1	N = 192
Semen parameters									
Concentration of leucocytes in seminal fluid, in M/ml * (Paper III).	0.2; (0.0–1.4); 0.1; 0.7	NA	0.4; (0.0–10.7); 0.1; 2.6 [G]	NA	NA	NA	NA	NA	0.1; (0.0–68.3); 0.0; 0.3 [G]
Number (N) of study subjects	N = 14		N = 16						N = 1345
Concentration of IL-6 in seminal plasma, in ng/L * (Paper III).	24.8; (3.0–1330.0); 20.8; 66.2 [@@]		30.6; (9.2–406.0); 17.6; 84.5 [H]						18.2; (2.0–4928.0); 11.1; 32.3 [H, @]
Number (N) of study subjects	N = 14		N = 16						N = 1345

CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; MG, *Mycoplasma genitalium*; TV, *Trichomonas vaginalis*; STI, sexually transmitted infections; M, million; FVU, first-voided urine; IL-6, interleukin-6

* The parameter is represented as median (range), 25th centile, 75th centile, as data are with non-parametric distribution.

**Because the dipstick analysis gives the results as factor parameter (leucocytes concentration: 0 WBC/µl; 25 WBC/µl; 100 WBC/µl; 500 WBC/µl), the median value was calculated.

A, B, C, D, E, F – p-value <0.005 (Mann-Whitney test with Bonferroni correction for 10 tests [Control *vs. N. gonorrhoeae*, Control *vs. C. trachomatis*, Control *vs. M. genitalium*, Control *vs. T. vaginalis*, *N. gonorrhoeae vs. C. trachomatis*, *N. gonorrhoeae vs. M. genitalium*, *N. gonorrhoeae vs. T. vaginalis*, *C. trachomatis vs. M. genitalium*, *C. trachomatis vs. T. vaginalis*, *M. genitalium vs. T. vaginalis*]). G – p-value = 0.006 (Mann-Whitney test with Bonferroni correction for three tests [Control *vs. M. genitalium*, Control *vs. C. trachomatis*, *C. trachomatis vs. M. genitalium*]). H – p-value = 0.024 (Mann-Whitney test with Bonferroni correction for three tests [Control *vs. M. genitalium*, Control *vs. C. trachomatis, C. trachomatis vs. M. genitalium*]).

[@] Data accounting for IL-6 in seminal plasma are not available for 36 patients.

[@@] Data accounting for IL-6 in seminal plasma are not available for one patient.

Table 7. The distribution of STI-positive cases according to the inflammatory reaction in first-voided urine.

STI agent	Percentage (number) of patients with inflammation in FVU (dipstick test[#], Paper I)	Percentage (number) of patients with inflammation in FVU (flow-cytometry[##], Paper II)
Monoinfections and combinations	73,6% (142) [A]	92.5% (283) [A]
Monoinfections only	72.5% (132) [B]	92.7% (268) [B]
N. gonorrhoeae	100.0% (22)	95.8% (23)
C. trachomatis	72.0% (85) [C]	93.9% (184) [C]
M. genitalium	63.9% (23) [D]	92.2% (59) [D]
T. vaginalis	33.3% (2)	40.0% (2)
Combinations only	90.9% (10) *	88.2% (15) **

FVU, first-voide urine; STI, sexually transmitted infection
* These combinations included seven CT+NG, two NG+MG, and one CT+MG.
** These combinations included ten CT+NG, four CT+MG, and one MG+NG.
# For dipstick test, FVU was considered inflammatory if ≥25 WBC/µl.
For flow-cytometry, FVU was considered inflammatory if WBC ≥ 15/µl and bacteria ≥ 20/µl.
P-values were calculated using Fisher's exact test.
A – p-value = 1.29×10^{-8}
B – p-value = 4.77×10^{-9}
C – p-value = 1.99×10^{-7}
D – p-value = 7.79×10^{-4}

While a urine dipstick test gives only limited quantitative information about an inflammatory reaction in urine, dividing the results into five discrete categories, a flow-cytometry method gives continuous quantitative information about an inflammatory reaction in the urine as well as counts of bacteria.

In general, flow-cytometry indicated inflammation in a significantly higher number of patients than a dipstick test in the case of *C. trachomatis* (21.9% more sensitive), *M genitalium* (28.3% more sensitive) and *T. vaginalis* (6.7% more sensitive). In the case of *N. gonorrhoeae* and combinations, a minor insignificant superiority of the dipstick test was seen (Table 7).

The ranking of STI pathogens according to the magnitude of inflammation in FVU is the same for the flow-cytometry and for the dipstick test (Table 6): the strongest inflammatory reaction is seen in the case of *N. gonorrhoeae*, followed by *C. trachomatis* and *M. genitalium*, the weakest inflammatory reaction is observed in the case of *T. vaginalis*. What stands out in Table 7 is absolutely the

same order for STI pathogens independent of diagnostic method used: the percentage of patients with confirmed inflammatory reaction in FVU is the highest for *N. gonorrhoeae*, followed by *C. trachomatis* and *M. genitalium*, and the lowest for *T. vaginalis*. This is a rather remarkable outcome that STI pathogens can not only be ranked similarly for potency to provoke the inflammation in FVU but also for the proportion of patients involved in inflammatory reaction in FVU, independently of the study technique used (dipstick test or flow-cytometry) (see Figure 3).

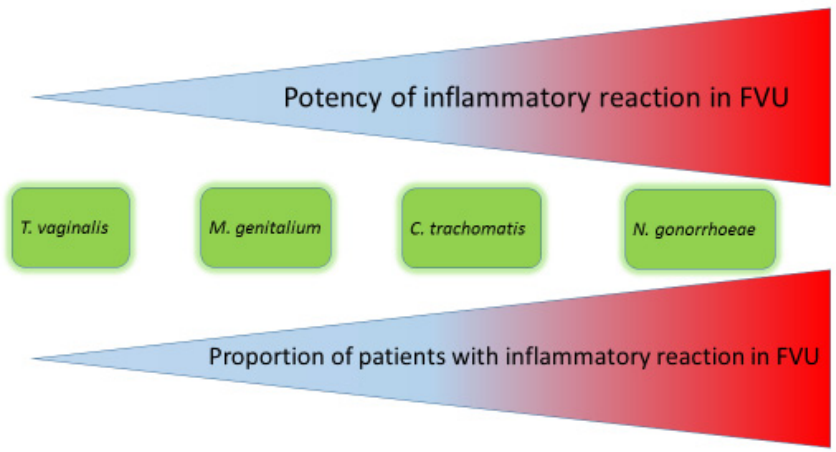

Figure 3. Rating of STI agents according to potency of inflammatory reaction in FVU and proportion of patients with inflammatory reaction in FVU.

3.3. Proposed cut-off values for seminal inflammatory parameters to predict STIs

We tried to find the best cut-off value to predict *C. trachomatis* and/or *M. genitalium* infection using semen concentration of neutrophils as a descriptive variable (Paper III). Using different previously proposed (Punab, et al., 2003; Gdoura, et al., 2008, b) cut-off levels for seminal neutrophils' concentrations, the proportion of STI-positive/-negative patients changed but not significantly (Supplementary Table S1 in Paper III).

We found the best cut-off value for semen neutrophils at a concentration of 0.28 million/ml for predicting *C. trachomatis* and/or *M. genitalium* infection with sensitivity 58.0%, specificity 71.3%, positive prognostic value 1.3%, negative prognostic value 95.6%, and area under the curve 0.691. For seminal IL-6, the optimal cut-off value predicting presence or absence of *C. trachomatis* and/or *M. genitalium* infection was 17.5 ng/l with sensitivity 75.5%, specificity 47.8%, positive prognostic value 1.2%, negative prognostic value 96.8%, and area under the curve 0.644 (Figure 4).

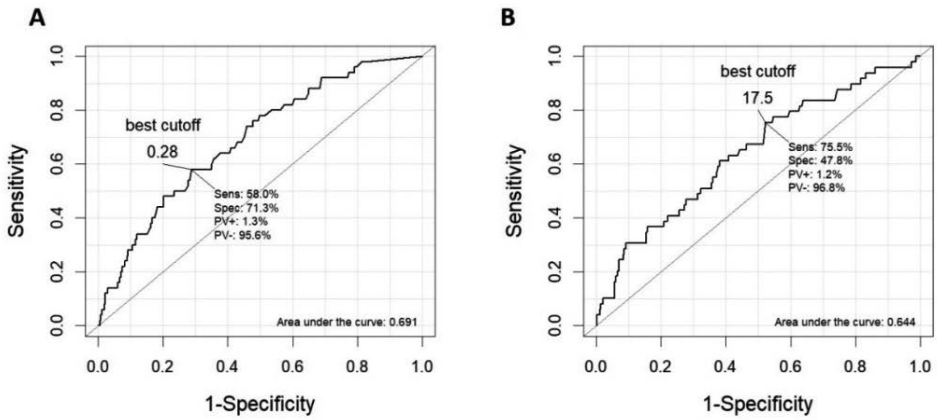

Figure 4. Receiver operating curves for semen leucocyte concentration (A) and semen IL-6 concentration (B). Both unselected fertile and infertile group, without exclusion of patients for hormonal, testicular volume and infertility causal factors.
Abbreviations: Sens, sensitivity; Spec, specificity; PV+, positive prognostic value; PV–, negative prognostic value.

The cut-off level for predicting *M. genitalium* and/or *C. trachomatis* infection proposed by us (0.28 million leukocytes per ml) is lower than 1 million/ml proposed by the WHO (World Health Organization, 2010). The suggestion to lower the cut-off level for leucocytospermia was also supported by our previous study (Punab, et al., 2003) as well as by some other studies (Gdoura, et al., 2008, b; Kopa, et al., 2005). Although the prevailing number of papers have been written on seminal IL-6, practically none have included a section relating to STI directly. Minor information regarding IL-6 and STIs is available in the literature (Kokab, et al., 2010; Dehghan Marvast, et al., 2016). To the best of our knowledge, there are no official proposals for seminal IL-6 cut-off values in regards to STI diagnosing.

3.4. Detection of inflammation in semen with different methods

The detection of inflammation in semen with different methods (detection of WBC count and IL-6 level) was addressed in Paper III. We made these analyses of both infertile and fertile groups and analysed these groups together because the total number of STI-positive patients among fertile patients was considerably low (only four *C. trachomatis* cases).

Both methods, measuring seminal IL-6 and counting semen leucocyte concentration, were able to evaluate the inflammatory reaction in the semen but they did not perfectly indicate the possibility of STI presence (Table 8). Nearly 40% of STI cases could remain undiagnosed as there is no elevation of seminal WBC concentration or seminal IL-6 level or only one parameter is elevated

while another parameter is in the normal range. Despite a good positive correlation between semen leucocyte concentration and seminal plasma IL-6 concentration (rho = 0.26, p-value < 2.2^{-16}), both markers of inflammation in semen tend to be unreliable for urethritis-associated STI diagnosis as nearly 40% of STI cases could be missed.

It has formerly been suggested that there is virtually no preference of the method chosen for the detection of inflammatory reaction in the semen as semen leucocyte concentration and seminal plasma IL-6 concentration are closely correlated (Krause, et al., 2003; Aghazarian, et al., 2013, 2015, 2019; Ausmees, et al., 2013; Comhaire, et al., 1994; Dehghan Marvast, et al., 2016; Eggert-Kruse, et al., 2001; Eldamnhoury, et al., 2018; Kokab, et al., 2010; Kopa, et al., 2005).

Table 8. Distribution of STIs according to semen leucocyte concentration and seminal IL-6 level (data is taken from Paper III).

STI status	WBCs in semen ≥ 0.2 M/ml		WBCs in semen < 0.2 M/ml	
	Seminal plasma IL-6 ≥ 17.5 ng/l	Seminal plasma IL-6 < 17.5 ng/l	Seminal plasma IL-6 ≥ 17.5 ng/l	Seminal plasma IL-6 < 17.5 ng/l
STI negatives (N = 2133) #	22.8% (486)	15.3% (327)	29.7% (634)	32.2% (686)
***C. trachomatis* positives (N = 27) ##**	55.6% (15)	3.7% (1)	18.5% (5)	22.2% (6)
***M. genitalium* positives (N = 22)**	59.1% (13)	9.1% (2)	18.2% (4)	13.6% (3)
***C. trachomatis* AND/OR *M. genitalium* positives (N = 49) ##**	57.1% (28)	6.1% (3)	18.4% (9)	18.4% (9)

M, million; WBCs, white blood cells; STI, sexually transmitted infection; IL-6, interleukin-6

The proposed seminal WBC concentration cut-off is taken from Punab *et al.* (2003). The proposed seminal IL-6 cut-off is taken from Paper III (see also section 7.4 in the text of the dissertation).

Data about inflammatory reaction in semen are not available for 65 out of 2198 STI negative patients (four patients have no information about semen WBC and seminal plasma IL-6 concentration; one patient has no information about semen WBC concentration only; 60 patients have no information about seminal plasma IL-6 concentration only). Therefore, these patients are not included into the calculations.

Data about seminal IL-6 concentration are not available for one out of 28 *C. trachomatis*-positive patient with concentration of WBC in semen ≥ 0.2 M/ml. Therefore, this person is not included into the calculations.

3.5. Levels of inflammation in case of different causative agents

It was very interesting to observe ranking of STI pathogens according to the magnitude of inflammation in FVU (Table 6). The inflammatory reaction was always weak in the case of *T. vaginalis* monoinfection, followed by *M. genitalium* monoinfection with quite moderate inflammatory reaction in FVU. The stronger inflammatory reaction in FVU was observed in the case of *C. trachomatis* monoinfection. *N. gonorrhoeae* monoinfection finalises the list, being the undisputed leader in the magnitude of inflammatory reaction in FVU. The STI ranking persists unchanged irrespective of the measurement method used for FVU (flow-cytometry or dipstick test).

Unfortunately, we cannot make such a ranking using the parameters of the seminal fluid. Nevertheless, one observation still deserves to be highlighted. In the semen, the ranking of *M. genitalium* and *C. trachomatis* is different compared with FVU specimens, with *M. genitalium* having higher values of inflammatory markers in semen (both concentration of leucocytes in seminal fluid and concentration of IL-6 in seminal plasma).

4. Impact of STIs on semen quality

We addressed the issue of STIs' impact on semen quality in Paper III. The inflammatory process in semen attributed to a particular STI can be confounded by other factors. In order to eliminate possible confounding with known factors influencing semen quality, we additionally excluded 625 male patients from the main study group. The reasons and order of decision to additionally eliminate study subjects were defined according to the following criteria: (I) defined causal factors of male infertility according to Punab et al. (2017): genetic causes, secondary hypogonadism, congenital anomalies: systemic and/or in urogenital tract, serious sexual dysfunctions, oncological diseases, seminal tract obstruction, other testicular factors that include acquired testicular damage (TD) [exposure to high dose of radiation in Chernobyl, testis trauma with volume change, mumps orchitis, other orchitis, epididymitis, testicular torsion, hernia operation with ipsilateral TD, epididymal cyst operation with ipsilateral TD, hydrocele operation with ipsilateral TD, other testis operation with ipsilateral TD] and secondary testicular damage [anabolic steroids, medication (salasopyrin, trexan), status diagnosed post kidney transplantation]; (II) bitesticular volume < 30 ml for unexplained reason; (III) hormonal changes: FSH ≥ 8.0 U/l and/or LH > 9.4 U/l and/or FSH < 1.0 U/l and/or LH < 1.0 U/l and/or total testosterone < 10.5 nmol/l). Detailed information with references for elimination criteria is provided in Supplementary Tables S2 and S3 in Paper III.

After performing the abovementioned exclusion, there remained 1345 STI-negative, 14 *C. trachomatis*-positive and 16 *M. genitalium*-positive patients in the selected infertility group.

STI-positive patients (*C. trachomatis* and *M. genitalium* cases together) had significantly lower total counts of spermatozoa (151.1 million *vs.* 226.2 million,

p-value = 0.028) and total counts of spermatozoa with progressive motility (68.6 million *vs.* 101.8 million, p-value = 0.034) compared to STI-negative patients (Table 2 in Paper III). The total count of spermatozoa with normal morphology was also lower among STI-positive patients (7.9 million *vs.* 16.4 million), however, the observed difference was over the significance level (p-value = 0.105).

When STI-positive cases were analysed separately, all these differences were slightly over the significance level, due to small groups having one or the other infection.

5. Impact of STIs on blood PSA level

The impact of STI on blood PSA level was addressed in Paper III. We made the data analysis after exclusion of patients with possible confounding factors, such as hormonal, testicular volume, and infertility causal factors, as described in section 8. After performing the abovementioned data correction, we did not reveal any impact of *M. genitalium* and *C. trachomatis* on blood levels of PSA (Supplementary Table S4 in Paper III). Median (range) of PSA in case of *M. genitalium* was 0.89 (0.17–1.65 μg/l), in the case of *C. trachomatis* 0.67 (0.19–2.1 μg/l) and in STI-negative men 0.71 (0.12–5.43 μg/l).

Our data correspond with the data of Motrich *et al.* (2006) who did not find any impact of *C. trachomatis* on the PSA level. We did not find any studies evaluating the impact of *M. genitalium* on PSA.

GENERAL DISCUSSION

1. Diverse clinical findings in urethritis patients as a diagnostic challenge

In our STI prevalence study of high-risk heterosexual males (Paper I), two pathogens were associated with a higher number of complaints than others: *N. gonorrhoeae* and *T. vaginalis*. The main complaints in the case of gonorrhoea included persistent cloudy discharge (72.7%), followed by unpleasant sensations in the urethra during micturition (63.6%), and, in the case of trichomoniasis – persistent discomfort in the urethra (50%) and redness of the urethral opening (50%). The majority of these patients had at least one complaint (95.5% in the case of NG and 50% in the case of TV) but it should be noted that 22.7% of NG and 33.3% of TV patients had mild symptoms and 4.5% of NG and 50% of TV cases did not have any symptoms at all. Therefore, it is impossible to confidently predict a particular STI agent simply by relying on profile of patient complaints.

Conflicting results have been revealed by previous studies. In the case of *N. gonorrhoeae*, very low prevalence or absence of asymptomatic cases is reported in some studies (Taylor-Robinson, et al., 2009; Gaydos, et al., 2009; Gottesman, et al., 2017), while not in others (Bowden, et al., 1998; Carne, et al., 2013; Detels, et al., 2011). Different results from other studies in the case of *T. vaginalis* (Gimenes, et al., 2014, b; Poole & McClelland, 2013; Schwebke & Hook, 2003; Seña, et al., 2007) could be associated with a very low number of *T. vaginalis*-positive patients in our study. Yet half of these patients were asymptomatic in our research. This infection can be asymptomatic even in 70% of men, because of which a small proportion of men would seek the control in an andrological, urological or dermatological clinic (Gimenes, et al., 2014, b; Poole & McClelland, 2013; Schwebke & Hook, 2003; Seña, et al., 2007).

Complaint profiles for *M. genitalium* and *C. trachomatis* were similar to each other and milder than in the case of gonorrhoeae. Again, the clinical picture in the case of these atypical bacteria varied significantly, and some patients (18.6% of CT and 22.2% of MG cases) were symptom-free. The small proportion of asymptomatic patients in the case of *C. trachomatis* and *M. genitalium* is in accordance with some previous studies (Taylor-Robinson, et al., 2009; Bowden, et al., 1998; Bradshaw, et al., 2006; Falk, et al., 2004; Gottesman, et al., 2017; Högdahl & Kihlström, 2007), but differs from others (Carne, et al., 2013; Detels, et al., 2011; Coble, et al., 2006; Sonnenberg, et al., 2015; Zheng, et al., 2014).

Macroscopic signs of urethritis were also not specific for particular STI agents. Namely, clear or cloudy urethral discharge, redness of the urethral opening, and balanitis/balanoposthitis presented virtually in every particular STI monoinfection and STI combinations, albeit in different proportions. This finding is in accordance with some previous studies (Jordan, et al., 2020), while

it contradicts other studies (Wetmore, et al., 2011; Ito, et al., 2016). In the case of combined STI, macroscopic signs of urethritis also varied.

In addition to asymptomatic patients who appeared to be STI positive, our study also revealed some STI-negative patients having different clinical findings (complaints, signs) and/or inflammation in first-voided and/or mid-stream urine. It is possible that these STI-negative patients could have some rare causes such as mechanical factors (Péc, et al., 1992) or another microbiological agents such as urinary tract pathogens (*Escherichia coli*, *Enterococcus*, *Klebsiella*, *Enterobacter* or *Proteus*), *Ureaplasma urealyticum*, herpesvirus, adenovirus, some respiratory tract pathogen (*Haemophilus*, *Neisseria meningitidis*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*) or bacterial vaginosis-associated organisms (Bachmann, et al., 2015; Horner, et al., 2016; Tan, et al., 2016; Dan, et al., 2012) that were not analysed in frame of this study.

Thus, clinical findings may vary considerably in the case of all four diseases studied by us. Therefore, the presence of specific complaints and macroscopic signs of urethritis does not automatically provide a clue to the particular STI agent nor does the absence of signs and symptoms guarantee absence of urethritis-associated STI in sexually active males, especially in the context of risky sexual behaviour. Hence, estimation of patients' sexual behaviour, and laboratory analyses with etiological evaluation of different STI agents are essential prerequisites for appropriate evaluation of patients in doubt of urethritis.

2. Methodological issues and proposals for revealing inflammatory reaction in urethritis patients

2.1. Inflammation in first-voided urine

Prior to the recently developed dipstick test and flow cytometry, the historically firstly applied microscopic method has generally been used for detection of inflammation in the urethra. The 2016 European guideline on the management of non-gonococcal urethritis suggests ≥5 polymorphonuclear leukocytes (PMNL) per microscopic HPF threshold for Gram-stained smear (GSS) (Horner, et al., 2016). Geisler *et al.* (2005) found that 6% of chlamydia-infected men had 1 to 4 and 12% had zero PMNL per oil-immersion field while these numbers were 1% and 5% for the men with gonorrhoea. The same ≥5 PMN/HPF threshold was only 63% sensitive and 77% specific for *C. trachomatis* infection according to Haddow *et al.* (2004). In a recent study by Sarier *et al*., the same threshold value demonstrated 92.9% sensitivity in the diagnosis of gonococcal urethritis (GU) while only 55.6% sensitivity in the diagnosis of non-gonococcal urethritis (NGU), but a threshold value of ≥2 PMNL/HPF reached 100% sensitivity for GU and 92.6% sensitivity for NGU (Sarier, et al., 2018). A study performed by Rietmeijer *et al.* showed that a significant 2.5-fold increase in chlamydia positivity (from 6.5% to 16.2%) occurred between the 1 and 2 PMNL/HPF strata supporting lowering the diagnostic criteria of the GSS

diagnosis of male urethritis (Rietmeijer & Mettenbrink, 2012). The results of this study were incorporated into the Sexually Transmitted Diseases Treatment Guidelines 2015 by the Centers for Disease Control and Prevention – the threshold for diagnosing urethritis using GSS was lowered from ≥5 to ≥2 PMNL/HPF (Workowski, et al., 2015). However, a subsequent large-scale study by Moi *et al.* did not support lowering the cut-off to ≥2 PMNL/HPF (Moi, et al., 2017, a). At the same time, a marked degree of both intra-observer and inter-observer variation has been found in the interpretation of the urethral GSS (Willcox, et al., 1981; Smith, et al., 2003), while the sensitivity of this method is also dependent upon the collection technique (the experience of the provider and the device utilised) (Bachmann, et al., 2015). Although both methylene blue/gentian violet stain and Gram stain can not only be used for detection of inflammation but also for direct microscopic gonococcal diagnostics, the specificity for *N. gonorrhoeae* is not 100%, as other *Neisseria* species may have an identical microscopic appearance (Genders, et al., 2013).

The microscopic method described above has some drawbacks – significant intra-observer and inter-observer variation (Willcox, et al., 1981; Smith, et al., 2003) and time consumption exists using this method. We therefore used dipstick test and flow cytometry. Our study revealed that flow cytometry of first-voided urine can be considered as a rapid and objective screening method in the case of suspected male urethritis. In addition, we showed that FVU flow-cytometry indicated inflammation in a significantly higher number of patients than the dipstick test in the case of *C. trachomatis* (21.9% more sensitive), *M genitalium* (28.3% more sensitive) and *T. vaginalis* (6.7% more sensitive). In comparison with a dipstick test that gives limited quantitative information about an inflammatory reaction in urine, dividing the results into five discrete categories (Leighton & Little, 1985; Tyndall, et al., 1994; Fraser, et al., 1995), the flow-cytometry method gives continuous quantitative information about an inflammatory reaction in the urine. The scarcity of studies examining the performance of flow cytometry in the diagnosis of urethritis precludes the establishment of international consensus on universal technique and threshold levels for this laboratory method (Grosso, et al., 2012; Ito, et al., 2014, a; Ito, et al., 2016; Pond, et al., 2015). UF-1000i flow cytometer has been found to be more sensitive in predicting the results of visual microscopy examination for leucocytes and bacteria than automated dipstick reflectometer Clinitek Atlas (Bayer Corp, Elkhart, USA) for first-morning, mid-stream urine (Jiang, et al., 2011).

Here we present to the reader some studies investigating the performance of flow cytometry of FVU in diagnostics of urethritis. In a study by Grosso *et al.* (2012), a urethral secretion mixed with transport medium was used that is not comparable with our method. In a study by Ito *et al.*, a cut-off point of leukocyte counts of 12.5 WBCs/µl resulted in a sensitivity of 86.9% and specificity of 88.6% for predicting chlamydial infection in asymptomatic men (Ito, et al., 2014, a). Men positive for *C. trachomatis* (n = 84) had the range 1.8–1666.9 WBCs/µl (median, 43.3 WBCs/µl) in their FVU, which is lower

compared with our results (median 222.3 WBCs/µl, range 0.9–2400.2 WBCs/µl). In another study assessing men with symptoms and signs compatible with acute urethritis the inflammatory reaction in FVU assessed by Sysmex UF-1000i flow-cytometer was the following (range and median): 3.9–1918.1 WBCs/µl and 199.0 WBCs/µl for *C. trachomatis* (n = 110), 6.9–580.3 WBCs/µl and 185.3 WBCs/µl for *M. genitalium* (n = 22) (Ito, et al., 2016). We found a wider WBC range (1.4–2034.1 WBCs/µl) and a lower median value (108.9 WBCs/µl) for *M. genitalium* in our study. In addition, the results regarding gonococcal urethritis were not presented in the flow-cytometric study by Ito *et al.* (2016). Pond *et al.* (2015) have tested this method in MG and CT patients and found its sensitivity to be slightly lower than in our study. Therefore, our research is the first to evaluate the performance of flow-cytometry for diagnosis of STI-related urethritis from first-voided urine, while taking into consideration four causative agents. The study gave strong evidence that first-voided urine is a suitable specimen for flow-cytometry analysis. The latter can be considered as a preliminary diagnosing method of urethritis, being a rapid and objective method with considerable sensitivity, devoid of intra-observer and inter-observer variation inherent to microscopic diagnostics, recommended for busy outpatient clinics.

We succeeded in showing that NG caused the highest inflammatory reaction in first-voided urine measured both by flow cytometry and dipstick method while CT and MG displayed moderate and TV showed weak inflammatory reaction in FVU measured by both methods. This implies that diagnosing urethritis-associated STIs relying only on inflammatory reaction in FVU can be misleading as some STI-positive cases may show no inflammatory reaction. In our study, up to 7.5% and 26.4% of all STI-positive cases using flow-cytometry or dipstick test, respectively, could have been missed if we had relied on our empirical diagnostic threshold (leucocytes < 15/µl and bacteria < 20/µl for flow cytometry, and WBC < 25/µl for dipstick test).

We have demonstrated that urethritis-associated STI pathogens were not only ranked similarly for potency to provoke the inflammation in FVU but also for the proportion of patients displaying inflammatory reaction in FVU, independently of the study technique used (dipstick test or flow-cytometry): the strongest inflammatory reaction and the largest percentage of patients with FVU inflammation was seen in the case of *N. gonorrhoeae*, followed by *C. trachomatis* and *M. genitalium*, the weakest inflammatory reaction and the smallest percentage of patients with FVU inflammation was observed in the case of *T. vaginalis*.

2.2. Inflammation in the semen

We now guide the attention of the reader to another aspect of STI – inflammatory changes in semen. We found higher semen leucocyte concentration among STI-positive patients compared with STI-negative patients. This difference principally arose from the group of *M. genitalium*-positive cases, while

among *C. trachomatis* cases there was only an elevated percentage of neutrophils among round cells in semen.

In the semen, the ranking of *M. genitalium* and *C. trachomatis* was different compared with FVU specimens, with *M. genitalium* having higher values of inflammatory markers in semen (both concentration of leucocytes in seminal fluid and concentration of IL-6 in seminal plasma). The differences observed could imply different pathogenic mechanisms of different urethritis-associated STI agents. Other studies have also shown different clinical and laboratory patterns of particular urethritis-associated STI agents in different specimens (Shahmanesh, 1989; Pate, et al., 2001; Falk, et al., 2004; Wiggins, et al., 2006), including semen (Al-Sweih, et al., 2012).

Our results showing a positive association between *M. genitalium* and leucocytospermia are contradictory to the study by Kjaergaard *et al.*, who did not find such an association (Kjaergaard, et al., 1997). The possibility of partial (methodical) artefact in the context of this study cannot be excluded (e.g. *M. genitalium* detection by in-house PCR, detection of *C. trachomatis* and *T. vaginalis* by culture methods).

Both our own and some other studies (Kjaergaard, et al., 1997; de Barbeyrac, et al., 2006) do not support the association of *C. trachomatis* with leucocytospermia. The only observed significant finding in *C. trachomatis*-positive men in our study was a higher percentage of neutrophils in seminal fluid among selected infertile group patients, but there was no significance for concentration of neutrophils in seminal fluid. Percentage of neutrophils in seminal fluid is a ratio parameter and there is no officially accepted reference level for this parameter assessing seminal inflammatory process. For this reason, we cannot use this parameter for a reliable investigation of seminal inflammation. However, some other researchers found an association between leucocytospermia and *C. trachomatis* infection (Hosseinzadeh, et al., 2004). One possible explanation for the observed differences in the association of chlamydia with pyospermia across different studies could be the inability to discriminate between recent and old chlamydia infection because a large proportion of *C. trachomatis*-positive patients can be asymptomatic. The addition of systemic serological tests into diagnostic armamentarium is also not useful for the same reason – antibodies elicited by *C. trachomatis* are long-lived and a positive antibody test will not distinguish a previous from a current infection (Schuppe, et al., 2017; Wagenlehner, et al., 2006).

Based on our study results we proposed the optimal cut-off level for predicting *M. genitalium* and/or *C. trachomatis* infection to be 0.28 million leucocytes per ml of semen. This value is lower than 1 million/ml proposed by the WHO (World Health Organization, 2010). The suggestion to lower the cut-off level for leucocytospermia was also supported by some other studies (Punab, et al., 2003; Gdoura, et al., 2008, b).

Besides neutrophils' count, we also assessed seminal interleukin-6 as a potential additional inflammatory marker in our study. In some previous studies, seminal IL-6 has been positively correlated with seminal WBCs (Aus-

mees, et al., 2013; Kopa, et al., 2005). We observed significantly higher IL-6 levels for *M. genitalium*-positive patients compared with STI-negative patients. According to our best knowledge, our study is the first to report such an association. In addition, we found only two studies that observed the relationship between seminal IL-6 and *C. trachomatis* in men, and both studies found elevated IL-6 in *C. trachomatis*-positive men (Kokab, et al., 2010; Dehghan Marvast, et al., 2016). We could not find any studies analysing seminal IL-6 in case of TV on NG infection.

Thus, taking into consideration the results of our studies and the previous studies, we can suggest that flow-cytometry analysis of first-voided urine can be considered as a rapid and objective diagnosis method of urethritis in men. At the same time, the absence of inflammatory reaction in FVU still does not fully exclude the possibility of urethritis-associated STI. Therefore, STI PCR etiological diagnosis is recommended in this situation. As concerns semen analysis then following the current WHO cut-off level for the diagnosis of seminal inflammation does not permit us to reliably exclude the possibility of infection. The presence of seminal leucocytes in concentration of ≥ 0.28 million leucocytes per ml of semen should guide the physician to additional diagnostic tests in order to eliminate the possibility of infection in the male genital tract, including urethritis-associated STIs.

3. Impact of urethritis-causing STIs on male reproductive system

Impact of STIs on the female reproductive system has been investigated significantly more than that of the male reproductive system. To date, there are insufficient data to negotiate a clear consensus on this topic.

Previous *in vitro* studies have confirmed the impact of chlamydia on sperm cells while *in vivo* studies are controversial (Redgrove & McLaughlin, 2014). Some studies (Dieterle, et al., 1995; de Barbeyrac, et al., 2006; Hosseinzadeh, et al., 2004; Dehghan Marvast, et al., 2016; Motrich, et al., 2006; Gimenes, et al., 2014, a) do not support an impact of *C. trachomatis* on sperm motility while other studies have revealed the negative impact of *C. trachomatis* on sperm concentration, motility, and morphology (Witkin, et al., 1993; Rybar, et al., 2012). In our study, we found a non-significantly lowered count of spermatozoa while no effect on sperms' progressive motility.

As concerns MG, there was also a clear trend in our study toward lower concentration, lower total count of spermatozoa, lower percentage of spermatozoa with progressive motility, and lower percentage of sperms with normal morphology. However, the observed difference between the groups was once again slightly over the significance level. Perhaps the reason for this can be a relatively small number of *M. genitalium* patients, inherently high variability of semen parameters, and decreased power of statistical tests consequently. Impact of *M. genitalium* on semen parameters was assessed in previous studies and the results again tend to be controversial. There are only a few studies from the

western world addressing the impact of MG on semen parameters. Some studies have revealed no impact of *M. genitalium* on semen parameters (Kjaergaard, et al., 1997; Gimenes, et al., 2014, a), while Yan *et al.* (2018) reported its negative impact on the progressive motility of spermatozoa.

We performed an additional analysis handling both atypical bacteria together. This analysis gave statistically significant results: there was a lower total count of spermatozoa and a lower count of spermatozoa with progressive motility among CT- or MG-positive subjects. It is a significant indication that STIs do have negative impact on spermatozoa, however further investigations should be conducted in larger cohorts or other study populations where the prevalence of STIs is supposed to be higher (i.e. young sexually active men).

We could not assess the impact of NG and TV on semen parameters as there were no cases in our substudy. There are a few *in vivo* studies concerning the impact of *N. gonorrhoeae* on semen parameters. No significant impact was seen in semen analysis (semen volume, sperm count, motility, velocity, and normal morphology) of asymptomatic men with gonorrhoea in the study by Pérez-Plaza et al. (1982) and in the study by Qing et al. (2017). One contradictory study has also been published – Rivera *et al.* (2022) found that the samples positive for *N. gonorrhoeae* had a significantly greater percentage of spermatozoa with progressive motility.

Clinical *in vivo* studies about TV's impact on semen are also scarce. Gopalkrishnan *et al.* (1990) showed that the seminal fluid viscosity and percentage particulate debris was increased significantly in the *T. vaginalis* group. Motility of spermatozoa and number of morphologically normal forms were also decreased significantly, spermatozoa viability was altered, and there was a significant change in membrane integrity as shown by the hypoosmotic swelling test. Gimenes *et al.* found a statistically insignificant tendency between *T. vaginalis* and necrospermia (Gimenes, et al., 2014, a).

4. Limitations of the study and future research

There were several limitations to our study.

We did not investigate some rare causes of urethritis such as mechanical factors (Péc, et al., 1992) or other microbiological agents such as *Escherichia coli* (Dan, et al., 2012), *Ureaplasma urealyticum*, viruses, some respiratory tract pathogens (*Haemophilus species*, *Neisseria meningitides*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*) or bacterial vaginosis-associated organisms (Bachmann, et al., 2015; Horner, et al., 2016).

In the STI prevalence study of high-risk heterosexual males (Paper I) we did use the dipstick test for assessing inflammatory reaction in FVU. This technique can sometimes give false-positive results caused by low urine specific gravity and presence of oxalic acid or traces of oxidising agents (Horner & Taylor-Robinson, 2007), and its sensitivity is lower in comparison with flow cytometry.

In our flow cytometry evaluation case-control study (Paper II), the control group was quite small due to difficulties in performing the studies at the prostate level. The patients in the control group were somewhat older than the patients in the group of cases; however, this difference should not be clinically important. In addition, the number of cases with combined STI and TV mono-infection was too small to draw any meaningful conclusions. Despite the fact that the volume of first-voided urine could not be registered in the case of 87 individuals in the group of cases, we believe that this should not significantly distort the information about the total flow-cytometric count of bacteria and leucocytes. We could also observe that the median volume of first-voided urine in both groups was higher than recommended (despite the desired volume of 15–20 ml, the actual volume was 54–58 ml), but this reflects the fact that collecting a smaller volume of urine is difficult for patients. To mitigate the effects of this difficulty, we analysed both concentrations and total counts of WBCs and bacteria and obtained similar results. As we set up empirical flow-cytometric cut-off values for first-voided urine (inflammatory, if leucocytes $\geq$15/µl and bacteria $\geq$20/µl) to form the control group, we could not assess the specificity and make the analysis of the receiver operating characteristic curve (ROC-curve) for flow-cytometry in diagnosing urethritis-associated STIs. In cases where inflammation and/or bacterial concentration were low in first-voided urine (but the subsequent PCR test confirmed an STI agent) we could not fully exclude a patient-related (not reported) pre-analytical error in urine collection, e.g. mid-stream urine was collected instead of first-voided urine.

A major disadvantage of our STI prevalence study among male partners of infertile couples (Paper III) was the relatively small groups of *C. trachomatis*- and *M. genitalium*-positive patients, however we had some major advantages compared to the previous ones. First, we included the control group of fertile men. Secondly, to analyse the influence of STIs on semen parameters we formed a group of clearly defined idiopathic infertile cases where all related diseases, anatomical and hormonal factors with a potential effect or relation to semen parameters were filtered out. Thirdly, the diagnosis of STI was made with nucleic acid amplification tests. In addition, the low prevalence of STIs indicates a beneficial epidemiological situation in our country and points to targeting other groups with a higher prevalence of STIs (i.e. young sexually active men) to disentangle the multifactorial impact of particular infective agents on semen parameters. At the same time, the groups with fertility issues could serve as acceptable controls.

PSA has been proposed as a possible surrogate marker on studying causality for STI impact on prostate inflammation (Sutcliffe, et al., 2006, 2011). However, our attempt to use the PSA to that end failed. Some possible explanations for this may include the large age range of patients (18–50 years), elusive temporal factor (it is virtually impossible to distinct between recent and old infection because a large proportion of cases are asymptomatic; timeframes between urethral and prostatic infection, prostatic infection and blood PSA response), high variability of PSA value (partly because of the large age range)

and possibly, the factor of prostate volume (higher PSA values in patients with larger prostate), and low number of STI-positive patients. Due to these reasons, thorough statistical adjustment with enough power was impossible.

There is an urgent need to compare all the available laboratory methods for the diagnosis of urethritis (dipstick test, flow cytometry, and microscopy of urethral smears or FVU) to establish the contemporary gold standard. Perhaps, stratifying immune cells' subpopulations using flow cytometry could advance our understanding of the pathogenesis of urethritis. Also, new scientific methods such as metaproteomic analysis of urine sediments could help in disentangling this conundrum (Yu, et al., 2015).

The fact that we found *M. genitalium* cases among the male partners of infertile couples and the impact of this bacterium on semen parameters suggests the role of this bacterium in male impaired fertility and deserves further investigations in larger cohorts and/or other study populations with a higher prevalence of STIs. Additionally, some researchers have associated *M. genitalium* with chronic prostatitis (Mo, et al., 2016; Krieger & Riley, 2002; Mändar, et al., 2005), hence pointing to the need for further detailed investigations addressing possible caveats.

CONCLUSIONS

1) *C. trachomatis* has the highest prevalence among Estonian high-risk men but *M. genitalium* holds an important second place. Prevalence of NG, TV and combined STIs is lower. The prevalence of urethritis-associated STIs among male partners of infertile couples and male partners of pregnant women is low. *M. genitalium* should be obligatorily incorporated in standard diagnostic panel of male urethritis.
2) The highest number of complaints in male patients is associated with *N. gonorrhoeae* while half of *T. vaginalis* cases and nearly a fifth of *M. genitalium* and *C. trachomatis* cases are asymptomatic. Neither of the macroscopic signs of urethritis is pathognomonic for any particular disease or STI agent. Therefore, the abscence of symptoms alone does not eliminate the possibility of urethritis-associated STI. Behind symptomatology, the patient's sexual behaviour patterns should also be accounted for when making decision about STI testing.
3) *N. gonorrhoeae* has the highest potency to generate urethral inflammation followed by *C. trachomatis* and *M. genitalium*, while *T. vaginalis* shows nearly absent urethral inflammation. *M. genitalium* does provoke an inflammatory reaction in semen, but in most cases far below the WHO's proposed limits. This means that the current reference values for assessing seminal inflammation do not permit us to reliably exclude the possibility of infection while it could mislead the clinical handling of patient.
4) Based on our research, two recommendations can be made in order to improve the diagnostics of urethritis:
   a. flow cytometry of first-voided urine can be considered as a rapid and objective screening method in case of suspected male urethritis;
   b. the present WHO cut-off level for seminal inflammation diagnosis should be lowered in order to find out more patients who need treatment.
5) *M. genitalium* and *C. trachomatis* possess negative impact on semen parameters. Therefore, the impact of these atypical bacteria on male fertility cannot be ruled out. The impact of *N. gonorrhoeae* and *T. vaginalis* on seminal parameters could not be assessed in the frame of this research due to the absence of these pathogens in the male partners of infertile couples and male partners of pregnant women. No impact of STIs on PSA could be demonstrated. The more precise effect of these microorganisms on male reproductive function still requires further investigation.

REFERENCES

1. Abdul Gaffoor, P. M. (1986). Gonococcal tysonitis. *Postgraduate Medical Journal, 62*(731), 869–870. doi:https://doi.org/10.1136/pgmj.62.731.869
2. Abusarah, E. A., Awwad, Z. M., Charvalos, E., & Shehabi, A. A. (2013). Molecular detection of potential sexually transmitted pathogens in semen and urine specimens of infertile and fertile males. *Diagnostic Microbiology and Infectious Disease, 77*(4), 283–286. doi:https://doi.org/10.1016/j.diagmicrobio.2013.05.018
3. Adu-Sarkodie Y. (1995). Trichomonas vaginalis transmission in a family. *Genitourinary Medicine*, *71*(3), 199–200. doi:https://doi.org/10.1136/sti.71.3.199
4. Aghazarian, A., Huf, W., Pflüger, H., & Klatte, T. (2019). The association of seminal leucocytes, interleukin-6 and interleukin-8 with sperm DNA fragmentation: A prospective study. *Andrologia, 51*(11), e13428. doi:https://doi.org/10.1111/and.13428
5. Aghazarian, A., Plas, E., Stancik, I., Pflüger, H., & Lackner, J. (2011). New method for differentiating chronic prostatitis/chronic pelvic pain syndrome IIIA from IIIB involving seminal macrophages and monocytes. *Urology*, *78*(4), 918–923. doi:https://doi.org/10.1016/j.urology.2011.06.028
6. Aghazarian, A., Stancik, I., Huf, W., & Pflüger, H. (2015). Evaluation of Leukocyte Threshold Values in Semen to Detect Inflammation Involving Seminal Interleukin-6 and Interleukin-8. *Urology, 86*(1), 52–56. doi:https://doi.org/10.1016/j.urology.2015.04.012
7. Aghazarian, A., Stancik, I., Pflüger, H., & Lackner, J. (2013). Influence of pathogens and moderate leukocytes on seminal interleukin (IL)-6, IL-8, and sperm parameters. *International Urology and Nephrology, 45*(2), 359–365. doi:https://doi.org/10.1007/s11255-013-0400-8
8. Ahmadi, M. H., Mirsalehian, A., & Bahador, A. (2016). Association of Chlamydia trachomatis with infertility and clinical manifestations: a systematic review and meta-analysis of case-control studies. *Infectious Diseases, 48*(7), 517–523. doi:https://doi.org/10.3109/23744235.2016.1160421
9. Ahmadi, M. H., Mirsalehian, A., Gilani, M., Bahador, A., & Talebi, M. (2018). Improvement of semen parameters after antibiotic therapy in asymptomatic infertile men infected with Mycoplasma genitalium. *Infection, 46*(1), 31–38. doi:https://doi.org/10.1007/s15010-017-1075-3
10. Akira, S., Taga, T., & Kishimoto, T. (1993). Interleukin-6 in biology and medicine. *Advances in Immunology*, *54*, 1–78. doi:https://doi.org/10.1016/s0065-2776(08)60532-5
11. Ala-Almohadesin, A., Mohammadbeygi, M., Bahavar, A., Mohammadi, M. A., Mohamadzadeh, N., Abolhasani, M., & Dabiri, H. (2019). Molecular Detection of Pathogens Causing Sexually Transmissible Infections in Patients with Prostate Cancer and Hyperplasia by Quantitative TaqMan Real-Time PCR Assay. *Clinical Laboratory, 65*(7). doi:https://doi.org/10.7754/Clin.Lab.2019.181243
12. Al-Sweih, N. A., Al-Fadli, A. H., Omu, A. E., & Rotimi, V. O. (2012). Prevalence of Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma urealyticum infections and seminal quality in infertile and fertile men in Kuwait. *Journal of Andrology, 33*(6), 1323–1329. doi:https://doi.org/10.2164/jandrol.111.013821
13. Amar A. D. (1967). Probable Trichomonas vaginalis epididymitis. *JAMA*, *200*(5), 417–418.

14. Attia, H., Finocchi, F., Orciani, M., Mehdi, M., Zidi Jrah, I., Lazzarini, R., Balercia, G., & Mattioli Belmonte, M. (2021). Pro-inflammatory cytokines and microRNAs in male infertility. *Molecular Biology Reports*, *48*(8), 5935–5942. doi:https://doi.org/10.1007/s11033-021-06593-6
15. Azariah, S., & Reid, M. (2000). Adenovirus and non-gonococcal urethritis. *International Journal of STD & AIDS, 11*(8), 548–550. doi:https://doi.org/10.1258/0956462001916308
16. Aumüller, G., & Riva, A. (1992). Morphology and functions of the human seminal vesicle. *Andrologia, 24*(4), 183–196. doi:https://doi.org/10.1111/j.1439-0272.1992.tb02636.x
17. Ausmees, K., Korrovits, P., Timberg, G., Erm, T., Punab, M., & Mändar, R. (2014). Semen quality in middle-aged males: associations with prostate-specific antigen and age-related prostate conditions. *Human Fertility*, *17*(1), 60–66. doi:https://doi.org/10.3109/14647273.2014.881563
18. Ausmees, K., Korrovits, P., Timberg, G., Punab, M., & Mändar, R. (2013). Semen quality and associated reproductive indicators in middle-aged males: the role of non-malignant prostate conditions and genital tract inflammation. *World Journal of Urology, 31*(6), 1411–1425. doi:https://doi.org/10.1007/s00345-013-1078-3
19. Avolio, M., De Rosa, R., Modolo, M. L., Stano, P., & Camporese, A. (2014). When should adenoviral non-gonococcal urethritis be suspected? Two case reports. *New Microbiologica, 37*(1), 109–112.
20. Bachmann, L. H., Manhart, L. E., Martin, D. H., Seña, A. C., Dimitrakoff, J., Jensen, J. S., & Gaydos, C. A. (2015). Advances in the Understanding and Treatment of Male Urethritis. *Clinical Infectious Diseases, 61*(Suppl. 8), S763–S769. doi:https://doi.org/10.1093/cid/civ755
21. Badalyan, R. R., Fanarjyan, S. V., & Aghajanyan, I. G. (2003). Chlamydial and ureaplasmal infections in patients with nonbacterial chronic prostatitis. *Andrologia, 35*(5), 263–265. doi: https://doi.org/10.1046/j.1439-0272.2003.00582.x
22. Bai, S., Li, Y., Hu, M. H., Wu, L., Shui, L. J., Wang, X. H., Liu, Y. X., Yue, Q. L., Yu, L. N., Fu, K. Q., Tong, X. H., Hu, X. C., & Xu, B. (2022). Association of sexually transmitted infection with semen quality in men from couples with primary and secondary infertility. *Asian Journal of Andrology*, *24*(3), 317–322. doi:https://doi.org/10.4103/aja202164
23. Bai, S., Li, Y., Wan, Y., Guo, T., Jin, Q., Liu, R., Tang, W., Sang, M., Tao, Y., Xie, B., Zhao, Y., Li, W., Xu, X., Yue, Q., Hu, X., & Xu, B. (2021). Sexually transmitted infections and semen quality from subfertile men with and without leukocytospermia. *Reproductive Biology and Endocrinology*, *19*(1), 92. doi:https://doi.org/10.1186/s12958-021-00769-2
24. Banyra, O., Nikitin, O., & Ventskivska, I. (2019). Acute epididymo-orchitis: relevance of local classification and partner's follow-up. *Central European Journal of Urology*, *72*(3), 324–329. doi:https://doi.org/10.5173/ceju.2019.1973
25. Barbonetti, A., Calogero, A. E., Balercia, G., Garolla, A., Krausz, C., La Vignera, S., Lombardo, F., Jannini, E. A., Maggi, M., Lenzi, A., Foresta, C., & Ferlin, A. (2018). The use of follicle stimulating hormone (FSH) for the treatment of the infertile man: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS). *Journal of Endocrinological Investigation*, *41*(9), 1107–1122. doi:https://doi.org/10.1007/s40618-018-0843-y
26. Bartoletti, R., Wagenlehner, F. M., Bjerklund Johansen, T. E., Köves, B., Cai, T., Tandogdu, Z., & Bonkat, G. (2019). Management of Urethritis: Is it Still the Time

for Empirical Antibiotic Treatments? *European Urology Focus, 5*(1), 29–35. doi:https://doi.org/10.1016/j.euf.2018.10.006
27. Basu, S., Aballa, T. C., Ferrell, S. M., Lynne, C. M., & Brackett, N. L. (2004). Inflammatory cytokine concentrations are elevated in seminal plasma of men with spinal cord injuries. *Journal of Andrology*, *25*(2), 250–254. doi:https://doi.org/10.1002/j.1939-4640.2004.tb02785.x
28. Batra, S. K. (1974). Sperm transport through vas deferens: review of hypotheses and suggestions for a quantitative model. *Fertility and Sterility, 25*(2), 186–202. doi:https://doi.org/10.1016/s0015-0282(16)40220-7
29. Beeton, M. L., Payne, M. S., & Jones, L. (2019). The Role of Ureaplasma spp. in the Development of Nongonococcal Urethritis and Infertility among Men. *Clinical Microbiology Reviews, 32*(4), e00137-18. doi:https://doi.org/10.1128/CMR.00137-18
30. Benchimol, M., de Andrade Rosa, I., da Silva Fontes, R., & Burla Dias, A. J. (2008). Trichomonas adhere and phagocytose sperm cells: adhesion seems to be a prominent stage during interaction. *Parasitology Research, 102*(4), 597–604. doi:https://doi.org/10.1007/s00436-007-0793-3
31. Berger, R. E., Alexander, E. R., Harnisch, J. P., Paulsen, C. A., Monda, G. D., Ansell, J., & Holmes, K. K. (1979). Etiology, manifestations and therapy of acute epididymitis: prospective study of 50 cases. *Journal of Urology, 121*(6), 750–754. doi:https://doi.org/10.1016/s0022-5347(17)56978-5
32. Berger, R. E., Alexander, E. R., Monda, G. D., Ansell, J., McCormick, J., & Holmes, K. K. (1978). Chlamydia trachomatis as a cause of acute "idiopathic" epididymitis. *The New England Journal of Medicine, 298*(6), 301–304. doi:https://doi.org/10.1056/NEJM197802092980603
33. Berger, R. E., Holmes, K. K., Mayo, M. E., & Reed, R. (1980). The clinical use of epididymal aspiration cultures in the management of selected patients with acute epididymitis. *The Journal of Urology, 1*, 60–61. doi:https://doi.org/10.1016/s0022-5347(17)55294-5
34. Berger, R. E., Kessler, D., & Holmes, K. K. (1987). Etiology and manifestations of epididymitis in young men: correlations with sexual orientation. *The Journal of Infectious Diseases, 155*(6), 1341–1343. doi:https://doi.org/10.1093/infdis/155.6.1341
35. Berntsson, M., Löwhagen, G. B., Bergström, T., Dubicanac, L., Welinder-Olsson, C., Alvengren, G., & Tunbäck, P. (2010). Viral and bacterial aetiologies of male urethritis: findings of a high prevalence of Epstein-Barr virus. *International Journal of STD & AIDS, 21*(3), 191–194. doi:https://doi.org/10.1258/ijsa.2009.009262
36. Bhasin, S., Brito, J. P., Cunningham, G. R., Hayes, F. J., Hodis, H. N., Matsumoto, A. M., Snyder, P. J., Swerdloff, R. S., Wu, F. C., & Yialamas, M. A. (2018). Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism*, *103*(5), 1715–1744. doi:https://doi.org/10.1210/jc.2018-00229
37. Bielecki, R., Ostaszewska-Puchalska, I., Zdrodowska-Stefanow, B., Baltaziak, M., Skawrońska, M., & Sokołowska, M. (2020). The presence of Chlamydia trachomatis infection in men with chronic prostatitis. *Central European Journal of Urology, 73*(3), 362–368. doi:https://doi.org/10.5173/ceju.2020.0040
38. Bishop, M. C. (2006). *Prostatitis. In: Schill W.-B., Comhaire F.H., Hargreave T. B. Andrology for the Clinician.* Berlin Heidelberg: Springer-Verlag.

39. Blahd, M. E. (1918). The Surgical Treatment of Gonorrheal Epididymitis. *The Journal of Urology, 2*(4), 321–324. doi:https://doi.org/10.1016/S0022-5347(17)74208-5
40. Boeri, L., Pederzoli, F., Capogrosso, P., Abbate, C., Alfano, M., Mancini, N., Clementi, M., Montanari, E., Montorsi, F., & Salonia, A. (2020). Semen infections in men with primary infertility in the real-life setting. *Fertility and Sterility*, *113*(6), 1174–1182. doi:https://doi.org/10.1016/j.fertnstert.2020.01.034
41. Boisvert, J. F., Koutsky, L. A., Suchland, R. J., & Stamm, W. E. (1999). Clinical features of Chlamydia trachomatis rectal infection by serovar among homosexually active men. *Sexually Transmitted Diseases*, *26*(7), 392–398. doi:https://doi.org/10.1097/00007435-199908000-00006
42. Boivin, J., Bunting, L., Collins, J. A., & Nygren, K. G. (2007). International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Human Reproduction, 22*(6), 1506–1512. doi:https://doi.org/10.1093/humrep/dem046
43. Bonner, M., Sheele, J. M., Cantillo-Campos, S., & Elkins, J. M. (2021). A Descriptive Analysis of Men Diagnosed With Epididymitis, Orchitis, or Both in the Emergency Department. *Cureus*, *13*(6), e15800. doi:https://doi.org/10.7759/cureus.15800
44. Boscolo-Berto, R., Iafrate, M., & Viel, G. (2010). Forensic implications in self-insertion of urethral foreign bodies. *The Canadian Journal of Urology, 17*(1), 5026–5027.
45. Boscolo-Berto, R., Siracusano, S., Porzionato, A., Polguj, M., Porcaro, A. B., Stecco, C., Macchi, V., & De Caro, R. (2020). The underestimated posterior lymphatic drainage of the prostate: An historical overview and preliminary anatomical study on cadaver. *The Prostate*, *80*(2), 153–161. doi:https://doi.org/10.1002/pros.23927
46. Bowden, F. J. (1998). Reappraising the value of urine leukocyte esterase testing in the age of nucleic acid amplification. *Sexually Transmitted Diseases, 25*(6), 322–326. doi:https://doi.org/10.1097/00007435-199807000-00010
47. Brackett, N. L., Cohen, D. R., Ibrahim, E., Aballa, T. C., & Lynne, C. M. (2007). Neutralization of cytokine activity at the receptor level improves sperm motility in men with spinal cord injuries. *Journal of Andrology*, *28*(5), 717–721. doi:https://doi.org/10.2164/jandrol.106.002022
48. Bradshaw, C. S., Denham, I. M., & Fairley, C. K. (2002). Characteristics of adenovirus associated urethritis. *Sexually Transmitted Infections, 78*(6), 445–447. doi:https://doi.org/10.1136/sti.78.6.445
49. Bradshaw, C. S., Tabrizi, S. N., Read, T. R., Garland, S. M., Hopkins, C. A., Moss, L. M., & Fairley, C. K. (2006). Etiologies of nongonococcal urethritis: bacteria, viruses, and the association with orogenital exposure. *The Journal of Infectious Diseases, 193*(3), 336–345. doi:https://doi.org/10.1086/499434
50. Breyer, B. N., Huang, W. Y., Rabkin, C. S., Alderete, J. F., Pakpahan, R., Beason, T. S., Kenfield, S. A., Mabie, J., Ragard, L., Wolin, K. Y., Grubb, R. L., 3rd, Andriole, G. L., & Sutcliffe, S. (2016). Sexually transmitted infections, benign prostatic hyperplasia and lower urinary tract symptom-related outcomes: results from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. *BJU International*, *117*(1), 145–154. do:https://doi.org/10.1111/bju.13050
51. Brooks, T., Zreick, J., & Iocca, A. (2013). Urinary obstruction from sexual practice involving magnetized beads inserted in the male urethra. *CMAJ: Canadian*

Medical Association Journal = Journal de l'Association Medicale Canadienne, 185(18), 1597–1598. doi:https://doi.org/10.1503/cmaj.130397

52. Bryan, E. R., Kim, J., Beagley, K. W., & Carey, A. J. (2020). Testicular inflammation and infertility: Could chlamydial infections be contributing? *American Journal of Reproductive Immunology, 84*(3), e13286. doi:https://doi.org/10.1111/aji.13286
53. Bryan, E. R., McLachlan, R. I., Rombauts, L., Katz, D. J., Yazdani, A., Bogoevski, K., Chang, C., Giles, M. L., Carey, A. J., Armitage, C. W., Trim, L. K., McLaughlin, E. A., & Beagley, K. W. (2019). Detection of chlamydia infection within human testicular biopsies. *Human Reproduction*, *34*(10), 1891–1898. doi:https://doi.org/10.1093/humrep/dez169
54. Burgess, J. A. (1971). Gonococcal Tysonitis without urethritis after prophylactic post-coital urination. *The British Journal of Venereal Diseases, 47*(1), 40–41. doi:https://doi.org/10.1136/sti.47.1.40
55. Caini, S., Gandini, S., Dudas, M., Bremer, V., Severi, E., & Gherasim, A. (2014). Sexually transmitted infections and prostate cancer risk: a systematic review and meta-analysis. *Cancer Epidemiology, 38*(4), 329–338. doi:https://doi.org/10.1016/j.canep.2014.06.002
56. Camejo, M. I., Segnini, A., & Proverbio, F. (2001). Interleukin-6 (IL-6) in seminal plasma of infertile men, and lipid peroxidation of their sperm. *Archives of Andrology*, *47*(2), 97–101. doi:https://doi.org/10.1080/014850101316901280
57. Camejo M. I. (2003). Relation between immunosuppressive PGE(2) and IL-10 to pro-inflammatory IL-6 in seminal plasma of infertile and fertile men. *Archives of Andrology*, *49*(2), 111–116. doi:https://doi.org/10.1080/01485010390129232
58. Campbell, M. F. (1927). Gonococcus Epididymitis: observations in three thousand cases from the urological service of Bellevue hospital. *Annals of Surgery, 86*(4), 577–590. doi:https://doi.org/10.1097/00000658-192710000-00013
59. Campbell, M. F. (1931). Complications of gonorrhea: Periurethral abscess, stricture, arthritis. *The American Journal of Surgery, 12*(2), 277-281. doi:https://doi.org/10.1016/S0002-9610(31)90053-6.
60. Campos, S. C., Elkins, J. M., & Sheele, J. M. (2021). Descriptive analysis of prostatitis in the emergency department. *The American Journal of Emergency Medicine, 44*, 143–147. doi:https://doi.org/10.1016/j.ajem.2021.01.054
61. Carne, C. A., Gibbs, J., Delaney, A., Sonnex, C., Verlander, N. Q., Smielewska, A., Skeggs, E., Bentley, N., & Jalal, H. (2013). Prevalence, clinical features and quantification of genital non-viral infections. *International Journal of STD & AIDS, 24*(4), 273–277. doi:https://doi.org/10.1177/0956462412472306
62. Carter, J. D., & Hudson, A. P. (2010). The evolving story of Chlamydia-induced reactive arthritis. *Current Opinion in Rheumatology*, *22*(4), 424–430. doi:https://doi.org/10.1097/BOR.0b013e32833a43a2
63. Castiglione, R., Salemi, M., Vicari, L. O., & Vicari, E. (2014). Relationship of semen hyperviscosity with IL-6, TNF-α, IL-10 and ROS production in seminal plasma of infertile patients with prostatitis and prostato-vesiculitis. *Andrologia*, *46*(10), 1148–1155. doi:https://doi.org/10.1111/and.12207
64. Cazanave, C., Manhart, L. E., & Bébéar, C. (2012). Mycoplasma genitalium, an emerging sexually transmitted pathogen. *Medecine et Maladies Infectieuses, 42*(9), 381–392. doi:https://doi.org/10.1016/j.medmal.2012.05.006

65. Charles S. X. (1991). Epidemiology of trichomonas vaginalis (TV) in rural adolescent and juvenile children. *Journal of Tropical Pediatrics*, *37*(2), 90. doi:https://doi.org/10.1093/tropej/37.2.90
66. Chen, J. Z., Gratrix, J., Brandley, J., Smyczek, P., Parker, P., Read, R., & Singh, A. E. (2017). Retrospective Review of Gonococcal and Chlamydial Cases of Epididymitis at 2 Canadian Sexually Transmitted Infection Clinics, 2004-2014. *Sexually Transmitted Diseases, 44*(6), 359–361. doi:https://doi.org/10.1097/OLQ.0000000000000602
67. Chen, M. Y., & Donovan, B. (2003). Screening for genital chlamydia trachomatis infection: are men the forgotten reservoir?. *The Medical Journal of Australia*, *179*(3), 124–125. doi:https://doi.org/10.5694/j.1326-5377.2003.tb05466.x
68. Chirwa, M., Davies, O., Castelino, S., Mpenge, M., Nyatsanza, F., Sethi, G., Shabbir, M., & Rayment, M. (2021). United Kingdom British Association for Sexual Health and HIV national guideline for the management of epididymo-orchitis, 2020. *International Journal of STD & AIDS*, *32*(10), 884–895. doi:https://doi.org/10.1177/09564624211003761
69. Chrisment, D., Machelart, I., Wirth, G., Lazaro, E., Greib, C., Pellegrin, J. L., Bébéar, C., & Peuchant, O. (2013). Reactive arthritis associated with Mycoplasma genitalium urethritis. *Diagnostic Microbiology and Infectious Disease, 77*(3), 278–279. doi:https://doi.org/10.1016/j.diagmicrobio.2013.07.015
70. Chughtai, B., Sawas, A., O'Malley, R. L., Naik, R. R., Ali Khan, S., & Pentyala, S. (2005). A neglected gland: a review of Cowper's gland. *International Journal of Andrology, 28*(2), 74–77. doi:https://doi.org/10.1111/j.1365-2605.2005.00499.x
71. Coble, B. I., Nordahl-Akesson, E., Vinnerberg, A., & Kihlström, E. (2006). Urine-based testing for Chlamydia trachomatis using polymerase chain reaction, leucocyte esterase and urethral and cervical smears. *Scandinavian Journal of Clinical and Laboratory Investigation, 66*(4), 269–277. doi:https://doi.org/10.1080/00365510600608266
72. Cohen, D. R., Basu, S., Randall, J. M., Aballa, T. C., Lynne, C. M., & Brackett, N. L. (2004). Sperm motility in men with spinal cord injuries is enhanced by inactivating cytokines in the seminal plasma. *Journal of Sndrology*, *25*(6), 922–925. doi:https://doi.org/10.1002/j.1939-4640.2004.tb03162.x
73. Cohen, M. S., Hoffman, I. F., Royce, R. A., Kazembe, P., Dyer, J. R., Daly, C. C., Zimba, D., Vernazza, P. L., Maida, M., Fiscus, S. A., & Eron, J. J., Jr (1997). Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. *Lancet, 349*(9069), 1868–1873. doi:https://doi.org/10.1016/s0140-6736(97)02190-9
74. Cole, S. (2020). Herpes Simplex Virus: Epidemiology, Diagnosis, and Treatment. *The Nursing Clinics of North America, 55*(3), 337–345. doi:https://doi.org/10.1016/j.cnur.2020.05.004
75. Comhaire, F., Bosmans, E., Ombelet, W., Punjabi, U., & Schoonjans, F. (1994). Cytokines in semen of normal men and of patients with andrological diseases. *American Journal of Reproductive Immunology, 31*(2–3), 99–103. doi:https://doi.org/10.1111/j.1600-0897.1994.tb00853.x
76. Cornelisse, V. J., Williamson, D., Zhang, L., Chen, M. Y., Bradshaw, C., Hocking, J. S., Hoy, J., Howden, B. P., Chow, E. P. F., & Fairley, C. K. (2019). Evidence for a new paradigm of gonorrhoea transmission: cross-sectional analysis of *Neisseria gonorrhoeae* infections by anatomical site in both partners in 60 male

couples. *Sexually Transmitted Infections*, *95*(6), 437–442. doi:https://doi.org/10.1136/sextrans-2018-053803
77. Corona, G., Goulis, D. G., Huhtaniemi, I., Zitzmann, M., Toppari, J., Forti, G., Vanderschueren, D., & Wu, F. C. (2020). European Academy of Andrology (EAA) guidelines on investigation, treatment and monitoring of functional hypogonadism in males: Endorsing organization: European Society of Endocrinology. *Andrology*, *8*(5), 970–987. doi:https://doi.org/10.1111/andr.12770
78. Corradi, G., Bucsek, M., Pánovics, J., Verebélyi, A., Kardos, M., Kádár, A., & Frang, D. (1996). Detection of Chlamydia trachomatis in the prostate by in-situ hybridization and by transmission electron microscopy. *International Journal of Andrology, 19*(2), 109–112. doi:https://doi.org/10.1111/j.1365-2605.1996.tb00446.x
79. Couldwell, D. L., & Lewis, D. A. (2015). Mycoplasma genitalium infection: current treatment options, therapeutic failure, and resistance-associated mutations. *Infection and Drug Resistance, 8*, 147–161. doi:https://doi.org/10.2147/IDR.S48813
80. Couldwell, D. L., Gidding, H. F., Freedman, E. V., McKechnie, M. L., Biggs, K., Sintchenko, V., & Gilbert, G. L. (2010). Ureaplasma urealyticum is significantly associated with non-gonococcal urethritis in heterosexual Sydney men. *International Journal of STD & AIDS, 21*(5), 337–341. doi:https://doi.org/10.1258/ijsa.2009.009499
81. Cox, C., McKenna, J. P., Watt, A. P., & Coyle, P. V. (2016). Ureaplasma parvum and Mycoplasma genitalium are found to be significantly associated with microscopy-confirmed urethritis in a routine genitourinary medicine setting. *International Journal of STD & AIDS, 27*(10), 861–867. doi:https://doi.org/10.1177/0956462415597620
82. Crespillo-Andujar, C., Díaz-Menéndez, M., & Mora-Rillo, M. (2018). Evidence for Previously Unidentified Sexual Transmission of Protozoan Parasites. *Emerging Infectious Diseases, 24*(3), 602–603. doi:https://doi.org/10.3201/eid2403.171838
83. Criss, A. K., & Seifert, H. S. (2012). A bacterial siren song: intimate interactions between Neisseria and neutrophils. *Nature Reviews Microbiology*, *10*(3), 178–190. doi:https://doi.org/10.1038/nrmicro2713
84. Crucitti, T., Jespers, V., Mulenga, C., Khondowe, S., Vandepitte, J., & Buvé, A. (2011). Non-sexual transmission of Trichomonas vaginalis in adolescent girls attending school in Ndola, Zambia. *PloS ONE*, *6*(1), e16310. doi:https://doi.org/10.1371/journal.pone.0016310
85. Cunningham, K. A., & Beagley, K. W. (2008). Male genital tract chlamydial infection: implications for pathology and infertility. *Biology of Reproduction, 79*(2), 180–189. doi:https://doi.org/10.1095/biolreprod.108.067835
86. Cunningham, K. A., & Beagley, K. W. (2008). Male genital tract chlamydial infection: implications for pathology and infertility. *Biology of Reproduction, 79*(2), 180–189. doi:https://doi.org/10.1095/biolreprod.108.067835
87. Daly, J. J., Sherman, J. K., Green, L., & Hostetler, T. L. (1989). Survival of Trichomonas vaginalis in human semen. *Genitourinary Medicine, 65*(2), 106–108. doi:https://doi.org/10.1136/sti.65.2.106
88. Damania, B., Kenney, S. C., & Raab-Traub, N. (2022). Epstein-Barr virus: Biology and clinical disease. *Cell, 185*(20), 3652–3670. doi:https://doi.org/10.1016/j.cell.2022.08.026

89. Dan, M., Gottesman, T., Schwartz, O., Tsivian, A., Gophna, U., & Rokney, A. (2012). Sexually transmitted Escherichia coli urethritis and orchiepididymitis. *Sexually Transmitted Diseases, 39*(1), 16–17. doi:https://doi.org/10.1097/OLQ.0b013e31823156a0
90. de Barbeyrac, B., Papaxanthos-Roche, A., Mathieu, C., Germain, C., Brun, J. L., Gachet, M., Mayer, G., Bébéar, C., Chene, G., & Hocké, C. (2006). Chlamydia trachomatis in subfertile couples undergoing an in vitro fertilization program: a prospective study. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, *129*(1), 46–53. https://doi.org/10.1016/j.ejogrb.2006.02.014
91. De Jong, Z., Pontonnier, F., Plante, P., Gautier, J. R., Ioualalen, A., Archambaud, M., & Chabanon, G. (1988). The frequency of Chlamydia trachomatis in acute epididymitis. *British Journal of Urology, 62*(1), 76–78. doi:https://doi.org/10.1111/j.1464-410x.1988.tb04271.x
92. Depuydt, C. E., Bosmans, E., Zalata, A., Schoonjans, F., & Comhaire, F. H. (1996). The relation between reactive oxygen species and cytokines in andrological patients with or without male accessory gland infection. *Journal of Andrology*, *17*(6), 699–707. doi:https://doi.org/10.1002/j.1939-4640.1996.tb01855.x
93. Depuydt, C., Zalata, A., Christophe, A., Mahmoud, A., & Comhaire, F. (1998). Mechanisms of sperm deficiency in male accessory gland infection. *Andrologia*, *30*(Suppl. 1), 29–33. doi:https://doi.org/10.1111/j.1439-0272.1998.tb02823.x
94. Derbel, R., Sellami, H., Sakka, R., Ben Slima, A., Mkaddem, I., Gdoura, R., Mcelreavey, E., & Ammar-Keskes, L. (2021). Relationship between nuclear DNA fragmentation, mitochondrial DNA damage and standard sperm parameters in spermatozoa of infertile patients with leukocytospermia. *Journal of Gynecology Obstetrics and Human Reproduction*, *50*(5), 102101. doi:https://doi.org/10.1016/j.jogoh.2021.102101
95. de Souza, L. S., Sardinha, J. C., Talhari, S., Heibel, M., Santos, M. N., & Talhari, C. (2021). Main etiological agents identified in 170 men with urethritis attended at the Fundação Alfredo da Matta, Manaus, Amazonas, Brazil. *Anais Brasileiros de Dermatologia, 96*(2), 176–183. doi:https://doi.org/10.1016/j.abd.2020.07.007
96. Deardourff, S. L., Deture, F. A., Drylie, D. M., Centifano, Y., & Kaufman, H. (1974). Association between herpes hominis type 2 and the male genitourinary tract. *The Journal of Urology, 112*(1), 126–127. doi:https://doi.org/10.1016/s0022-5347(17)59659-7
97. Deguchi, T., Kanematsu, E., Iwata, H., Komeda, H., Okano, M., Ito, Y., Saito, A., Ban, Y., & Kawada, Y. (1992). Chlamydial epididymitis diagnosed by genetic detection of Chlamydia trachomatis from epididymal aspirate by polymerase chain reaction. *Kansenshogaku zasshi, 66*(7), 991–994. doi:https://doi.org/10.11150/kansenshogakuzasshi1970.66.991
98. Deguchi, T., Kikuchi, M., Yasuda, M., & Ito, S. (2016). Multidrug-Resistant Mycoplasma genitalium Is Increasing. *Clinical Infectious Diseases, 62*(3), 405–406. doi:https://doi.org/10.1093/cid/civ898
99. Deguchi, T., Shimada, Y., Horie, K., Mizutani, K., Seike, K., Tsuchiya, T., Yokoi, S., Yasuda, M., & Ito, S. (2015). Bacterial loads of Ureaplasma parvum contribute to the development of inflammatory responses in the male urethra. *International Journal of STD & AIDS, 26*(14), 1035–1039. doi:https://doi.org/10.1177/0956462414565796
100. Deguchi, T., Yoshida, T., Miyazawa, T., Yasuda, M., Tamaki, M., Ishiko, H., & Maeda, S. (2004). Association of Ureaplasma urealyticum (biovar 2) with non-

gonococcal urethritis. *Sexually Transmitted Diseases, 31*(3), 192–195. doi:https://doi.org/10.1097/01.olq.0000114653.26951.71
101. Dehghan Marvast, L., Aflatoonian, A., Talebi, A. R., Eley, A., & Pacey, A. A. (2017). Relationship between Chlamydia trachomatis and Mycoplasma genitalium infection and pregnancy rate and outcome in Iranian infertile couples. *Andrologia, 49*(9). doi:https://doi.org/10.1111/and.12747
102. Dehghan Marvast, L., Aflatoonian, A., Talebi, A. R., Ghasemzadeh, J., & Pacey, A. A. (2016). Semen inflammatory markers and Chlamydia trachomatis infection in male partners of infertile couples. *Andrologia, 48*(7), 729–736. doi:https://doi.org/10.1111/and.12501
103. Densen P. (1989). Interaction of complement with Neisseria meningitidis and Neisseria gonorrhoeae. *Clinical Microbiology Reviews*, *2*(Suppl.), S11–S17. doi:https://doi.org/10.1128/CMR.2.Suppl.S11
104. Detels, R., Green, A. M., Klausner, J. D., Katzenstein, D., Gaydos, C., Handsfield, H., Pequegnat, W., Mayer, K., Hartwell, T. D., & Quinn, T. C. (2011). The incidence and correlates of symptomatic and asymptomatic Chlamydia trachomatis and Neisseria gonorrhoeae infections in selected populations in five countries. *Sexually Transmitted Diseases*, *38*(6), 503–509.
105. Dieterle, S., Mahony, J. B., Luinstra, K. E., & Stibbe, W. (1995). Chlamydial immunoglobulin IgG and IgA antibodies in serum and semen are not associated with the presence of Chlamydia trachomatis DNA or rRNA in semen from male partners of infertile couples. *Human Reproduction, 10*(2), 315–319. doi:https://doi.org/10.1093/oxfordjournals.humrep.a135934
106. Doble, A., Taylor-Robinson, D., Thomas, B. J., Jalil, N., Harris, J. R., & Witherow, R. O. (1989). Acute epididymitis: a microbiological and ultrasonographic study. *British Journal of Urology, 63*(1), 90–94. doi:https://doi.org/10.1111/j.1464-410x.1989.tb05132.x
107. Domes, T., Lo, K. C., Grober, E. D., Mullen, J. B., Mazzulli, T., & Jarvi, K. (2012). The utility and cost of Chlamydia trachomatis and Neisseria gonorrhoeae screening of a male infertility population. *Fertility and Sterility, 97*(2), 299–305. doi:https://doi.org/10.1016/j.fertnstert.2011.11.024
108. Dousset, B., Hussenet, F., Daudin, M., Bujan, L., Foliguet, B., & Nabet, P. (1997). Seminal cytokine concentrations (IL-1beta, IL-2, IL-6, sR IL-2, sR IL-6), semen parameters and blood hormonal status in male infertility. *Human Reproduction, 12*(7), 1476–1479. doi:https://doi.org/10.1093/humrep/12.7.1476
109. Darville T. (2005). Chlamydia trachomatis infections in neonates and young children. *Seminars in Pediatric Infectious Diseases*, *16*(4), 235–244. doi:https://doi.org/10.1053/j.spid.2005.06.004
110. Dubin, L., & Amelar, R. D. (1970). Varicocele size and results of varicocelectomy in selected subfertile men with varicocele. *Fertility and Sterility, 21*(8), 606–609. doi:https://doi.org/10.1016/s0015-0282(16)37684-1
111. Dutta, S., Sengupta, P., Slama, P., & Roychoudhury, S. (2021). Oxidative Stress, Testicular Inflammatory Pathways, and Male Reproduction. *International Journal of Molecular Sciences, 22*(18), 10043. doi:https://doi.org/10.3390/ijms221810043
112. Eggert-Kruse, W., Boit, R., Rohr, G., Aufenanger, J., Hund, M., & Strowitzki, T. (2001). Relationship of seminal plasma interleukin (IL) -8 and IL-6 with semen quality. *Human Reproduction, 16*(3), 517–528. doi:https://doi.org/10.1093/humrep/16.3.517

113. Eickhoff, J. H., Frimodt-Møller, N., Walter, S., & Frimodt-Møller, C. (1999). A double-blind, randomized, controlled multicentre study to compare the efficacy of ciprofloxacin with pivampicillin as oral therapy for epididymitis in men over 40 years of age. *BJU International, 84*(7), 827–834. doi:https://doi.org/10.1046/j.1464-410x.1999.00252.x
114. Eldamnhoury, E. M., Elatrash, G. A., Rashwan, H. M., & El-Sakka, A. I. (2018). Association between leukocytospermia and semen interleukin-6 and tumor necrosis factor-alpha in infertile men. *Andrology, 6*(5), 775–780. doi:https://doi.org/10.1111/andr.12513
115. Elfassy, Y., Bongrani, A., Levy, P., Foissac, F., Fellahi, S., Faure, C., McAvoy, C., Capeau, J., Dupont, J., Fève, B., Levy, R., Bastard, J. P., & Metasperme group (2020). Relationships between metabolic status, seminal adipokines, and reproductive functions in men from infertile couples. *European Journal of Endocrinology*, *182*(1), 67–77. doi:https://doi.org/10.1530/EJE-19-0615
116. European Association of Urology. (2020). *EAU Guidelines. Edn. presented at the EAU Annual Congress Amsterdam the Netherlands 2020.* EAU Guidelines Office, Arnhem, the Netherlands. Source: http://uroweb.org/guidelines/compilations-of-all-guidelines/
117. Falk, L., Fredlund, H., & Jensen, J. S. (2004). Symptomatic urethritis is more prevalent in men infected with Mycoplasma genitalium than with Chlamydia trachomatis. *Sexually Transmitted Infections, 80*(4), 289–293. doi:https://doi.org/10.1136/sti.2003.006817
118. Fan, W., & Zhang, Q. (2013). Bilateral inflammation of the paraurethral glands around the external urethral orifice due to Chlamydia trachomatis in a male. *International Journal of Dermatology, 52*(12), 1567–1568. doi:https://doi.org/10.1111/j.1365-4632.2011.05160.x
119. Fan, W., Zhang, Q., & Jiang, T. (2014). Pathogen profile in men with inflammation of paraurethral glands. *Sexually Transmitted Infections, 90*(1), 52–54. doi:https://doi.org/10.1136/sextrans-2013-051180
120. Fan, W., Zhang, Q., Wang, L., & Jiang, T. (2019). Is Gonococcal Paraurethral Duct Infection a Local Complication of Urethral Gonorrhea in Men? *American Journal of Men's Health, 13*(3), 1557988319849134. doi:https://doi.org/10.1177/1557988319849134
121. Fan, W., Zhang, Q., Wang, L., Ye, X., & Jiang, T. (2016). Risk Factors associated with Paraurethral Duct Dilatation following Gonococcal Paraurethral Duct Infection in Men. *PloS ONE, 11*(11), e0166355. doi:https://doi.org/10.1371/journal.pone.0166355
122. Fan, W., Zhang, Q., Wei, M., Ai, M., Fan, Z., & Jiang, T. (2022). Gonococcal tysonitis, a rare local complication of gonorrhea: a clinical study of 15 cases. *European Journal of Clinical Microbiology & Infectious Diseases, 41*(5), 787–792. doi:https://doi.org/10.1007/s10096-022-04434-3
123. Farahani, L., Tharakan, T., Yap, T., Ramsay, J. W., Jayasena, C. N., & Minhas, S. (2021). The semen microbiome and its impact on sperm function and male fertility: A systematic review and meta-analysis. *Andrology, 9*(1), 115–144. doi:https://doi.org/10.1111/andr.12886
124. Fathy, A., Chen, S. J., Novak, N., Schuppe, H. C., Haidl, G., & Allam, J. P. (2014). Differential leucocyte detection by flow cytometry improves the diagnosis of genital tract inflammation and identifies macrophages as proinflammatory cytokine-

producing cells in human semen. *Andrologia*, *46*(9), 1004–1012. doi:https:// doi.org/ 10.1111/and.12188
125. Fichorova, R. N. (2009). Impact of T. vaginalis infection on innate immune responses and reproductive outcome. *Journal of Reproductive Immunology, 83*(1-2), 185–189. doi:https://doi.org/10.1016/j.jri.2009.08.007
126. Figueroa-Angulo, E. E., Rendón-Gandarilla, F. J., Puente-Rivera, J., Calla-Choque, J. S., Cárdenas-Guerra, R. E., Ortega-López, J., Quintas-Granados, L. I., Alvarez-Sánchez, M. E., & Arroyo, R. (2012). The effects of environmental factors on the virulence of Trichomonas vaginalis. *Microbes and Infection*, *14*(15), 1411–1427. doi:https://doi.org/10.1016/j.micinf.2012.09.004
127. Fisher, I., & Morton, R. S. (1969). Epididymitis due to Trichomonas vaginalis. *The British journal of venereal diseases*, *45*(3), 252–253. doi:https://doi.org/ 10.1136/sti.45.3.252
128. Fiumara, N. J. (1977). Gonococcal tysonitis. *The British Journal of Venereal Diseases, 53*(2), 145. doi:https://doi.org/10.1136/sti.53.2.145
129. Fode, M., Fusco, F., Lipshultz, L., & Weidner, W. (2016). Sexually Transmitted Disease and Male Infertility: A Systematic Review. *European Urology Focus, 2*(4), 383–393. doi:https://doi.org/10.1016/j.euf.2016.08.002
130. Forde, J. C., Casey, R. G., & Grainger, R. (2009). An unusual penpal: case report and literature review of posterior urethral injuries secondary to foreign body insertion. *The Canadian Journal of Urology, 16*(4), 4757–4759.
131. Fraser, P. A., Teasdale, J., Gan, K. S., Eglin, R., Scott, S. C., & Lacey, C. J. (1995). Neutrophil enzymes in urine for the detection of urethral infection in men. *Genitourinary Medicine*, *71*(3), 176–179. https://doi.org/10.1136/sti.71.3.176
132. Frick, J., & Aulitzky, W. (1991). Physiology of the prostate. *Infection, 19*(Suppl. 3), S115–S118. doi:https://doi.org/10.1007/BF01643679
133. Friebe, K., Bohring, C., Skrzypek, J., & Krause, W. (2003). Levels of interleukin-6 and interleukin-8 in seminal fluid of men attending an andrological clinic. *Andrologia*, *35*(2), 126–129. doi:https://doi.org/10.1046/j.1439-0272.2003.00544.x
134. Frølund, M., Falk, L., Ahrens, P., & Jensen, J. S. (2019). Detection of ureaplasmas and bacterial vaginosis associated bacteria and their association with non-gonococcal urethritis in men. *PloS ONE, 14*(4), e0214425. doi:https://doi.org/10.1371/ journal.pone.0214425
135. Frølund, M., Lidbrink, P., Wikström, A., Cowan, S., Ahrens, P., & Jensen, J. S. (2016). Urethritis-associated Pathogens in Urine from Men with Non-gonococcal Urethritis: A Case-control Study. *Acta Dermato-venereologica, 96*(5), 689–694. doi:https://doi.org/10.2340/00015555-2314
136. Furness, G., Kamat, M. H., Kaminski, Z., & Seebode, J. J. (1971). The etiology of idiopathic epididymitis. *The Journal of Urology, 106*(3), 387–392. doi:https:// doi.org/10.1016/s0022-5347(17)61297-7
137. Furuya, R., Takahashi, S., Furuya, S., Kunishima, Y., Takeyama, K., & Tsukamoto, T. (2004). Is seminal vesiculitis a discrete disease entity? Clinical and microbiological study of seminal vesiculitis in patients with acute epididymitis. *The Journal of Urology, 171*(4), 1550–1553. doi:https://doi.org/10.1097/01.ju.0000116288.59223.e9
138. Furuya, R., Takahashi, S., Furuya, S., Saitoh, N., Ogura, H., Kurimura, Y., & Tsukamoto, T. (2009). Is urethritis accompanied by seminal vesiculitis? *International Journal of Urology, 16*(7), 628–631. doi:https://doi.org/10.1111/j.1442-2042.2009.02314.x

139. Furuya, R., Takahashi, S., Furuya, S., Takeyama, K., & Tsukamoto, T. (2005). A patient with seminal vesiculitis prior to acute chlamydial epididymitis. *Journal of Infection and Chemotherapy, 11*(5), 250–252. doi:https://doi.org/10.1007/s10156-005-0404-0
140. Furuya, R., Takahashi, S., Furuya, S., Takeyama, K., Masumori, N., & Tsukamoto, T. (2006). Chlamydial seminal vesiculitis without symptomatic urethritis and epididymitis. *International Journal of Urology, 13*(4), 466–467. doi:https://doi.org/10.1111/j.1442-2042.2006.01317.x
141. Furuya, Y., Akashi, T., & Fuse, H. (2003). Soluble Fas and interleukin-6 and interleukin-8 levels in seminal plasma of infertile men. *Archives of Andrology*, *49*(6), 449–452. doi:https://doi.org/10.1080/01485010390219926
142. García-Morales, L., González-González, L., Querol, E., & Piñol, J. (2016). A minimized motile machinery for Mycoplasma genitalium. *Molecular Microbiology, 100*(1), 125–138. doi:https://doi.org/10.1111/mmi.13305
143. Gaydos, C., Maldeis, N. E., Hardick, A., Hardick, J., & Quinn, T. C. (2009). Mycoplasma genitalium compared to chlamydia, gonorrhoea and trichomonas as an aetiological agent of urethritis in men attending STD clinics. *Sexually Transmitted Infections, 85*(6), 438–440. doi:https://doi.org/10.1136/sti.2008.035477
144. Gdoura, R., Kchaou, W., Ammar-Keskes, L., Chakroun, N., Sellemi, A., Znazen, A., Rebai, T., & Hammami, A. (2008, a). Assessment of Chlamydia trachomatis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, and Mycoplasma genitalium in semen and first void urine specimens of asymptomatic male partners of infertile couples. *Journal of Andrology, 29*(2), 198–206. doi:https://doi.org/10.2164/jandrol.107.003566
145. Gdoura, R., Kchaou, W., Chaari, C., Znazen, A., Keskes, L., Rebai, T., & Hammami, A. (2007). Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis and Mycoplasma genitalium infections and semen quality of infertile men. *BMC Infectious Diseases, 7*, 129. doi:https://doi.org/10.1186/1471-2334-7-129
146. Gdoura, R., Kchaou, W., Znazen, A., Chakroun, N., Fourati, M., Ammar-Keskes, L., & Hammami, A. (2008, b). Screening for bacterial pathogens in semen samples from infertile men with and without leukocytospermia. *Andrologia, 40*(4), 209–218. doi:https://doi.org/10.1111/j.1439-0272.2008.00845.x
147. Geisler, W. M., Yu, S., & Hook, E. W. (2005). Chlamydial and gonococcal infection in men without polymorphonuclear leukocytes on gram stain: implications for diagnostic approach and management. *Sexually Transmitted Diseases, 32*(10), 630–634. doi:https://doi.org/10.1097/01.olq.0000175390.45315.a1
148. Genders, R. E., Spitaels, D., Jansen, C. L., van den Akker, T. W., & Quint, K. D. (2013). A misleading urethral smear with polymorphonuclear leucocytes and intracellular diplococci; case report of urethritis caused by Neisseria meningitidis. *Journal of Medical Microbiology, 62*(Pt 12), 1905–1906. doi:https://doi.org/10.1099/jmm.0.059378-0
149. Getman, D., Jiang, A., O'Donnell, M., & Cohen, S. (2016). Mycoplasma genitalium Prevalence, Coinfection, and Macrolide Antibiotic Resistance Frequency in a Multicenter Clinical Study Cohort in the United States. *Journal of Clinical Microbiology, 54*(9), 2278–2283. doi:https://doi.org/10.1128/JCM.01053-16
150. Ghaly, A. F., Taylor, P. M., Goorney, B. P., & Haye, K. R. (1994). Transrectal ultrasound in male urethritis. *Genitourinary Medicine, 70*(6), 399–402. doi:https://doi.org/10.1136/sti.70.6.399

151. Gimenes, F., Medina, F. S., Abreu, A. L., Irie, M. M., Esquiçati, I. B., Malagutti, N., Vasconcellos, V. R., Discacciati, M. G., Bonini, M. G., Maria-Engler, S. S., & Consolaro, M. E. (2014, a). Sensitive simultaneous detection of seven sexually transmitted agents in semen by multiplex-PCR and of HPV by single PCR. *PloS ONE*, *9*(6), e98862. doi:https://doi.org/10.1371/journal.pone.0098862
152. Gimenes, F., Souza, R. P., Bento, J. C., Teixeira, J. J., Maria-Engler, S. S., Bonini, M. G., & Consolaro, M. E. (2014, b). Male infertility: a public health issue caused by sexually transmitted pathogens. *Nature Reviews Urology, 11*(12), 672–687. doi:https://doi.org/10.1038/nrurol.2014.285
153. Gnanadurai, R., & Fifer, H. (2020). Mycoplasma genitalium: A Review. *Microbiology, 166*(1), 21–29. doi:https://doi.org/10.1099/mic.0.000830
154. Gomez, C. I., Stenback, W. A., James, A. N., Criswell, B. S., & Williams, R. P. (1979). Attachment of Neisseria gonorrhoeae to human sperm. Microscopical study of trypsin and iron. *The British Journal of Venereal Diseases, 55*(4), 245–255. doi:https://doi.org/10.1136/sti.55.4.245
155. Gong, Y. H., Liu, Y., Li, P., Zhu, Z. J., Hong, Y., Fu, G. H., Xue, Y. J., Xu, C., & Li, Z. (2018). A nonobstructive azoospermic patient with *Trichomonas vaginalis* infection in testes. *Asian Journal of Andrology*, *20*(1), 97–98. doi:https://doi.org/10.4103/1008-682X.195561
156. Gonzales, G. F. (1989). Functional structure and ultrastructure of seminal vesicles. *Archives of Andrology, 22*(1), 1–13. doi:https://doi.org/10.3109/01485018908986745
157. Gopalkrishnan, K., Hinduja, I. N., & Kumar, T. C. (1990). Semen characteristics of asymptomatic males affected by Trichomonas vaginalis. *Journal of In Vitro Fertilization and Embryo Transfer, 7*(3), 165–167. doi:https://doi.org/10.1007/BF01135682
158. Gottesman, T., Yossepowitch, O., Samra, Z., Rosenberg, S., & Dan, M. (2017). Prevalence of Mycoplasma genitalium in men with urethritis and in high risk asymptomatic males in Tel Aviv: a prospective study. *International Journal of STD & AIDS, 28*(2), 127–132. doi:https://doi.org/10.1177/0956462416630675
159. Gratrix, J., Plitt, S., Turnbull, L., Smyczek, P., Brandley, J., Scarrott, R., Naidu, P., Bertholet, L., Chernesky, M., Read, R., & Singh, A. E. (2017). Trichomonas vaginalis Prevalence and Correlates in Women and Men Attending STI Clinics in Western Canada. *Sexually Transmitted Diseases*, *44*(10), 627–629. doi:https://doi.org/10.1097/OLQ.0000000000000650
160. Greenberg, S. H. (1979). Male reproductive tract sequelae of gonococcal and non-gonococcal urethritis. *Archives of Andrology, 3*(4), 317–319. doi:https://doi.org/10.3109/01485017908988422
161. Grosso, S., Bruschetta, G., & Camporese, A. (2012). Utilizzo sperimentale di Sysmex UF-1000i nello screening di uretrite non gonococcica [Experimental evaluation of the Sysmex UF-1000i for ruling out non-gonococcal urethritis]. *Le Infezioni in Medicina, 20*(3), 188–194.
162. Gruschwitz, M. S., Brezinschek, R., & Brezinschek, H. P. (1996). Cytokine levels in the seminal plasma of infertile males. *Journal of Andrology*, *17*(2), 158–163. doi: https://doi.org/10.1002/j.1939-4640.1996.tb01765.x
163. Gubish, E. R., Jr, James, A. N., Adlan, A., & Williams, R. P. (1979). Comparative attachment of Neisseria gonorrhoeae and of gonococcal pili to various mammalian sperm. *Comparative Immunology, Microbiology and Infectious Diseases, 2*(4), 559–563. doi:https://doi.org/10.1016/0147-9571(79)90098-5

164. Haanpää, M., & Paavonen, J. (2004). Transient urinary retention and chronic neuropathic pain associated with genital herpes simplex virus infection. *Acta Obstetricia et Gynecologica Scandinavica, 83*(10), 946–949. doi:https://doi.org/10.1111/j.0001-6349.2004.00500.x
165. Habib, F. S., Metwally, D. M., & Habib, K. S. (2004). Cryopreservation of Trichomonas vaginalis: a trial of using four different cryoprotectants. *Journal of the Egyptian Society of Parasitology*, *34*(3), 931–940.
166. Haddow, L. J., Bunn, A., Copas, A. J., Gilson, R., Prince, M., Ridgway, G. L., & Sadiq, S. T. (2004). Polymorph count for predicting non-gonococcal urethral infection: a model using Chlamydia trachomatis diagnosed by ligase chain reaction. *Sexually Transmitted Infections, 80*(3), 198–200. doi:https://doi.org/10.1136/sti.2003.006924
167. Haidl, F., Haidl, G., Oltermann, I., & Allam, J. P. (2015). Seminal parameters of chronic male genital inflammation are associated with disturbed sperm DNA integrity. *Andrologia*, *47*(4), 464–469. doi:https://doi.org/10.1111/and.12408
168. Hamasuna R. (2012). Editorial Comment from Dr Hamasuna to Prevalence of genital mycoplasmas and ureaplasmas in men younger than 40 years-of-age with acute epididymitis. *International Journal of Urology*, *19*(3), 239. https://doi.org/10.1111/j.1442-2042.2011.02927.x
169. Hanaoka, N., Ito, S., Konagaya, M., Nojiri, N., Yasuda, M., Fujimoto, T., & Deguchi, T. (2019). Infectious human adenoviruses are shed in urine even after disappearance of urethral symptoms. *PloS ONE, 14*(3), e0212434. doi:https://doi.org/10.1371/journal.pone.0212434
170. Hanaoka, N., Ito, S., Nojiri, N., Konagaya, M., Yasuda, M., Deguchi, T., & Fujimoto, T. (2020). Human Adenovirus B7d-Associated Urethritis after Suspected Sexual Transmission, Japan. *Emerging Infectious Diseases, 26*(10), 2444–2447. doi:https://doi.org/10.3201/eid2610.191538
171. Harnett, G. B., & Newnham, W. A. (1981). Isolation of adenovirus type 19 from the male and female genital tracts. *The British Journal of Venereal Diseases, 57*(1), 55–57. doi:https://doi.org/10.1136/sti.57.1.55
172. Harnisch, J. P., Berger, R. E., Alexander, E. R., Monda, G., & Holmes, K. K. (1977). Aetiology of acute epididymitis. *Lancet, 1*(8016), 819–821. doi:https://doi.org/10.1016/s0140-6736(77)92773-8
173. Harris, S. R., Clarke, I. N., Seth-Smith, H. M., Solomon, A. W., Cutcliffe, L. T., Marsh, P., Skilton, R. J., Holland, M. J., Mabey, D., Peeling, R. W., Lewis, D. A., Spratt, B. G., Unemo, M., Persson, K., Bjartling, C., Brunham, R., de Vries, H. J., Morré, S. A., Speksnijder, A., Bébéar, C. M., … Thomson, N. R. (2012). Whole-genome analysis of diverse Chlamydia trachomatis strains identifies phylogenetic relationships masked by current clinical typing. *Nature Genetics*, *44*(4), 413–S1. doi:https://doi.org/10.1038/ng.2214
174. Hawkins, D. A., Taylor-Robinson, D., Thomas, B. J., & Harris, J. R. (1986). Microbiological survey of acute epididymitis. *Genitourinary Medicine, 62*(5), 342–344. doi:https://doi.org/10.1136/sti.62.5.342
175. Henkel, R. (2021). Long-term consequences of sexually transmitted infections on men's sexual function: A systematic review. *Arab Journal of Urology, 19*(3), 411–418. doi:https://doi.org/10.1080/2090598X.2021.1942414
176. Henry, C. H., Hughes, C. V., Gérard, H. C., Hudson, A. P., & Wolford, L. M. (2000). Reactive arthritis: preliminary microbiologic analysis of the human

temporomandibular joint. *Journal of Oral and Maxillofacial Surgery, 58*(10), 1137–1144. doi:https://doi.org/10.1053/joms.2000.9575
177. Heyward, V. H., & Stolarczyk, L. M. (1996). *Applied Body Composition Assessment.* Champaign: Human Kinetics.
178. Hiroi, S., Furubayashi, K., Kawahata, T., Morikawa, S., & Kase, T. (2012). A case of urethritis caused by human adenovirus type 56. *Japanese Journal of Infectious Diseases, 65*(3), 273–274. doi:https://doi.org/10.7883/yoken.65.273
179. Hiroi, S., Kawahata, T., & Furubayashi, K. (2020). First isolation of human adenovirus type 85 by molecular analysis of adenoviruses in cases of urethritis. *Journal of Medical Microbiology, 69*(2), 265–269. doi:https://doi.org/10.1099/jmm.0.001149
180. Hirsch, E. W. (1927). Comparative Histology of the Urethral Mucosa and Its Relation to Gonococcal Infections. *The Journal of Urology, 17*(6), 575-580. doi:https://doi.org/10.1016/S0022-5347(17)73378-2.
181. Hobbs, M. M., & Seña, A. C. (2013). Modern diagnosis of Trichomonas vaginalis infection. *Sexually Transmitted Infections, 89*(6), 434–438. doi:https://doi.org/10.1136/sextrans-2013-051057
182. Holmes, K. K., Berger, R. E., & Alexander, E. R. (1979). Acute epididymitis: etiology and therapy. *Archives of Andrology, 3*(4), 309–316. doi:https://doi.org/10.3109/01485017908988421
183. Hori, S., & Tsutsumi, Y. (1995). Histological differentiation between chlamydial and bacterial epididymitis: nondestructive and proliferative versus destructive and abscess forming – immunohistochemical and clinicopathological findings. *Human Pathology, 26*(4), 402–407. doi:https://doi.org/10.1016/0046-8177(95)90141-8
184. Horner, P. J., & Martin, D. H. (2017). Mycoplasma genitalium Infection in Men. *The Journal of Infectious Diseases, 216*(Suppl. 2), S396–S405. doi:https://doi.org/10.1093/infdis/jix145
185. Horner, P. J., & Taylor-Robinson, D. (2007). Is there a role for leucocyte esterase testing in non-invasive screening using nucleic acid amplification tests of asymptomatic men? *International Journal of STD & AIDS, 18*(2), 73–74. doi:https://doi.org/10.1258/095646207779949718
186. Horner, P. J., Blee, K., Falk, L., van der Meijden, W., & Moi, H. (2016). 2016 European guideline on the management of non-gonococcal urethritis. *International Journal of STD & AIDS, 27*(11), 928–937. doi:https://doi.org/10.1177/0956462416648585
187. Horner, P., Donders, G., Cusini, M., Gomberg, M., Jensen, J. S., & Unemo, M. (2018). Should we be testing for urogenital Mycoplasma hominis, Ureaplasma parvum and Ureaplasma urealyticum in men and women? – a position statement from the European STI Guidelines Editorial Board. *Journal of the European Aca demy of Dermatology and Venereology: JEADV, 32*(11), 1845–1851. doi:https://doi.org/10.1111/jdv.15146
188. Hosseinzadeh, S., Eley, A., & Pacey, A. A. (2004). Semen quality of men with asymptomatic chlamydial infection. *Journal of Andrology, 25*(1), 104–109. doi:https://doi.org/10.1002/j.1939-4640.2004.tb02764.x
189. Hrbacek, J., Urban, M., Hamsikova, E., Tachezy, R., & Heracek, J. (2013). Thirty years of research on infection and prostate cancer: no conclusive evidence for a link. A systematic review. *Urologic Oncology, 31*(7), 951–965. doi:https://doi.org/10.1016/j.urolonc.2012.01.013

190. Hrbacek, J., Urban, M., Hamsikova, E., Tachezy, R., Eis, V., Brabec, M., & Heracek, J. (2011). Serum antibodies against genitourinary infectious agents in prostate cancer and benign prostate hyperplasia patients: a case-control study. *BMC Cancer, 11*(53). doi:https://doi.org/10.1186/1471-2407-11-53
191. Hu, V. H., Harding-Esch, E. M., Burton, M. J., Bailey, R. L., Kadimpeul, J., & Mabey, D. C. (2010). Epidemiology and control of trachoma: systematic review. *Tropical Medicine & International Health*, *15*(6), 673–691. doi:https://doi.org/10.1111/j.1365-3156.2010.02521.x
192. Huang, C., Zhu, H. L., Xu, K. R., Wang, S. Y., Fan, L. Q., & Zhu, W. B. (2015). Mycoplasma and ureaplasma infection and male infertility: a systematic review and meta-analysis. *Andrology, 3*(5), 809–816. doi:https://doi.org/10.1111/andr.12078
193. Huleihel, M., Lunenfeld, E., Levy, A., Potashnik, G., & Glezerman, M. (1996). Distinct expression levels of cytokines and soluble cytokine receptors in seminal plasma of fertile and infertile men. *Fertility and Sterility*, *66*(1), 135–139. doi:https://doi.org/10.1016/S0015-0282(16)58401-5
194. Högdahl, M., & Kihlström, E. (2007). Leucocyte esterase testing of first-voided urine and urethral and cervical smears to identify Mycoplasma genitalium-infected men and women. *International Journal of STD & AIDS, 18*(12), 835–838. doi:https://doi.org/10.1258/095646207782716983
195. Ibáñez-Escribano, A., Nogal-Ruiz, J. J., Pérez-Serrano, J., Gómez-Barrio, A., Escario, J. A., & Alderete, J. F. (2015). Sequestration of host-CD59 as potential immune evasion strategy of Trichomonas vaginalis. *Acta Tropica*, *149*, 1–7. doi:https://doi.org/10.1016/j.actatropica.2015.05.003
196. Ingram, P. C. (1926). Suppurating Gonococcal Epididymitis: Reports of Three Cases. *British Medical Journal, 1*(3406), 653. doi:https://doi.org/10.1136/bmj.1.3406.653
197. Iqbal, J., Al-Rashed, J., & Kehinde, E. O. (2016). Detection of Trichomonas vaginalis in prostate tissue and serostatus in patients with asymptomatic benign prostatic hyperplasia. *BMC Infectious Diseases, 16*(1), 506. doi:https://doi.org/10.1186/s12879-016-1843-1
198. Israele, V., Shirley, P., & Sixbey, J. W. (1991). Excretion of the Epstein-Barr virus from the genital tract of men. *The Journal of Infectious Diseases, 163*(6), 1341–1343. doi:https://doi.org/10.1093/infdis/163.6.1341
199. Ito, S., Hanaoka, N., Shimuta, K., Seike, K., Tsuchiya, T., Yasuda, M., Yokoi, S., Nakano, M., Ohnishi, M., & Deguchi, T. (2016). Male non-gonococcal urethritis: From microbiological etiologies to demographic and clinical features. *International Journal of Urology, 23*(4), 325–331. doi:https://doi.org/10.1111/iju.13044
200. Ito, S., Horie, K., Seike, K., Yasuda, M., Tsuchiya, T., Yokoi, S., Nakano, M., & Deguchi, T. (2014, a). Usefulness of quantifying leukocytes in first-voided urine to predict positivity for Chlamydia trachomatis in asymptomatic men at high risk for chlamydial infection. *Journal of Infection and Chemotherapy, 20*(12), 748–751. doi:https://doi.org/10.1016/j.jiac.2014.08.002
201. Ito, S., Kikuchi, M., Seike, K., Tsuchiya, T., Yasuda, M., Yokoi, S., Nakano, M., & Deguchi, T. (2014, b). Prevalence of genital mycoplasmas in asymptomatic male partners of women diagnosed as having chlamydial infections. *Journal of Infection and Chemotherapy, 20*(2), 143–145. doi:https://doi.org/10.1016/j.jiac.2013.07.011

202. Ito, S., Tsuchiya, T., Yasuda, M., Yokoi, S., Nakano, M., & Deguchi, T. (2012). Prevalence of genital mycoplasmas and ureaplasmas in men younger than 40 years-of-age with acute epididymitis. *International Journal of Urology, 19*(3), 234–238. doi:https://doi.org/10.1111/j.1442-2042.2011.02917.x
203. Ito, S., Yasuda, M., Kondo, H., Yamada, Y., Nakane, K., Mizutani, K., Tsuchiya, T., Yokoi, S., Nakano, M., & Deguchi, T. (2017). Clinical courses of herpes simplex virus-induced urethritis in men. *Journal of Infection and Chemotherapy, 23*(10), 717–719. doi:https://doi.org/10.1016/j.jiac.2017.03.017
204. Ito, Y. (1989). [The role of Chlamydia trachomatis and Ureaplasma urealyticum in patients with acute epididymitis]. *Kansenshogaku Zasshi, 63*(4), 293–304. doi:https://doi.org/10.11150/kansenshogakuzasshi1970.63.293
205. Jalil, N., Doble, A., Gilchrist, C., & Taylor-Robinson, D. (1988). Infection of the epididymis by Ureaplasma urealyticum. *Genitourinary Medicine, 64*(6), 367–368. doi:https://doi.org/10.1136/sti.64.6.367
206. James, A. N., Knox, J. M., & Williams, R. P. (1976). Attachment of gonococci to sperm. Influence of physical and chemical factors. *The British Journal of Venereal Diseases, 52*(2), 128–135. doi:https://doi.org/10.1136/sti.52.2.128
207. James-Holmquest, A. N., Swanson, J., Buchanan, T. M., Wende, R. D., & Williams, R. P. (1974). Differential attachment by piliated and nonpiliated Neisseria gonorrhoeae to human sperm. *Infection and Immunity, 9*(5), 897–902. doi:https://doi.org/10.1128/iai.9.5.897-902.1974
208. Janssenswillen, C., Tournaye, H., Pierard, D., Devroey, P., & Van Steirteghem, A. (1997). Microsurgical epididymal sperm aspiration with motile trophozoite cells but no spermatozoa. *Human Reproduction, 12*(10), 2217–2219. doi:https://doi.org/10.1093/humrep/12.10.2217
209. Jarecki-Black, J. C., Lushbaugh, W. B., Golosov, L., & Glassman, A. B. (1988). Trichomonas vaginalis: preliminary characterization of a sperm motility inhibiting factor. *Annals of Clinical & Laboratory Science, 18*(6), 484–489.
210. Jenniskens, M. L., Veerbeek, J. H., Deurloo, K. L., van Hannen, E. J., & Thijsen, S. F. (2017). Routine testing of Mycoplasma genitalium and Trichomonas vaginalis. *Infectious Diseases, 49*(6), 461–465. doi:https://doi.org/10.1080/23744235.2017.1290271
211. Jensen, J. S., Cusini, M., Gomberg, M., & Moi, H. (2016). 2016 European guideline on Mycoplasma genitalium infections. *Journal of the European Academy of Dermatology and Venereology, 30*(10), 1650–1656. doi:https://doi.org/10.1111/jdv.13849
212. Jensen, J. S., Cusini, M., Gomberg, M., Moi, H., Wilson, J., & Unemo, M. (2022). 2021 European guideline on the management of Mycoplasma genitalium infections. *Journal of the European Academy of Dermatology and Venereology, 36*(5), 641–650. doi:https://doi.org/10.1111/jdv.17972
213. Ježek, D. (2013). *Atlas on the Human Testis. Normal Morphology and Pathology.* London: Springer-Verlag. doi:10.1007/978-1-4471-2763-5
214. Jiang, T., Chen, P., Ouyang, J., Zhang, S., & Cai, D. (2011). Urine particles analysis: performance evaluation of Sysmex UF-1000i and comparison among urine flow cytometer, dipstick, and visual microscopic examination. *Scandinavian Journal of Clinical and Laboratory Investigation, 71*(1), 30–37. doi:https://doi.org/10.3109/00365513.2010.535011

215. Jírovec, O., & Petrů, M. (1968). Trichomonas vaginalis and trichomoniasis. *Advances in Parasitology*, *6*, 117–188. doi:https://doi.org/10.1016/s0065-308x(08)60473-x
216. John, H., Maake, C., Barghorn, A., Zbinden, R., Hauri, D., & Joller-Jemelka, H. I. (2003). Immunological alterations in the ejaculate of chronic prostatitis patients: clues for autoimmunity. *Andrologia*, *35*(5), 294–299. doi:https://doi.org/10.1046/j.1439-0272.2003.00573.x
217. Jordan, S. J., Toh, E., Williams, J. A., Fortenberry, L. J., LaPradd, M., Ryan, J. D., Nelson, D. E., & Batteiger, T. A. (2020). No Pathogen-Specific Sign or Symptom Predicts the Etiology of Monomicrobial Nongonococcal Urethritis in Men. *Sexually Transmitted Diseases*, *47*(5), 329–331. https://doi.org/10.1097/OLQ.0000000000001158
218. Jordan, S. J., Toh, E., Williams, J. A., Fortenberry, L., LaPradd, M. L., Katz, B. P., Batteiger, B. E., Nelson, D. E., & Batteiger, T. A. (2020). Aetiology and prevalence of mixed-infections and mono-infections in non-gonococcal urethritis in men: a case-control study. *Sexually Transmitted Infections, 96*(4), 306–311. doi:https://doi.org/10.1136/sextrans-2019-054121
219. Jungwirth, A., Giwercman, A., Tournaye, H., Diemer, T., Kopa, Z., Dohle, G., Krausz, C., & European Association of Urology Working Group on Male Infertility (2012). European Association of Urology guidelines on Male Infertility: the 2012 update. *European Urology*, *62*(2), 324–332. https://doi.org/10.1016/j.eururo.2012.04.048
220. Jungwirth, A., Straberger, A., Esterbauer, B., Fink, K., & Schmeller, N. (2003). Acrosome reaction in Chlamydia-positive and negative patients. *Andrologia, 35*(5), 314–316. doi:https://doi.org/10.1046/j.1439-0272.2003.00583.x
221. Kamarkhani, Z., Rafiei-Sefiddashti, R., Haghighi, L., Badirzadeh, A., & Hadighi, R. (2021). Molecular Examination of Trichomonas vaginalis Infection and Risk of Prostate Cancer in the Biopsy of Patients with Different Prostate Lesions. *Ethiopian Journal of Health Sciences, 31*(2), 237–240. doi:https://doi.org/10.4314/ejhs.v31i2.5
222. Kampmeier, R. H. (1978). Description of Trichomonas vaginalis by M. A. Donné. *Sexually Transmitted Diseases, 5*(3), 119. doi:https://doi.org/10.1097/00007435-197807000-00008
223. Kawaguchi, S., Shigehara, K., Sasagawa, T., Shimamura, M., Nakashima, T., Sugimoto, K., Nakashima, K., Furubayashi, K., & Namiki, M. (2012). Liquid-based urine cytology as a tool for detection of human papillomavirus, Mycoplasma spp., and Ureaplasma spp. in men. *Journal of Clinical Microbiology, 50*(2), 401–406. doi:https://doi.org/10.1128/JCM.05219-11
224. Kenfak-Foguena, A., Zarkik, Y., Wisard, M., Praz, V., Darling, K. E., Jaton-Ogay, K., Jichlinski, P., & Cavassini, M. (2010). Periurethral abscess complicating gonococcal urethritis: case report and literature review. *Infection, 38*(6), 497–500. doi:https://doi.org/10.1007/s15010-010-0050-z
225. Khosropour, C. M., Manhart, L. E., Gillespie, C. W., Lowens, M. S., Golden, M. R., Jensen, N. L., Kenny, G. E., & Totten, P. A. (2015). Efficacy of standard therapies against Ureaplasma species and persistence among men with non-gonococcal urethritis enrolled in a randomised controlled trial. *Sexually Transmitted Infections, 91*(5), 308–313. doi:https://doi.org/10.1136/sextrans-2014-051859
226. Kim, H. J., Park, J. K., Park, S. C., Kim, Y. G., Choi, H., Ko, J. I., Kim, M. K., Jeong, Y. B., & Shin, Y. S. (2017). The prevalence of causative organisms of

community-acquired urethritis in an age group at high risk for sexually transmitted infections in Korean Soldiers. *Journal of the Royal Army Medical Corps, 163*(1), 20–22. doi:https://doi.org/10.1136/jramc-2015-000488
227. Kim, J. H., Moon, H. S., Kim, K. S., Hwang, H. S., Ryu, J. S., & Park, S. Y. (2019). Comparison of Seropositivity to Trichomonas vaginalis between Men with Prostatic Tumor and Normal Men. *The Korean Journal of Parasitology, 57*(1), 21–25. doi:https://doi.org/10.3347/kjp.2019.57.1.21
228. King Wade, H. (1927). Treatment of Acute Gonorrheal Epididymitis with Special Attention to Prevention of Azoospermia. *The Journal of Urology, 18*(4), 427-432. doi:https://doi.org/10.1016/S0022-5347(17)73296-X
229. Kissinger, P. (2015). Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues. *BMC Infectious Diseases, 15*. doi:https://doi.org/10.1186/s12879-015-1055-0
230. Kissinger, P. J., Gaydos, C. A., Seña, A. C., Scott McClelland, R., Soper, D., Secor, W. E., Legendre, D., Workowski, K. A., & Muzny, C. A. (2022). Diagnosis and Management of Trichomonas vaginalis: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections Treatment Guidelines. *Clinical Infectious Diseases, 74*(Suppl. 2), S152–S161. doi:https://doi.org/10.1093/cid/ciac030
231. Kiviat, M. D., Kiviat, N. B., & Berger, R. E. (1987). Chlamydia trachomatis epididymitis diagnosed by fluorescent monoclonal antibody. *Urology, 30*(4), 395–397. doi:https://doi.org/10.1016/0090-4295(87)90313-x
232. Kjaergaard, N., Kristensen, B., Hansen, E. S., Farholt, S., Schønheyder, H. C., Uldbjerg, N., & Madsen, H. (1997). Microbiology of semen specimens from males attending a fertility clinic. *PMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica, 105*(7), 566–570. doi:https://doi.org/10.1111/j.1699-0463.1997.tb05054.x
233. Koçak, I., Yenisey, C., Dündar, M., Okyay, P., & Serter, M. (2002). Relationship between seminal plasma interleukin-6 and tumor necrosis factor alpha levels with semen parameters in fertile and infertile men. *Urological Research*, *30*(4), 263–267. doi:https://doi.org/10.1007/s00240-002-0269-y
234. Kokab, A., Akhondi, M. M., Sadeghi, M. R., Modarresi, M. H., Aarabi, M., Jennings, R., Pacey, A. A., & Eley, A. (2010). Raised inflammatory markers in semen from men with asymptomatic chlamydial infection. *Journal of Andrology, 31*(2), 114–120. doi:https://doi.org/10.2164/jandrol.109.008300
235. Kopa, Z., Wenzel, J., Papp, G. K., & Haidl, G. (2005). Role of granulocyte elastase and interleukin-6 in the diagnosis of male genital tract inflammation. *Andrologia, 37*(5), 188–194. doi:https://doi.org/10.1111/j.1439-0272.2005.00676.x
236. Korrovits, P., Ausmees, K., Mändar, R., & Punab, M. (2008). Prevalence of asymptomatic inflammatory (National Institutes of Health Category IV) prostatitis in young men according to semen analysis. *Urology*, *71*(6), 1010–1015. doi:https://doi.org/10.1016/j.urology.2007.12.082
237. Korrovits, P., Ausmees, K., Mändar, R., & Punab, M. (2011). Seminal interleukin-6 and serum prostate-specific antigen as possible predictive biomarkers in asymptomatic inflammatory prostatitis. *Urology*, *78*(2), 442–446. doi:https://doi.org/10.1016/j.urology.2011.02.013
238. Korrovits, P., Punab, M., Türk, S., & Mändar, R. (2006). Seminal microflora in asymptomatic inflammatory (NIH IV category) prostatitis. *European Urology*, *50*(6), 1338–1346. doi:https://doi.org/10.1016/j.eururo.2006.05.013

239. Korytny, A., Nasser, R., Geffen, Y., Friedman, T., Paul, M., & Ghanem-Zoubi, N. (2017). Ureaplasma parvum causing life-threatening disease in a susceptible patient. *BMJ Case Reports*, bcr2017220383. doi:https://doi.org/10.1136/bcr-2017-220383
240. Koslov, D. S., & Andersson, K. E. (2013). Physiological and pharmacological aspects of the vas deferens-an update. *Frontiers in Pharmacology, 4*. doi:https://doi.org/10.3389/fphar.2013.00101
241. Kratzsch, J., Paasch, U., Grunewald, S., Mueller, M. A., Thiery, J., & Glander, H. J. (2008). Resistin correlates with elastase and interleukin-6 in human seminal plasma. *Reproductive Biomedicine Online*, *16*(2), 283–288. doi:https://doi.org/10.1016/s1472-6483(10)60587-1
242. Krause, W., & Bohring, C. (2003). Male infertility and genital chlamydial infection: victim or perpetrator? *Andrologia, 35*(4), 209–216. doi:https://doi.org/10.1046/j.1439-0272.2003.00561.x
243. Krause, W., Bohring, C., Gueth, A., Hörster, S., Krisp, A., & Skrzypek, J. (2003). Cellular and biochemical markers in semen indicating male accessory gland inflammation. *Andrologia, 35*(5), 279–282.
244. Krieger, J. N., & Riley, D. E. (2002). Prostatitis: what is the role of infection. *International Journal of Antimicrobial Agents, 19*(6), 475–479. doi:https://doi.org/10.1016/s0924-8579(02)00086-9
245. Krieger, J. N., Jacobs, R., & Ross, S. O. (2000). Detecting urethral and prostatic inflammation in patients with chronic prostatitis. *Urology, 55*(2), 186–192. doi:https://doi.org/10.1016/s0090-4295(99)00437-9
246. Krieger, J. N., Riley, D. E., Roberts, M. C., & Berger, R. E. (1996). Prokaryotic DNA sequences in patients with chronic idiopathic prostatitis. *Journal of Clinical Microbiology, 34*(12), 3120–3128. doi:https://doi.org/10.1128/jcm.34.12.3120-3128.1996
247. Krishnan, R., & Heal, M. R. (1991). Study of the seminal vesicles in acute epididymitis. *British Journal of Urology, 67*(6), 632–637. doi:https://doi.org/10.1111/j.1464-410x.1991.tb15229.x
248. Kristensen, J. K., & Scheibel, J. H. (1984). Etiology of acute epididymitis presenting in a venereal disease clinic. *Sexually Transmitted Diseases, 11*(1), 32–33. doi:https://doi.org/10.1097/00007435-198401000-00008
249. Kullisaar, T., Türk, S., Punab, M., Korrovits, P., Kisand, K., Rehema, A., Zilmer, K., Zilmer, M., & Mändar, R. (2008). Oxidative stress in leucocytospermic prostatitis patients: preliminary results. *Andrologia*, *40*(3), 161–172. doi:https://doi.org/10.1111/j.1439-0272.2007.00816.x
250. Kummer, W., & Deckmann, K. (2017). Brush cells, the newly identified gatekeepers of the urinary tract. *Current Opinion in Urology, 27*(2), 85–92. doi:https://doi.org/10.1097/MOU.0000000000000361
251. Kusdian, G., & Gould, S. B. (2014). The biology of Trichomonas vaginalis in the light of urogenital tract infection. *Molecular and Biochemical Parasitology, 198*(2), 92–99. doi:https://doi.org/10.1016/j.molbiopara.2015.01.004
252. Kutia, S. A., Radkovskij, V. A., Astafurov, D. D., Lugin, I. A., & Yarovaya, O. Y. (2021). Современные представления о бульбоуретральных железах [Modern ideas about bulbourethral glands]. *Urologiia, 2*, 128–134. doi:https://dx.doi.org/10.18565/urology.2021.2.128-134
253. Lampiao, F., & du Plessis, S. S. (2009). Effects of tumour necrosis factor alpha and interleukin-6 on progesterone and calcium ionophore-induced acrosome re-

action. *International Journal of Andrology*, *32*(3), 274–277. doi:https://doi.org/10.1111/j.1365-2605.2008.00922.x
254. Langston, M. E., Bhalla, A., Alderete, J. F., Nevin, R. L., Pakpahan, R., Hansen, J., Elliott, D., De Marzo, A. M., Gaydos, C. A., Isaacs, W. B., Nelson, W. G., Sokoll, L. J., Zenilman, J. M., Platz, E. A., & Sutcliffe, S. (2019). Trichomonas vaginalis infection and prostate-specific antigen concentration: Insights into prostate involvement and prostate disease risk. *The Prostate*, *79*(14), 1622–1628. doi:https://doi.org/10.1002/pros.23886
255. Langston, M. E., Pakpahan, R., Nevin, R. L., De Marzo, A. M., Elliott, D. J., Gaydos, C. A., Isaacs, W. B., Nelson, W. G., Sokoll, L. J., Zenilman, J. M., Platz, E. A., & Sutcliffe, S. (2018). Sustained influence of infections on prostate-specific antigen concentration: An analysis of changes over 10 years of follow-up. *The Prostate, 78*(13), 1024–1034. doi:https://doi.org/10.1002/pros.23660
256. Lanjouw, E., Ouburg, S., de Vries, H. J., Stary, A., Radcliffe, K., & Unemo, M. (2016). 2015 European guideline on the management of Chlamydia trachomatis infections. *International Journal of STD & AIDS, 27*(5), 333–348. doi:https://doi.org/10.1177/0956462415618837
257. Lautenschlager, S., & Eichmann, A. (2002). Urethritis: an underestimated clinical variant of genital herpes in men? *Journal of the American Academy of Dermatology, 46*(2), 307–308. doi:https://doi.org/10.1067/mjd.2002.117857
258. Lawson, J. S., & Glenn, W. K. (2022). Multiple pathogens and prostate cancer. *Infectious Agents and Cancer, 17*(1), 23. doi:https://doi.org/10.1186/s13027-022-00427-1
259. Lee, C. H., Akin-Olugbade, O., & Kirschenbaum, A. (2011). Overview of prostate anatomy, histology, and pathology. *Endocrinology and Metabolism Clinics of North America*, *40*(3), 565–ix. doi:https://doi.org/10.1016/j.ecl.2011.05.012
260. Lee, J. C. (2000). Microbiology of the prostate. *Current Urology Reports, 1*(2), 159–163. doi:https://doi.org/10.1007/s11934-000-0052-y
261. Lee, J. S., Yeo, I. S., Lee, H. I., Park, J. A., Koh, K. S., & Song, W. C. (2020). Three-dimensional reconstruction of the luminal structure of human seminal vesicle. *Journal of Anatomy, 237*(6), 1006–1014. doi:https://doi.org/10.1111/joa.13269
262. Leighton, P. M., & Little, J. A. (1985). Leucocyte esterase determination as a secondary procedure for urine screening. *Journal of Clinical Pathology, 38*(2), 229–232. doi:https://doi.org/10.1136/jcp.38.2.229
263. Levy, R., Layani-Milon, M. P., Giscard D'Estaing, S., Najioullah, F., Lornage, J., Aymard, M., & Lina, B. (1999). Screening for Chlamydia trachomatis and Ureaplasma urealyticum infection in semen from asymptomatic male partners of infertile couples prior to in vitro fertilization. *International Journal of Andrology, 22*(2), 113–118. doi:https://doi.org/10.1046/j.1365-2605.1999.00157.x
264. Li, W. N., Shi, L., Long, X. Y., Li, Y., Zhu, W. B., & Liu, G. (2020, a). Mycoplasma genitalium incidence, treatment failure, and resistance: a retrospective survey of men of infertile couples from a hospital in China. *Andrology, 8*(1), 91–100. doi:https://doi.org/10.1111/andr.12646
265. Li, X., Ni, M., Xing, S., Yu, Y., Zhou, Y., Yang, S., Li, H., Zhu, R., & Han, M. (2020, b). Reactive Oxygen Species Secreted by Leukocytes in Semen Induce Self-Expression of Interleukin-6 and Affect Sperm Quality. *American journal of Men's Health*, *14*(5), 1557988320970053. doi:https://doi.org/10.1177/1557988320970053

266. Lian, W. Q., Luo, F., Song, X. L., Lu, Y. J., & Zhao, S. C. (2015). Gonorrhea and Prostate Cancer Incidence: An Updated Meta-Analysis of 21 Epidemiologic Studies. *Medical Science Monitor, 21*, 1902–1910. doi:https://doi.org/10.12659/MSM.893579
267. Liddle, O. L., Samuel, M. I., Sudhanva, M., Ellis, J., & Taylor, C. (2015). Adenovirus urethritis and concurrent conjunctivitis: a case series and review of the literature. *Sexually Transmitted Infections, 91*(2), 87–90. doi:https://doi.org/10.1136/sextrans-2014-051868
268. Liu, J. H., Li, H. Y., Cao, Z. G., Duan, Y. F., Li, Y., & Ye, Z. Q. (2002). Influence of several uropathogenic microorganisms on human sperm motility parameters in vitro. *Asian Journal of Andrology, 4*(3), 179–182.
269. Liu, J., Wang, Q., Ji, X., Guo, S., Dai, Y., Zhang, Z., Jia, L., Shi, Y., Tai, S., & Lee, Y. (2014). Prevalence of Ureaplasma urealyticum, Mycoplasma hominis, Chlamydia trachomatis infections, and semen quality in infertile and fertile men in China. *Urology, 83*(4), 795–799. doi:https://doi.org/10.1016/j.urology.2013.11.009
270. Lloyd, G., Case, J. R., De Frias, D., & Brannigan, R. E. (2003). Trichomonas vaginalis orchitis with associated severe oligoasthenoteratospermia and hypogonadism. *The Journal of Urology*, *170*(3), 924. doi:https://doi.org/10.1097/01.ju.0000080375.18547.cc
271. López-Hurtado, M., Velazco-Fernández, M., Pedraza-Sánchez, M. J., Flores-Salazar, V. R., Villagrana Zesati, R., & Guerra-Infante, F. M. (2018). Molecular detection of Chlamydia trachomatis and semen quality of sexual partners of infertile women. *Andrologia, 50*(1), 10.1111/and.12812. doi:https://doi.org/10.1111/and.12812
272. Lotti, F., & Maggi, M. (2015). Ultrasound of the male genital tract in relation to male reproductive health. *Human Reproduction Update, 21*(1), 56–83. doi:https://doi.org/10.1093/humupd/dmu042
273. Mackern-Oberti, J. P., Motrich, R. D., Breser, M. L., Sánchez, L. R., Cuffini, C., & Rivero, V. E. (2013). Chlamydia trachomatis infection of the male genital tract: an update. *Journal of Reproductive Immunology, 100*(1), 37–53. doi:https://doi.org/10.1016/j.jri.2013.05.002
274. Mackern-Oberti, J. P., Motrich, R. D., Damiani, M. T., Saka, H. A., Quintero, C. A., Sánchez, L. R., Moreno-Sosa, T., Olivera, C., Cuffini, C., & Rivero, V. E. (2017). Male genital tract immune response against Chlamydia trachomatis infection. *Reproduction, 154*(4), R99–R110. doi:https://doi.org/10.1530/REP-16-0561
275. Madeb, R., Nativ, O., Benilevi, D., Feldman, P. A., Halachmi, S., & Srugo, I. (2000). Need for diagnostic screening of Herpes simplex virus in patients with nongonococcal urethritis. *Clinical Infectious Diseases, 30*(6), 982–983. doi:https://doi.org/10.1086/313829
276. Malathi, J., Madhavan, H. N., Therese, K. L., Rinku, J. P., & Narendar, K. P. (2002). Prevalence of Chlamydia trachomatis & herpes simplex virus in males with urethritis & females with cervicitis attending STD clinic. *The Indian Journal of Medical Research, 116*, 58–63.
277. Manhart, L. E., Broad, J. M., & Golden, M. R. (2011). Mycoplasma genitalium: should we treat and how? *Clinical Infectious Diseases, 53*(Suppl. 3), S129–S142. doi:https://doi.org/10.1093/cid/cir702
278. Manhart, L. E., Jensen, J. S., Bradshaw, C. S., Golden, M. R., & Martin, D. H. (2015). Efficacy of Antimicrobial Therapy for Mycoplasma genitalium Infections.

Clinical Infectious Diseases, 61(Suppl. 8), S802–S817. doi:https://doi.org/10.1093/cid/civ785
279. Mårdh, P. A., & Colleen, S. (1975). Search for uro-genital tract infections in patients with symptoms of prostatitis. Studies on aerobic and strictly anaerobic bacteria, mycoplasmas, fungi, trichomonads and viruses. *Scandinavian Journal of Urology and Nephrology, 9*(1), 8–16. doi:https://doi.org/10.3109/00365597509139906
280. Martínez, P., Proverbio, F., & Camejo, M. I. (2007). Sperm lipid peroxidation and pro-inflammatory cytokines. *Asian Journal of Andrology*, *9*(1), 102–107. doi:https://doi.org/10.1111/j.1745-7262.2007.00238.x
281. Martínez-Prado, E., & Camejo Bermúdez, M. I. (2010). Expression of IL-6, IL-8, TNF-alpha, IL-10, HSP-60, anti-HSP-60 antibodies, and anti-sperm antibodies, in semen of men with leukocytes and/or bacteria. *American Journal of Reproductive Immunology*, *63*(3), 233–243. doi:https://doi.org/10.1111/j.1600-0897.2009.00786. x
282. Marty, M. S., Chapin, R. E., Parks, L. G., & Thorsrud, B. A. (2003). Development and maturation of the male reproductive system. *Birth defects research. Part B, Developmental and Reproductive Toxicology, 68*(2), 125–136. doi:https://doi.org/10.1002/bdrb.10015
283. Massari, P., Ram, S., Macleod, H., & Wetzler, L. M. (2003). The role of porins in neisserial pathogenesis and immunity. *Trends in Microbiology*, *11*(2), 87–93. doi:https://doi.org/10.1016/s0966-842x(02)00037-9
284. Melly, M. A., McGee, Z. A., & Rosenthal, R. S. (1984). Ability of monomeric peptidoglycan fragments from Neisseria gonorrhoeae to damage human fallopian-tube mucosa. *The Journal of Infectious Diseases*, *149*(3), 378–386. doi:https://doi.org/10.1093/infdis/149.3.378
285. McKechnie, M. L., Hillman, R., Couldwell, D., Kong, F., Freedman, E., Wang, H., & Gilbert, G. L. (2009). Simultaneous identification of 14 genital microorganisms in urine by use of a multiplex PCR-based reverse line blot assay. *Journal of Clinical Microbiology, 47*(6), 1871–1877. doi:https://doi.org/10.1128/JCM.00120-09
286. McNeal J. E. (1988). Normal histology of the prostate. *The American Journal of Surgical Pathology*, *12*(8), 619–633. doi:https://doi.org/10.1097/00000478-198808000-00003
287. Melekos, M. D., & Asbach, H. W. (1987). Epididymitis: aspects concerning etiology and treatment. *The Journal of Urology, 138*(1), 83–86. doi:https://doi.org/10.1016/s0022-5347(17)42999-5
288. Melekos, M. D., & Asbach, H. W. (1988). The role of chlamydiae in epididymitis. *International Urology and Nephrology, 20*(3), 293–297. doi:https://doi.org/10.1007/BF02549519
289. Matalliotakis, I., Kiriakou, D., Fragouli, I., Sifakis, S., Eliopoulos, G., & Koumantakis, E. (1998). Interleukin-6 in seminal plasma of fertile and infertile men. *Archives of Andrology*, *41*(1), 43–50. doi:https://doi.org/10.3109/ 01485019808988545
290. Micheli, L., Collodel, G., Cerretani, D., Menchiari, A., Noto, D., Signorini, C., & Moretti, E. (2019). Relationships between Ghrelin and Obestatin with MDA, Pro-inflammatory Cytokines, GSH/GSSG Ratio, Catalase Activity, and Semen Parameters in Infertile Patients with Leukocytospermia and Varicocele. *Oxidative Medicine and Cellular Longevity*, *2019*, 7261842. doi:https://doi.org/10.1155/2019/7261842

291. Midlej, V., & Benchimol, M. (2010). Trichomonas vaginalis kills and eats--evidence for phagocytic activity as a cytopathic effect. *Parasitology*, *137*(1), 65–76. doi:https://doi.org/10.1017/S0031182009991041
292. Mielczarek, E., & Blaszkowska, J. (2016). Trichomonas vaginalis: pathogenicity and potential role in human reproductive failure. *Infection, 44*(4), 447–458. doi:https://doi.org/10.1007/s15010-015-0860-0
293. Milbrandt, M., Winter, A. C., Nevin, R. L., Pakpahan, R., Bradwin, G., De Marzo, A. M., Elliott, D. J., Gaydos, C. A., Isaacs, W. B., Nelson, W. G., Rifai, N., Sokoll, L. J., Zenilman, J. M., Platz, E. A., & Sutcliffe, S. (2017). Insight into infection-mediated prostate damage: Contrasting patterns of C-reactive protein and prostate-specific antigen levels during infection. *The Prostate, 77*(13), 1325–1334. doi:https://doi.org/10.1002/pros.23392
294. Mitteregger, D., Aberle, S. W., Makristathis, A., Walochnik, J., Brozek, W., Marberger, M., & Kramer, G. (2012). High detection rate of Trichomonas vaginalis in benign hyperplastic prostatic tissue. *Medical Microbiology and Immunology, 201*(1), 113–116. doi:https://doi.org/10.1007/s00430-011-0205-2
295. Miyake, M., Ohnishi, K., Hori, S., Nakano, A., Nakano, R., Yano, H., Ohnishi, S., Owari, T., Morizawa, Y., Itami, Y., Nakai, Y., Inoue, T., Anai, S., Torimoto, K., Tanaka, N., Fujii, T., Furuya, H., Rosser, C. J., & Fujimoto, K. (2019). Mycoplasma genitalium Infection and Chronic Inflammation in Human Prostate Cancer: Detection Using Prostatectomy and Needle Biopsy Specimens. *Cells, 8*(3), 212. doi:https://doi.org/10.3390/cells8030212
296. Mo, X., Zhu, C., Gan, J., Wang, C., Wei, F., Gong, W., & Cai, Q. (2016). Prevalence and correlates of Mycoplasma genitalium infection among prostatitis patients. *Sexual Health,* 10.1071/SH15155. Advance online publication. doi:https://doi.org/10.1071/SH15155
297. Moi, H., Hartgill, U., Skullerud, K. H., Reponen, E. J., Syvertsen, L., & Moghaddam, A. (2017, a). Microscopy of Stained Urethral Smear in Male Urethritis; Which Cutoff Should be Used? *Sexually Transmitted Diseases, 44*(3), 189–194. doi:https://doi.org/10.1097/OLQ.0000000000000565
298. Moi, H., Reinton, N., Randjelovic, I., Reponen, E. J., Syvertsen, L., & Moghaddam, A. (2017, b). Urethral inflammatory response to ureaplasma is significantly lower than to Mycoplasma genitalium and Chlamydia trachomatis. *International Journal of STD & AIDS, 28*(8), 773–780. doi:https://doi.org/10.1177/0956462416666482
299. Molijn, G. J., & Bogdanowicz, J. F. (1997). Chlamydial epididymitis presenting as a solid asymptomatic scrotal mass. *British Journal of Urology, 80*(2), 354. doi:https://doi.org/10.1046/j.1464-410x.1997.00293.x
300. Molin, L., & Danielsson, D. (1970). Prostatic reservoir of gonococci. *British Medical Journal, 3*(5725), 767. doi:https://doi.org/10.1136/bmj.3.5725.767
301. Moretti, E., Cerretani, D., Noto, D., Signorini, C., Iacoponi, F., & Collodel, G. (2021). Relationship Between Semen IL-6, IL-33 and Malondialdehyde Generation in Human Seminal Plasma and Spermatozoa. *Reproductive Sciences*, *28*(8), 2136–2143. doi:https://doi.org/10.1007/s43032-021-00493-7
302. Moretti, E., Collodel, G., Mazzi, L., Campagna, M., Iacoponi, F., & Figura, N. (2014). Resistin, interleukin-6, tumor necrosis factor-alpha, and human semen parameters in the presence of leukocytospermia, smoking habit, and varicocele. *Fertility and Sterility*, *102*(2), 354–360. doi:https://doi.org/10.1016/j.fertnstert.2014.04.017

303. Moretti, E., Cosci, I., Spreafico, A., Serchi, T., Cuppone, A. M., & Collodel, G. (2009). Semen characteristics and inflammatory mediators in infertile men with different clinical diagnoses. *International Journal of Andrology*, *32*(6), 637–646. doi:https://doi.org/10.1111/j.1365-2605.2008.00911.x
304. Morgentaler A. (1998). Motile organisms in the epididymis?. *Human reproduction*, *13*(8), 2335. doi:https://doi.org/10.1093/oxfordjournals.humrep.a019720
305. Moridi, K., Ghazvini, K., Hemmaty, M., Hoseiniun, H., Torkaman, M., & Fallah Mehrabadi, M. H. (2021). Prevalence Determination of m. Hominis and m. Genitalium in the Semen Samples in the Northeast of Iran Using Culture and Multiplex Polymerase Chain Reaction. *Archives of Razi Institute, 76*(1), 41–49. doi:https://doi.org/10.22092/ari.2019.125966.1338
306. Morse S. A. (1978). The biology of the gonococcus. *CRC Critical Reviews in Microbiology*, *7*(2), 93–189. doi:https://doi.org/10.3109/10408417909083071
307. Morse S. A. (1979). Neisseria gonorrhoeae: physiology and metabolism. *Sexually Transmitted Diseases*, *6*(1), 28–37. doi:https://doi.org/10.1097/00007435-197901000-00009
308. Motrich, R. D., Cuffini, C., Oberti, J. P., Maccioni, M., & Rivero, V. E. (2006). Chlamydia trachomatis occurrence and its impact on sperm quality in chronic prostatitis patients. *The Journal of infection, 53*(3), 175–183. doi:https://doi.org/10.1016/j.jinf.2005.11.007
309. Mulcahy, F. M., Bignell, C. J., Rajakumar, R., Waugh, M. A., Hetherington, J. W., Ewing, R., & Whelan, P. (1987). Prevalence of chlamydial infection in acute epididymo-orchitis. *Genitourinary Medicine, 63*(1), 16–18. doi:https://doi.org/10.1136/sti.63.1.16
310. Munoz, J. L., & Goje, O. J. (2016). Mycoplasma genitalium: An Emerging Sexually Transmitted Infection. *Scientifica*, 7537318. doi:https://doi.org/10.1155/2016/7537318
311. Muro, S., Suriyut, J., & Akita, K. (2021). Anatomy of Cowper's gland in humans suggesting a secretion and emission mechanism facilitated by cooperation of striated and smooth muscles. *Scientific Reports, 11*(1), 16705. doi:https://doi.org/10.1038/s41598-021-96130-z
312. Mändar, R., Raukas, E., Türk, S., Korrovits, P., & Punab, M. (2005). Mycoplasmas in semen of chronic prostatitis patients. *Scandinavian Journal of Urology and Nephrology, 39*(6), 479–482. doi:https://doi.org/10.1080/00365590500199822
313. Nallella, K. P., Allamaneni, S. S., Pasqualotto, F. F., Sharma, R. K., Thomas, A. J., Jr, & Agarwal, A. (2004). Relationship of interleukin-6 with semen characteristics and oxidative stress in patients with varicocele. *Urology*, *64*(5), 1010–1013. doi:https://doi.org/10.1016/j.urology.2004.05.045
314. Nandipati, K. C., Pasqualotto, F. F., Thomas, A. J., Jr, & Agarwal, A. (2005). Relationship of interleukin-6 with semen characteristics and oxidative stress in vasectomy reversal patients. *Andrologia*, *37*(4), 131–134. doi:https://doi.org/10.1111/j.1439-0272.2005.00668.x
315. Najafi, A., Chaechi Nosrati, M. R., Ghasemi, E., Navi, Z., Yousefi, A., Majidiani, H., Ghaneialvar, H., Sayehmiri, K., Galvan-Ramirez, M. L., & Fakhar, M. (2019). Is there association between Trichomonas vaginalis infection and prostate cancer risk?: A systematic review and meta-analysis. *Microbial Pathogenesis, 137*, 103752. doi:https://doi.org/10.1016/j.micpath.2019.103752
316. Nasr El-Din, A., Sorour, H., Fattouh, M., & Abu El-Hamd, M. (2021). Evaluation of the role of Chlamydia trachomatis in primary male infertility. *International*

Journal of Clinical Practice, 75(10), e14702. doi:https://doi.org/10.1111/ijcp.14702
317. Naz, R. K., & Kaplan, P. (1994). Increased levels of interleukin-6 in seminal plasma of infertile men. *Journal of Andrology*, *15*(3), 220–227. doi:https://doi.org/10.1002/j.1939-4640.1994.tb00436.x
318. Ness, R. B., Markovic, N., Carlson, C. L., & Coughlin, M. T. (1997). Do men become infertile after having sexually transmitted urethritis? An epidemiologic examination. *Fertility and Sterility, 68*(2), 205–213. doi:https://doi.org/10.1016/s0015-0282(97)81502-6
319. Nieschlag, E., Behre, H. M., & Nieschlag, S. (2010). *Andrology. Male Reproductive Health and Dysfunction. 3rd Edition.* Berlin Heidelberg: Springer-Verlag.
320. Nistal, M., Santamaría, L., & Paniagua, R. (1992). The ampulla of the ductus deferens in man: morphological and ultrastructural aspects. *Journal of Anatomy, 180*(Pt. 1), 97–104.
321. Näher, H., Gissmann, L., Freese, U. K., Petzoldt, D., & Helfrich, S. (1992). Subclinical Epstein-Barr virus infection of both the male and female genital tract--indication for sexual transmission. *The Journal of Investigative Dermatology, 98*(5), 791–793. doi:https://doi.org/10.1111/1523-1747.ep12499958
322. Ochsendorf, F. R. (2008). Sexually transmitted infections: impact on male fertility. *Andrologia, 40*(2), 72–75. doi:https://doi.org/10.1111/j.1439-0272.2007.00825.x
323. Ohsawa, M., Mishima, K., Suzuki, A., Hagino, K., Doi, J., & Aozasa, K. (1994). Malignant lymphoma of the urethra: report of a case with detection of Epstein-Barr virus genome in the tumour cells. *Histopathology, 24*(6), 525–529. doi:https://doi.org/10.1111/j.1365-2559.1994.tb00570.x
324. O'Mahony, C. (2006). Adenoviral non-gonococcal urethritis. *International Journal of STD & AIDS, 17*(3), 203–204. doi:https://doi.org/10.1258/095646206775809312
325. Ondondo, R. O., Whittington, W. L., Astete, S. G., & Totten, P. A. (2010). Differential association of ureaplasma species with non-gonococcal urethritis in heterosexual men. *Sexually Transmitted Infections, 86*(4), 271–275. doi:https://doi.org/10.1136/sti.2009.040394
326. Ong, J. J., Morton, A. N., Henzell, H. R., Berzins, K., Druce, J., Fairley, C. K., Bradshaw, C. S., Read, T. R., Hocking, J. S., & Chen, M. Y. (2017). Clinical Characteristics of Herpes Simplex Virus Urethritis Compared With Chlamydial Urethritis Among Men. *Sexually Transmitted Diseases, 44*(2), 121–125. doi:https://doi.org/10.1097/OLQ.0000000000000547
327. Ozdemir, E., Keleştemur, N., & Kaplan, M. (2011). Trichomonas vaginalis as a rare cause of male factor infertility at a hospital in East Anatolia. *Andrologia, 43*(4), 283–285. doi:https://doi.org/10.1111/j.1439-0272.2010.01119.x
328. Paavonen J. (2012). Chlamydia trachomatis infections of the female genital tract: state of the art. *Annals of Medicine*, *44*(1), 18–28. doi:https://doi.org/10.3109/07853890.2010.546365
329. Palmer, C. J., Houlihan, M., Psutka, S. P., Ellis, K. A., Vidal, P., & Hollowell, C. M. (2016). Urethral Foreign Bodies: Clinical Presentation and Management. *Urology, 97*, 257–260. doi:https://doi.org/10.1016/j.urology.2016.05.045
330. Papeš, D., Pasini, M., Jeroničić, A., Vargović, M., Kotarski, V., Markotić, A., & Škerk, V. (2017). Detection of sexually transmitted pathogens in patients with chronic prostatitis/chronic pelvic pain: a prospective clinical study. *International Journal of STD & AIDS, 28*(6), 613–615. doi:https://doi.org/10.1177/0956462417691440

331. Paradisi, R., Mancini, R., Bellavia, E., Beltrandi, E., Pession, A., Venturoli, S., & Flamigni, C. (1997). T-helper 2 type cytokine and soluble interleukin-2 receptor levels in seminal plasma of infertile men. *American Journal of Reproductive Immunology*, *38*(2), 94–99. doi:https://doi.org/10.1111/j.1600-0897.1997.tb00282.x
332. Paralanov, V., Lu, J., Duffy, L. B., Crabb, D. M., Shrivastava, S., Methé, B. A., Inman, J., Yooseph, S., Xiao, L., Cassell, G. H., Waites, K. B., & Glass, J. I. (2012). Comparative genome analysis of 19 Ureaplasma urealyticum and Ureaplasma parvum strains. *BMC Microbiology, 12*. doi:https://doi.org/10.1186/1471-2180-12-88
333. Park, J. S., Lee, D., Koo, K. C., Chung, B. H., & Lee, K. S. (2020). The role of prostatic apex shape in voiding symptoms and urine flow: an exploratory and confirmatory study. *World Journal of Urology, 38*(5), 1275–1282. doi:https://doi.org/10.1007/s00345-019-02925-1
334. Pate, M. S., Hedges, S. R., Sibley, D. A., Russell, M. W., Hook, E. W., & Mestecky, J. (2001). Urethral cytokine and immune responses in Chlamydia trachomatis-infected males. *Infection and Immunity, 69*(11), 7178–7181. doi:https://doi.org/10.1128/IAI.69.11.7178-7181.2001
335. Patel, R., Kennedy, O. J., Clarke, E., Geretti, A., Nilsen, A., Lautenschlager, S., Green, J., Donders, G., van der Meijden, W., Gomberg, M., Moi, H., & Foley, E. (2017). 2017 European guidelines for the management of genital herpes. *International Journal of STD & AIDS, 28*(14), 1366–1379. doi:https://doi.org/10.1177/0956462417727194
336. Péc, J., Straka, S., Novomeský, F., Kliment, J., Péc, M., & Lazárová, Z. (1992). Mechanical urethritis and ascendent genitourinary infections due to sexual stimulation of the urethra by inserted foreign bodies. *Genitourinary Medicine, 68*(6), 399–400. doi:https://doi.org/10.1136/sti.68.6.399
337. Pellati, D., Mylonakis, I., Bertoloni, G., Fiore, C., Andrisani, A., Ambrosini, G., & Armanini, D. (2008). Genital tract infections and infertility. *European Journal of Obstetrics, Gynecology, and Reproductive Biology, 140*(1), 3–11. doi:https://doi.org/10.1016/j.ejogrb.2008.03.009
338. Pereira-Neves, A., & Benchimol, M. (2007). Phagocytosis by Trichomonas vaginalis: new insights. *Biology of the Cell*, *99*(2), 87–101. doi:https://doi.org/10.1042/BC20060084
339. Pereira-Neves, A., & Benchimol, M. (2008). Trichomonas vaginalis: in vitro survival in swimming pool water samples. *Experimental Parasitology*, *118*(3), 438–441. doi:https://doi.org/10.1016/j.exppara.2007.09.005
340. Pérez-Plaza, M., Padrón, R. S., Más, J., & Peralta, H. (1982). Semen analyses in men with asymptomatic genital gonorrhoea. *International Journal of Andrology, 5*(1), 6–10. doi:https://doi.org/10.1111/j.1365-2605.1982.tb00227.x
341. Perkins, M. J., & Decker, C. F. (2016). Non-gonococcal urethritis. *Disease-a-Month, 62*(8), 274–279. doi:https://doi.org/10.1016/j.disamonth.2016.03.011
342. Peterson, K., & Drame, D. (2010). Iatrogenic transmission of Trichomonas vaginalis by a traditional healer. *Sexually Transmitted Infections*, *86*(5), 353–354. doi:https://doi.org/10.1136/sti.2010.043125
343. Petrin, D., Delgaty, K., Bhatt, R., & Garber, G. (1998). Clinical and microbiological aspects of Trichomonas vaginalis. *Clinical Microbiology Reviews*, *11*(2), 300–317. doi:https://doi.org/10.1128/CMR.11.2.300
344. Pilatz, A., Hossain, H., Kaiser, R., Mankertz, A., Schüttler, C. G., Domann, E., Schuppe, H. C., Chakraborty, T., Weidner, W., & Wagenlehner, F. (2015). Acute

epididymitis revisited: impact of molecular diagnostics on etiology and contemporary guideline recommendations. *European Urology, 68*(3), 428–435. doi: https://doi.org/10.1016/j.eururo.2014.12.005

345. Pilatz, A., Hudemann, C., Wagenlehner, F., Schuppe, H. C., Diemer, T., Weidner, W., Renz, H., & Bschleipfer, T. (2013). Zytokine im Ejakulat : Ist die Bestimmung bei Urogenitalen Erkrankungen Sinnvoll? [Seminal cytokines: Is Quantification Useful in Urogenital Disorders?]. *Der Urologe. Ausg. A*, *52*(3), 359–366. doi: https://doi.org/10.1007/s00120-013-3141-5
346. Pilatz, A., Kilb, J., Kaplan, H., Fietz, D., Hossain, H., Schüttler, C. G., Diemer, T., Bergmann, M., Domann, E., Weidner, W., Wagenlehner, F., & Schuppe, H. C. (2019). High prevalence of urogenital infection/inflammation in patients with azoospermia does not impede surgical sperm retrieval. *Andrologia, 51*(10), e13401. doi:https://doi.org/10.1111/and.13401
347. Piludu, M., Hand, A. R., Cossu, M., & Piras, M. (2009). Immunocytochemical localization of MG1 mucin in human bulbourethral gland. *Journal of Anatomy, 214*(1), 179–182. doi:https://doi.org/10.1111/j.1469-7580.2008.01018.x
348. Plecko, V., Zele-Starcevic, L., Tripkovic, V., Skerlev, M., Ljubojevic, S., Plesko, S., Marekovic, I., & Jensen, J. S. (2014). Unusually low prevalence of Mycoplasma genitalium in urine samples from infertile men and healthy controls: a prevalence study. *BMJ Open*, *4*(8), e005372. doi:https://doi.org/10.1136/bmjopen-2014-005372
349. Pond, M. J., Nori, A. V., Patel, S., Laing, K., Ajayi, M., Copas, A. J., Butcher, P. D., Hay, P., & Sadiq, S. T. (2015). Performance evaluation of automated urine microscopy as a rapid, non-invasive approach for the diagnosis of non-gonococcal urethritis. *Sexually Transmitted Infections*, *91*(3), 165–170. doi:https://doi.org/10.1136/sextrans-2014-051761
350. Poole, D. N., & McClelland, R. S. (2013). Global epidemiology of Trichomonas vaginalis. *Sexually Transmitted Infections, 89*(6), 418–422. doi:https://doi.org/10.1136/sextrans-2013-051075
351. Pradidarcheep, W., Wallner, C., Dabhoiwala, N. F., & Lamers, W. H. (2011). Anatomy and histology of the lower urinary tract. *Handbook of Experimental Pharmacology,* (202), 117–148. doi:https://doi.org/10.1007/978-3-642-16499-6_7
352. Prasad Ray, R., Ghosh, B., & Pal, D. K. (2015). Urethral foreign body in an adolescent boy: report of two rare cases and review of literature. *International Journal of Adolescent Medicine and Health, 27*(4), 463–465. doi:https://doi.org/10.1515/ijamh-2014-0057
353. Pudney, J., & Anderson, D. (2011). Innate and acquired immunity in the human penile urethra. *Journal of Reproductive Immunology, 88*(2), 219–227. doi:https://doi.org/10.1016/j.jri.2011.01.006
354. Punab, M., Lõivukene, K., Kermes, K., & Mändar, R. (2003). The limit of leucocytospermia from the microbiological viewpoint. *Andrologia, 35*(5), 271–278. doi:https://doi.org/10.1046/j.1439-0272.2003.00555.x
355. Punab, M., Poolamets, O., Paju, P., Vihljajev, V., Pomm, K., Ladva, R., Korrovits, P., & Laan, M. (2017). Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. *Human Reproduction*, *32*(1), 18–31. https://doi.org/10.1093/humrep/dew284
356. Puppo, V., & Puppo, G. (2016). Comprehensive review of the anatomy and physiology of male ejaculation: Premature ejaculation is not a disease. *Clinical Anatomy, 29*(1), 111–119. doi:https://doi.org/10.1002/ca.22655

357. Qian, L., Zhou, Y., Du, C., Wen, J., Teng, S., & Teng, Z. (2014). IL-18 levels in the semen of male infertility: semen analysis. *International Journal of Biological Macromolecules*, *64*, 190–192. doi:https://doi.org/10.1016/j.ijbiomac.2013.12.005
358. Qing, L., Song, Q. X., Feng, J. L., Li, H. Y., Liu, G., & Jiang, H. H. (2017). Prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium and Ureaplasma urealyticum infections using a novel isothermal simultaneous RNA amplification testing method in infertile males. *Annals of Clinical Microbiology and Antimicrobials, 16*(1), 45. doi:https://doi.org/10.1186/s12941-017-0220-2
359. Quinn, T. C., Goodell, S. E., Mkrtichian, E., Schuffler, M. D., Wang, S. P., Stamm, W. E., & Holmes, K. K. (1981). Chlamydia trachomatis proctitis. *The New England Journal of Medicine*, *305*(4), 195–200. doi:https://doi.org/10.1056/NEJM198107233050404
360. Rahman, N., Featherstone, N. C., & DeCaluwe, D. (2010). Spider-man, magnets, and urethral-cutaneous fistula. *Urology, 76*(1), 162–163. doi:https://doi.org/10.1016/j.urology.2009.08.089
361. Ratkal, J. M., Raykar, R., & Shirol, S. S. (2015). Electric Wire as Foreign Body in the Bladder and Urethra-a Case Report and Review of Literature. *The Indian Journal of Surgery, 77*(Suppl. 3), 1323–1325. doi:https://doi.org/10.1007/s12262-014-1162-y
362. Rebhun J (1964). Pulmonary Trichomoniasis associated with a fever of unknown origin. *California Medicine*, *100*(6), 443–444.
363. Rechenchoski, D. Z., Faccin-Galhardi, L. C., Linhares, R. E., & Nozawa, C. (2017). Herpesvirus: an underestimated virus. *Folia Microbiologica, 62*(2), 151–156. doi:https://doi.org/10.1007/s12223-016-0482-7
364. Redgrove, K. A., & McLaughlin, E. A. (2014). The Role of the Immune Response in Chlamydia trachomatis Infection of the Male Genital Tract: A Double-Edged Sword. *Frontiers in Immunology, 5*, 534. doi:https://doi.org/10.3389/fimmu.2014.00534
365. Rietmeijer, C. A., & Mettenbrink, C. J. (2012). Recalibrating the Gram stain diagnosis of male urethritis in the era of nucleic acid amplification testing. *Sexually Tansmitted Diseases, 39*(1), 18–20. doi:https://doi.org/10.1097/OLQ.0b013e3182354da3
366. Rietmeijer, C. A., & Mettenbrink, C. J. (2017). The Diagnosis of Nongonococcal Urethritis in Men: Can There Be a Universal Standard? *Sexually Transmitted Diseases, 44*(3), 195–196. doi:https://doi.org/10.1097/OLQ.0000000000000594
367. Rivera, V. V., Cardona Maya, W. D., & Puerta-Suárez, J. (2022). The relationship between sexually transmitted microorganisms and seminal quality in asymptomatic men. *Asian Journal of Urology, 9*(4), 473–479. doi:https://doi.org/10.1016/j.ajur.2021.09.004
368. Roan, N. R., & Starnbach, M. N. (2008). Immune-mediated control of Chlamydia infection. *Cellular Microbiology, 10*(1), 9–19. doi:https://doi.org/10.1111/j.1462-5822.2007.01069.x
369. Roche. (2021, June). Combur¹⁰ Test UX, 08967369001.
370. Rohde, K. H., & Dyer, D. W. (2003). Mechanisms of iron acquisition by the human pathogens Neisseria meningitidis and Neisseria gonorrhoeae. *Frontiers in Bioscience: a Journal and Virtual Library*, *8*, d1186–d1218. doi:https://doi.org/10.2741/1133

371. Rose-John S. (2020). Interleukin-6 signalling in health and disease. *F1000Research*, *9*, F1000 Faculty Rev-1013. doi:https://doi.org/10.12688/f1000research.26058.1
372. Rossignol, L., Feuillepain, L., Ndeikoundam Ngangro, N., Souty, C., Fournet, N., Le Strat, Y., Baroux, N., Hanslik, T., Lot, F., & Blanchon, T. (2019). Estimate of male urethritis incidences in France between 2007 and 2017 with a specific focus on Neisseria gonorrhoeae, Chlamydia trachomatis, and Trichomonas vaginalis infections. *BMC Infectious Diseases, 19*(1). doi:https://doi.org/10.1186/s12879-019-4202-1
373. Rowley, J., Vander Hoorn, S., Korenromp, E., Low, N., Unemo, M., Abu-Raddad, L. J., Chico, R. M., Smolak, A., Newman, L., Gottlieb, S., Thwin, S. S., Broutet, N., & Taylor, M. M. (2019). Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates. *Bulletin of the World Health Organization*, *97*(8), 548–562. doi:https://doi.org/10.2471/BLT.18.228486
374. Rusz, A., Pilatz, A., Wagenlehner, F., Linn, T., Diemer, T., Schuppe, H. C., Lohmeyer, J., Hossain, H., & Weidner, W. (2012). Influence of urogenital infections and inflammation on semen quality and male fertility. *World Journal of Urology, 30*(1), 23–30. doi:https://doi.org/10.1007/s00345-011-0726-8
375. Ryan, C. M., de Miguel, N., & Johnson, P. J. (2011). Trichomonas vaginalis: current understanding of host-parasite interactions. *Essays in Biochemistry*, *51*, 161–175. doi:https://doi.org/10.1042/bse0510161
376. Rybar, R., Prinosilova, P., Kopecka, V., Hlavicova, J., Veznik, Z., Zajicova, A., & Rubes, J. (2012). The effect of bacterial contamination of semen on sperm chromatin integrity and standard semen parameters in men from infertile couples. *Andrologia, 44*(Suppl. 1), 410–418. doi:https://doi.org/10.1111/j.1439-0272.2011.01198.x
377. Salmeri, M., Santanocita, A., Toscano, M. A., Morello, A., Valenti, D., La Vignera, S., Bellanca, S., Vicari, E., & Calogero, A. E. (2010). Chlamydia trachomatis prevalence in unselected infertile couples. *Systems Biology in Reproductive Medicine*, *56*(6), 450–456. doi:https://doi.org/10.3109/19396361003792853
378. Samaraweera, G. R., Garcia, K., Druce, J., Williams, H., Bradshaw, C. S., Fairley, C. K., Chow, E. P., Denham, I. M., Read, T. R., & Chen, M. Y. (2016). Characteristics of adenovirus urethritis among heterosexual men and men who have sex with men: a review of clinical cases. *Sexually Transmitted Infections, 92*(3), 172–174. doi:https://doi.org/10.1136/sextrans-2015-052243
379. Sanches, B. D. A., Tamarindo, G. H., Maldarine, J. D. S., Da Silva, A. D. T., Dos Santos, V. A., Góes, R. M., Taboga, S. R., & Carvalho, H. F. (2021). Telocytes of the male urogenital system: Interrelationships, possible functions, and pathological implications. *Cell Biology International, 45*(8), 1613–1623. doi:https://doi.org/10.1002/cbin.11612
380. Sanders, C. J., & Mulder, M. M. (1998). Periurethral gland abscess: aetiology and treatment. *Sexually Transmitted Infections, 74*(4), 276–278. doi:https://doi.org/10.1136/sti.74.4.276
381. Sarier, M., & Kukul, E. (2019). Classification of non-gonococcal urethritis: a review. *International urology and nephrology, 51*(6), 901–907. doi:https://doi.org/10.1007/s11255-019-02140-2
382. Sarier, M., Sepin, N., Duman, I., Demir, M., Hizel, A., Göktaş, Ş., Emek, M., Kukul, E., & Soylu, A. (2018). Microscopy of Gram-stained urethral smear in the

diagnosis of urethritis: Which threshold value should be selected?. *Andrologia*, *50*(10), e13143. https://doi.org/10.1111/and.13143
383. Scheibel, J. H., Andersen, J. T., Brandenhoff, P., Geerdsen, J. P., Bay-Nielsen, A., Schultz, B. A., & Walter, S. (1983). Chlamydia trachomatis in acute epididymitis. *Scandinavian Journal of Urology and Nephrology, 17*(1), 47–50. doi:https://doi.org/10.3109/00365598309179780
384. Schuppe, H. C., Pilatz, A., Hossain, H., Diemer, T., Wagenlehner, F., & Weidner, W. (2017). Urogenital Infection as a Risk Factor for Male Infertility. *Deutsches Arzteblatt International, 114*(19), 339–346. doi:https://doi.org/10.3238/arztebl.2017.0339
385. Schwebke, J. R., & Hook, E. W. (2003). High rates of Trichomonas vaginalis among men attending a sexually transmitted diseases clinic: implications for screening and urethritis management. *The Journal of Infectious Diseases, 188*(3), 465–468. doi:https://doi.org/10.1086/376558
386. Seike, K., Maeda, S., Kubota, Y., Tamaki, M., Yasuda, M., & Deguchi, T. (2013). Prevalence and morbidity of urethral Trichomonas vaginalis in Japanese men with or without urethritis. *Sexually Transmitted Infections, 89*(6), 528–530. doi:https://doi.org/10.1136/sextrans-2012-050702
387. Sellami, H., Znazen, A., Sellami, A., Mnif, H., Louati, N., Ben Zarrouk, S., Keskes, L., Rebai, T., Gdoura, R., & Hammami, A. (2014). Molecular detection of Chlamydia trachomatis and other sexually transmitted bacteria in semen of male partners of infertile couples in Tunisia: the effect on semen parameters and spermatozoa apoptosis markers. *PloS ONE*, *9*(7). doi:https://doi.org/10.1371/journal.pone.0098903
388. Seña, A. C., Miller, W. C., Hobbs, M. M., Schwebke, J. R., Leone, P. A., Swygard, H., Atashili, J., & Cohen, M. S. (2007). Trichomonas vaginalis infection in male sexual partners: implications for diagnosis, treatment, and prevention. *Clinical Infectious Diseases*, *44*(1), 13–22. doi:https://doi.org/10.1086/511144
389. Sethi, S., Singh, G., Samanta, P., & Sharma, M. (2012). Mycoplasma genitalium: an emerging sexually transmitted pathogen. *The Indian Journal of Medical Research, 136*(6), 942–955.
390. Shahmanesh, M. (1989). Characteristics of inflammatory cells in urethral smears from men with non-gonococcal urethritis. *Genitourinary Medicine, 65*(1), 18–21. doi:https://doi.org/10.1136/sti.65.1.18
391. Sherman, J. K., Hostetler, T. L., McHenry, K., & Daly, J. J. (1991). Cryosurvival of Trichomonas vaginalis during cryopreservation of human semen. *Cryobiology*, *28*(3), 246–250. doi:https://doi.org/10.1016/0011-2240(91)90029-n
392. Sherrard, J. (2014). Gonorrhoea. *Medicine, 42*(6), 323–326. doi:https://doi.org/10.1016/j.mpmed.2014.03.011
393. Shiadeh, M. N., Niyyati, M., Fallahi, S., & Rostami, A. (2016). Human parasitic protozoan infection to infertility: a systematic review. *Parasitology Research, 115*(2), 469–477. doi:https://doi.org/10.1007/s00436-015-4827-y
394. Shigehara, K., Kawaguchi, S., Sasagawa, T., Furubayashi, K., Shimamura, M., Maeda, Y., Konaka, H., Mizokami, A., Koh, E., & Namiki, M. (2011). Prevalence of genital Mycoplasma, Ureaplasma, Gardnerella, and human papillomavirus in Japanese men with urethritis, and risk factors for detection of urethral human papillomavirus infection. *Journal of Infection and Chemotherapy, 17*(4), 487–492. doi:https://doi.org/10.1007/s10156-010-0203-0

395. Shimada, Y., Ito, S., Mizutani, K., Sugawara, T., Seike, K., Tsuchiya, T., Yokoi, S., Nakano, M., Yasuda, M., & Deguchi, T. (2014). Bacterial loads of Ureaplasma urealyticum contribute to development of urethritis in men. *International Journal of STD & AIDS, 25*(4), 294–298. doi:https://doi.org/10.1177/0956462413504556
396. Shimoya, K., Matsuzaki, N., Ida, N., Okada, T., Taniguchi, T., Sawai, K., Itoh, S., Ohashi, K., Saji, F., & Tanizawa, O. (1995). Detection of monocyte chemotactic and activating factor (MCAF) and interleukin (IL)-6 in human seminal plasma and effect of leukospermia on these cytokine levels. *American Journal of Reproductive Immunology*, *34*(5), 311–316. doi:https://doi.org/10.1111/j.1600-0897.1995.tb00957.x
397. Shipitsyna, E., Krasnoselskikh, T., Zolotoverkhaya, E., Savicheva, A., Krotin, P., Domeika, M., & Unemo, M. (2013). Sexual behaviours, knowledge and attitudes regarding safe sex, and prevalence of non-viral sexually transmitted infections among attendees of youth clinics in St. Petersburg, Russia. *Journal of the European Academy of Dermatology and Venereology, 27*(1), e75–e84. doi:https://doi.org/10.1111/j.1468-3083.2012.04512.x
398. Simms, A., Chappidi, M., Yang, H., Hampson, L., Breyer, B., & Cohen, A. J. (2020). Urethral Defect in Setting of Recurrent Urethral Foreign Body Insertion. *Urology, 137*, e12–e13. doi:https://doi.org/10.1016/j.urology.2019.11.024
399. Simos, P., & Stewart, A. G. (2022). Sexually acquired reactive arthritis secondary to macrolide-resistant Mycoplasma genitalium urethritis. *Internal Medicine Journal, 52*(2), 332–333. doi:https://doi.org/10.1111/imj.15684
400. Singh, M., & Blandy, J. P. (1976). The pathology of urethral stricture. *The Journal of Urology, 115*(6), 673–676. doi:https://doi.org/10.1016/s0022-5347(17)59331-3
401. Sivaraj, V., Ahamed, A., Artykov, R., & Menon-Johansson, A. (2021). Epididymitis and its aetiologies in a central London sexual health clinic. *International Journal of STD & AIDS, 32*(1), 96–99. doi:https://doi.org/10.1177/0956462420963879
402. Skerk, V., Krhen, I., Schonwald, S., Cajic, V., Markovinovic, L., Roglic, S., Zekan, S., Andracevic, A. T., & Kruzic, V. (2004). The role of unusual pathogens in prostatitis syndrome. *International Journal of Antimicrobial Agents, 24*(Suppl. 1), S53–S56. doi:https://doi.org/10.1016/j.ijantimicag.2004.02.010
403. Skerk, V., Markovinovic, L., Zekan, S., Jaksic, J., Zidovec Lepej, S., Markotic, A., Skerk, V., Radosevic, V., Cvitkovic, L., & Begovac, J. (2009). The significance of Chlamydia trachomatis in urethritis and prostatitis – differences in therapeutic approach – Croatian experience. *Journal of Chemotherapy, 21*(1), 63–67. doi:https://doi.org/10.1179/joc.2009.21.1.63
404. Skerk, V., Schönwald, S., Krhen, I., Markovinović, L., Beus, A., Kuzmanović, N. S., Kruzić, V., & Vince, A. (2002). Aetiology of chronic prostatitis. *International Journal of Antimicrobial Agents, 19*(6), 471–474. doi:https://doi.org/10.1016/s0924-8579(02)00087-0
405. Smith, R., Copas, A. J., Prince, M., George, B., Walker, A. S., & Sadiq, S. T. (2003). Poor sensitivity and consistency of microscopy in the diagnosis of low grade non-gonococcal urethritis. *Sexually Transmitted Infections, 79*(6), 487–490. doi:https://doi.org/10.1136/sti.79.6.487
406. Smolec, D., Ekiel, A., Kłuciński, P., & Kawecki, J. (2021). Occurrence of urogenital mycoplasmas in men with the common genitourinary diseases. *Brazilian Journal of Microbiology, 52*(4), 2013–2019. doi:https://doi.org/10.1007/s42770-021-00620-1

407. Sonnenberg, P., Ison, C. A., Clifton, S., Field, N., Tanton, C., Soldan, K., Beddows, S., Alexander, S., Khanom, R., Saunders, P., Copas, A. J., Wellings, K., Mercer, C. H., & Johnson, A. M. (2015). Epidemiology of Mycoplasma genitalium in British men and women aged 16–44 years: evidence from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3). *International Journal of Epidemiology*, *44*(6), 1982–1994. https://doi.org/10.1093/ije/dyv194
408. Sripada, S., Amezaga, M. R., Hamilton, M., McKenzie, H., Templeton, A., & Bhattacharya, S. (2010). Absence of chlamydial deoxyribonucleic acid from testicular and epididymal samples from men with obstructive azoospermia. *Fertility and Sterility, 93*(3), 833–836. doi:https://doi.org/10.1016/j.fertnstert.2008.10.063
409. Srugo, I., Steinberg, J., Madeb, R., Gershtein, R., Elias, I., Tal, J., & Nativ, O. (2003). Agents of non-gonococcal urethritis in males attending an Israeli clinic for sexually transmitted diseases. *The Israel Medical Association Journal, 5*(1), 24–27.
410. Strand, A., Vahlne, A., Svennerholm, B., Wallin, J., & Lycke, E. (1986). Asymptomatic virus shedding in men with genital herpes infection. *Scandinavian Journal of Infectious Diseases, 18*(3), 195–197. doi:https://doi.org/10.3109/00365548609032327
411. Strauss, M., Colodner, R., Sagas, D., Adawi, A., Edelstein, H., & Chazan, B. (2018). Detection of Ureaplasma Species by a Semi-Quantitative PCR Test in Urine Samples: Can It Predict Clinical Significance? *The Israel Medical Association Journal, 20*(1), 9–13.
412. Sturm, P. D., Moodley, P., Khan, N., Ebrahim, S., Govender, K., Connolly, C., & Sturm, A. W. (2004). Aetiology of male urethritis in patients recruited from a population with a high HIV prevalence. *International Journal of Antimicrobial Agents, 24*(Suppl. 1), S8–S14. doi:https://doi.org/10.1016/j.ijantimicag.2004.02.004
413. Subramanian, S. (1981). Gonococcal urethritis with bilateral tysonitis and periurethral abscess. *Sexually Transmitted Diseases, 8*(2), 77–78. doi:https://doi.org/10.1097/00007435-198104000-00009
414. Sutcliffe, S., Nevin, R. L., Pakpahan, R., Elliott, D. J., Cole, S. R., De Marzo, A. M., Gaydos, C. A., Isaacs, W. B., Nelson, W. G., Sokoll, L. J., Zenilman, J. M., Cersovsky, S. B., & Platz, E. A. (2011). Prostate involvement during sexually transmitted infections as measured by prostate-specific antigen concentration. *British Journal of Cancer, 105*(5), 602–605. doi:https://doi.org/10.1038/bjc.2011.271
415. Sutcliffe, S., Zenilman, J. M., Ghanem, K. G., Jadack, R. A., Sokoll, L. J., Elliott, D. J., Nelson, W. G., De Marzo, A. M., Cole, S. R., Isaacs, W. B., & Platz, E. A. (2006). Sexually transmitted infections and prostatic inflammation/cell damage as measured by serum prostate specific antigen concentration. *The Journal of Urology, 175*(5), 1937–1942. doi:https://doi.org/10.1016/S0022-5347(05)00892-X
416. Sysmex. (2011). *UF-500i – High-quality urinalysis at the touch of a button.* Retrieved October 13, 2019, from The 'second generation' urine Fluoresence Flow Cytometry: the key to modern screening for urinary tract infections. Sysmex Xtra: https://www.sysmex-europe.com/fileadmin/media/f100/Xtra/Xtra_article_The_key_to_ modern_screening__of_UTI.pdf
417. Zegers-Hochschild, F., Adamson, G. D., de Mouzon, J., Ishihara, O., Mansour, R., Nygren, K., Sullivan, E., Vanderpoel, S., International Committee for Monitoring

Assisted Reproductive Technology, & World Health Organization (2009). International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. *Fertility and Sterility*, *92*(5), 1520–1524. https://doi.org/10.1016/j.fertnstert.2009.09.009

418. Zhang, N., Wang, R., Li, X., Liu, X., Tang, Z., & Liu, Y. (2014). Are Ureaplasma spp. a cause of nongonococcal urethritis? A systematic review and meta-analysis. *PLOS One, 9*(12), e113771. doi:https://doi.org/10.1371/journal.pone.0113771
419. Zhang, Z., Li, F., Deng, Y., Li, Y., Sheng, W., Tian, X., Yang, Z., Wang, S., Guo, L., Hao, L., & Mei, X. (2023). Trichomonas vaginalis excretory secretory proteins reduce semen quality and male fertility. *Acta Tropica*, *238*, 106794. doi:https://doi.org/10.1016/j.actatropica.2022.106794
420. Zhang, Z., Li, Y., Lu, H., Li, D., Zhang, R., Xie, X., Guo, L., Hao, L., Tian, X., Yang, Z., Wang, S., & Mei, X. (2022). A systematic review of the correlation between Trichomonas vaginalis infection and infertility. *Acta Tropica, 236*, 106693. doi:https://doi.org/10.1016/j.actatropica.2022.106693
421. Zheng, B. J., Yin, Y. P., Xiang, Z., Han, Y., Shi, M. Q., Jiang, N., Yu, R. X., & Chen, X. S. (2014). An epidemiological study of Mycoplasma genitalium infections among males attending a sexually transmitted disease clinic in Guangxi, China. *Japanese Journal of Infectious Diseases*, *67*(1), 17–21. doi:https://doi.org/10.7883/yoken.67.17
422. Tabrizi, S. N., Ling, A. E., Bradshaw, C. S., Fairley, C. K., & Garland, S. M. (2007). Human adenoviruses types associated with non-gonococcal urethritis. *Sexual Health, 4*(1), 41–44. doi:https://doi.org/10.1071/sh06054
423. Tam Le, M., Nguyen, D., Bach Nguyen, H., Quynh Tram Ngo, V., & Quoc Huy Nguyen, V. (2022). Ureaplasma urealyticum and Mycoplasma genitalium detection and sperm quality: A cross-sectional study in Vietnam. *International Journal of Reproductive Biomedicine, 20*(3), 185–194. doi:https://doi.org/10.18502/ijrm.v20i3.10710
424. Tan, C. W., & Chlebicki, M. P. (2016). Urinary tract infections in adults. *Singapore Medical Journal*, *57*(9), 485–490. doi: https://doi.org/10.11622/smedj.2016153
425. Tantengco, O., Aquino, I., de Castro Silva, M., Rojo, R. D., & Abad, C. (2021). Association of mycoplasma with prostate cancer: A systematic review and meta-analysis. *Cancer Epidemiology, 75*. doi:https://doi.org/10.1016/j.canep.2021.102021
426. Taylor-Robinson, D., & Jensen, J. S. (2011). Mycoplasma genitalium: from Chrysalis to multicolored butterfly. *Clinical Microbiology Reviews, 34*(3), 498–514. doi:https://doi.org/10.1128/CMR.00006-11
427. Taylor-Robinson, D., & Schaeverbeke, T. (1996). Mycoplasmas in rheumatoid arthritis and other human arthritides. *Journal of Clinical Pathology, 49*(10), 781–782. doi:https://doi.org/10.1136/jcp.49.10.781
428. Taylor-Robinson, D., Gilroy, C. B., Horowitz, S., & Horowitz, J. (1994). Mycoplasma genitalium in the joints of two patients with arthritis. *European Journal of Clinical Microbiology & Infectious Diseases, 13*(12), 1066–1069. doi:https://doi.org/10.1007/BF02111830
429. Taylor-Robinson, D., Renton, A., Jensen, J. S., Ison, C. A., Filatova, E., Dmitriev, G., & Akovbian, V. (2009). Association of Mycoplasma genitalium with acute non-gonococcal urethritis in Russian men: a comparison with gonococcal and

chlamydial urethritis. *International Journal of STD & AIDS, 20*(4), 234–237. doi:https://doi.org/10.1258/ijsa.2008.008298
430. The American Society of Andrology. (2010). *Handbook of Andrology. 2nd Edition.* Lawrence: Allen Press Inc.
431. Thomas, R., Macsween, K. F., McAulay, K., Clutterbuck, D., Anderson, R., Reid, S., Higgins, C. D., Swerdlow, A. J., Harrison, N., Williams, H., & Crawford, D. H. (2006). Evidence of shared Epstein-Barr viral isolates between sexual partners, and low level EBV in genital secretions. *Journal of Medical Virology, 78*(9), 1204–1209. doi:https://doi.org/10.1002/jmv.20682
432. Tiplica, G. S., Radcliffe, K., Evans, C., Gomberg, M., Nandwani, R., Rafila, A., Nedelcu, L., & Salavastru, C. (2015). 2015 European guidelines for the management of partners of persons with sexually transmitted infections. *Journal of the European Academy of Dermatology and Venereology, 29*(7), 1251–1257. https://doi.org/10.1111/jdv.13181
433. Toktanis, G., Kaya-Sezginer, E., Yilmaz-Oral, D., & Gur, S. (2018). Potential therapeutic value of transient receptor potential channels in male urogenital system. *Pflugers Archiv: European Journal of Physiology, 470*(11), 1583–1596. doi:https://doi.org/10.1007/s00424-018-2188-y
434. Tønsberg, E., & Hartgill, U. (2014). The urethral smear as a tool in diagnosing adenovirus-induced urethritis. *International Journal of STD & AIDS, 25*(14), 1047–1049. doi:https://doi.org/10.1177/0956462414531561
435. Toth, M., Patton, D. L., Campbell, L. A., Carretta, E. I., Mouradian, J., Toth, A., Shevchuk, M., Baergen, R., & Ledger, W. (2000). Detection of chlamydial antigenic material in ovarian, prostatic, ectopic pregnancy and semen samples of culture-negative subjects. *American Journal of Reproductive Immunology, 43*(4), 218–222. doi:https://doi.org/10.1111/j.8755-8920.2000.430406.x
436. Tully, J. G., & Baseman, J. B. (1991). Mycoplasma. *Lancet, 337*(8752), 1296. doi:https://doi.org/10.1016/0140-6736(91)92971-4
437. Tully, J. G., Rose, D. L., Baseman, J. B., Dallo, S. F., Lazzell, A. L., & Davis, C. P. (1995). Mycoplasma pneumoniae and Mycoplasma genitalium mixture in synovial fluid isolate. *Journal of Clinical Microbiology, 33*(7), 1851–1855. doi:https://doi.org/10.1128/jcm.33.7.1851-1855.1995
438. Tully, J. G., Taylor-Robinson, D., Cole, R. M., & Rose, D. L. (1981). A newly discovered mycoplasma in the human urogenital tract. *Lancet, 1*(8233), 1288–1291. doi:https://doi.org/10.1016/s0140-6736(81)92461-2
439. Tummala, S., Everstine, A., Acharya, V., & Bhatia, S. (2020). Prostate Arterial Anatomy: A Primer for Interventional Radiologists. *Techniques in Vascular and Interventional Radiology*, *23*(3), 100689. doi:https://doi.org/10.1016/j.tvir.2020.100689
440. Tuttle, J. P., Holbrook, T. W., & Derrick, F. C. (1977). Interference of human spermatozoal motility by trichomonas vaginalis. *The Journal of Urology, 118*(6), 1024–1025. doi:https://doi.org/10.1016/s0022-5347(17)58285-3
441. Tyndall, M. W., Nasio, J., Maitha, G., Ndinya-Achola, J. O., Plummer, F. A., Sellors, J. W., Luinstra, K. E., Jang, D., Mahony, J. B., & Chernesky, M. A. (1994). Leukocyte esterase urine strips for the screening of men with urethritis--use in developing countries. *Genitourinary Medicine*, *70*(1), 3–6. doi:https://doi.org/10.1136/sti.70.1.3
442. Unemo, M., Ross, J., Serwin, A. B., Gomberg, M., Cusini, M., & Jensen, J. S. (2020). European guideline for the diagnosis and treatment of gonorrhoea in

adults. *International Journal of STD & AIDS*, 956462420949126. Advance online publication. doi:https://doi.org/10.1177/0956462420949126
443. Unemo, M., & Shafer, W. M. (2011). Antibiotic resistance in Neisseria gonorrhoeae: origin, evolution, and lessons learned for the future. *Annals of the New York Academy of Sciences*, *1230*, E19–E28. doi:https://doi.org/10.1111/j.1749-6632.2011.06215.x
444. Unemo, M., & Shafer, W. M. (2014). Antimicrobial resistance in Neisseria gonorrhoeae in the 21st century: past, evolution, and future. *Clinical Microbiology Reviews*, *27*(3), 587–613. doi:https://doi.org/10.1128/CMR.00010-14
445. Unemo, M., Seifert, H. S., Hook, E. W., Hawkes, S., Ndowa, F., & Dillon, J. R. (2019). Gonorrhoea. *Nature Reviews, 5*(1). doi:https://doi.org/10.1038/s41572-019-0128-6
446. Uusküla, A., & Raukas, E. (2004). Atypical genital herpes: report of five cases. *Scandinavian Journal of Infectious Diseases, 36*(1), 37–39. doi:https://doi.org/10.1080/00365540310017276
447. Wada, K., Hamasuna, R., Sadahira, T., Araki, M., & Yamamoto, S. (2021). UAA-AAUS guideline for M. genitalium and non-chlamydial non-gonococcal urethritis. *Journal of Infection and Chemotherapy, 27*(10), 1384–1388. doi:https://doi.org/10.1016/j.jiac.2021.07.007
448. Wagenlehner, F. M., Naber, K. G., & Weidner, W. (2006). Chlamydial infections and prostatitis in men. *BJU International, 97*(4), 687–690. doi:https://doi.org/10.1111/j.1464-410X.2006.06007.x
449. van der Veer, C., van Rooijen, M. S., Himschoot, M., de Vries, H. J., & Bruisten, S. M. (2016). Trichomonas vaginalis and Mycoplasma genitalium: age-specific prevalence and disease burden in men attending a sexually transmitted infections clinic in Amsterdam, the Netherlands. *Sexually Transmitted Infections, 92*(1), 83–85. doi:https://doi.org/10.1136/sextrans-2015-052118
450. Van Gerwen, O. T., Camino, A. F., Sharma, J., Kissinger, P. J., & Muzny, C. A. (2021). Epidemiology, Natural History, Diagnosis, and Treatment of Trichomonas vaginalis in Men. *Clinical Infectious Diseases, 73*(6), 1119–1124. doi:https://doi.org/10.1093/cid/ciab514
451. Vandenbroucke-Grauls, C. M., Rozenberg-Arska, M., den Hengst, C. W., & Verhoef, J. (1982). Prostatitis due to penicillinase-producing Neisseria gonorrhoeae. Case reports. *The British journal of venereal diseases, 58*(5), 311–313. doi:https://doi.org/10.1136/sti.58.5.311
452. van Rooijen, M. S., van der Loeff, M. F., Morré, S. A., van Dam, A. P., Speksnijder, A. G., & de Vries, H. J. (2015). Spontaneous pharyngeal Chlamydia trachomatis RNA clearance. A cross-sectional study followed by a cohort study of untreated STI clinic patients in Amsterdam, The Netherlands. *Sexually Transmitted Infections*, *91*(3), 157–164. doi:https://doi.org/10.1136/sextrans-2014-051633
453. Wasef, W., Hughes, S., Sugunendran, H., & Alawattegama, A. (2005). The value of testing urine in non-gonococcal urethritis. *International Journal of STD & AIDS, 16*(7). doi:https://doi.org/10.1258/0956462054308413
454. Weber, S. M., & Lamb, R. (2005). An unusual urethral foreign body: silicone caulk. *The Journal of Emergency Medicine, 29*(4), 475–476. doi:https://doi.org/10.1016/j.jemermed.2005.02.017
455. Weidner, W., Pilatz, A., Diemer, T., Schuppe, H. C., Rusz, A., & Wagenlehner, F. (2013). Male urogenital infections: impact of infection and inflammation on

ejaculate parameters. *World Journal of Urology, 31*(4), 717–723. doi:https://doi.org/10.1007/s00345-013-1082-7
456. Weidner, W., Schiefer, H. G., & Garbe, C. (1987). Acute nongonococcal epididymitis. Aetiological and therapeutic aspects. *Drugs, 34*(Suppl. 1), 111–117. doi:https://doi.org/10.2165/00003495-198700341-00024
457. Weidner, W., Schiefer, H. G., Krauss, H., Jantos, C., Friedrich, H. J., & Altmannsberger, M. (1991). Chronic prostatitis: a thorough search for etiologically involved microorganisms in 1,461 patients. *Infection, 19*(Suppl. 3), S119–S125. doi:https://doi.org/10.1007/BF01643680
458. Veiga, E., Treviño, M., Romay, A. B., Navarro, D., Trastoy, R., & Macía, M. (2020). Colonisation of the male reproductive tract in asymptomatic infertile men: Effects on semen quality. *Andrologia, 52*(7), e13637. doi:https://doi.org/10.1111/and.13637
459. Wein, A. J., Kavoussi, L. R., Novick, A. C., Partin, A. W., & Peters, C. A. (2012). *Campbell-Walsh Urology. 10th Edition.* Philadelphia: Elsevier Saunders.
460. Weinberger, M., Cytron, S., Servadio, C., Block, C., Rosenfeld, J. B., & Pitlik, S. D. (1988). Prostatic abscess in the antibiotic era. *Reviews of Infectious Diseases, 10*(2), 239–249. doi:https://doi.org/10.1093/clinids/10.2.239
461. Wendel, K. A., Erbelding, E. J., Gaydos, C. A., & Rompalo, A. M. (2003). Use of urine polymerase chain reaction to define the prevalence and clinical presentation of Trichomonas vaginalis in men attending an STD clinic. *Sexually Transmitted Infections, 79*(2), 151–153. doi:https://doi.org/10.1136/sti.79.2.151
462. Verze, P., Cai, T., & Lorenzetti, S. (2016). The role of the prostate in male fertility, health and disease. *Nature Reviews Urology, 13*(7), 379–386. doi:https://doi.org/10.1038/nrurol.2016.89
463. Veznik, Z., Pospisil, L., Svecova, D., Zajicova, A., & Unzeitig, V. (2004). Chlamydiae in the ejaculate: their influence on the quality and morphology of sperm. *Acta Obstetricia et Gynecologica Scandinavica, 83*(7), 656–660. doi:https://doi.org/10.1111/j.0001-6349.2004.00542.x
464. Wetmore, C. M., Manhart, L. E., Lowens, M. S., Golden, M. R., Whittington, W. L., Xet-Mull, A. M., Astete, S. G., McFarland, N. L., McDougal, S. J., & Totten, P. A. (2011). Demographic, behavioral, and clinical characteristics of men with nongonococcal urethritis differ by etiology: a case-comparison study.*Sexually Transmitted Diseases*, *38*(3), 180–186. https://doi.org/10.1097/OLQ.0b013e3182040de9
465. Whelan, J., Eeuwijk, J., Bunge, E., & Beck, E. (2021). Systematic Literature Review and Quantitative Analysis of Health Problems Associated with Sexually Transmitted Neisseria Gonorrhoeae Infection. *Infectious Diseases and Therapy, 10*(4), 1887–1905. doi:https://doi.org/10.1007/s40121-021-00481-z
466. Wiggins, R. C., Holmes, C. H., Andersson, M., Ibrahim, F., Low, N., & Horner, P. J. (2006). Quantifying leukocytes in first catch urine provides new insights into our understanding of symptomatic and asymptomatic urethritis. *International Journal of STD & AIDS, 17*(5), 289–295. doi:https://doi.org/10.1258/095646206776790268
467. Willcox, J. R., Adler, M. W., & Belsey, E. M. (1981). Observer variation in the interpretation of Gram-stained urethral smears: implications for the diagnosis of non-specific urethritis. *The British Journal of Venereal Diseases, 57*(2), 134–136. doi:https://doi.org/10.1136/sti.57.2.134
468. Wyrick P. B. (2000). Intracellular survival by Chlamydia. *Cellular Microbiology*, *2*(4), 275–282. doi:https://doi.org/10.1046/j.1462-5822.2000.00059.x

469. Villegas, H., Piñon, M., Shor, V., & Karchmer, S. (1991). Electron microscopy of Chlamydia trachomatis infection of the male genital tract. *Archives of Andrology, 27*(2), 117–126. doi:https://doi.org/10.3109/01485019108987663
470. Witkin, S. S., Jeremias, J., Grifo, J. A., & Ledger, W. J. (1993). Detection of Chlamydia trachomatis in semen by the polymerase chain reaction in male members of infertile couples. *American Journal of Obstetrics and Gynecology, 168*(5), 1457–1462. doi:https://doi.org/10.1016/s0002-9378(11)90781-9
471. Wiwanitkit, V. (2008). Counteraction during movement of spermatozoa by Trichomonas vaginalis observed by visual image analysis: a possible cause of female infertility. *Fertility and sterility, 90*(3), 528–530. doi:https://doi.org/10.1016/j.fertnstert.2007.07.1306
472. Workowski, K. A., Bolan, G. A., & Centers for Disease Control and Prevention. (2015). Sexually transmitted diseases treatment guidelines, 2015. *MMWR. Recommendations and reports: Morbidity and mortality weekly report. Recommendations and reports, 64*(RR-03), 1–137.
473. World Health Organization. (2010). *WHO laboratory manual for the Examination and processing of human semen. 5th Edition.* World Health Organization. Retrieved from https://apps.who.int/iris/handle/10665/44261
474. World Health Organization. (2021). *WHO laboratory manual for the examination and processing of human semen, 6th Edition*. Geneva: World Health Organization. Retrieved from https://www.who.int/publications/i/item/9789240030787
475. Xiao, J., Ren, L., Lv, H., Ding, Q., Lou, S., Zhang, W., & Dong, Z. (2013). Atypical microorganisms in expressed prostatic secretion from patients with chronic prostatitis/chronic pelvic pain syndrome: microbiological results from a case-control study. *Urologia Internationalis, 91*(4), 410–416. doi:https://doi.org/10.1159/000350934
476. Yan, Z. C., Shang, X. J., Liu, W., Wan, X. X., Wan, C. C., Xu, S., Zhong, Y., & Weng, Z. Q. (2018). [Impact of Mycoplasma genitalium infection on the semen quality of infertile males]. *Zhonghua Kan ke Xue = National journal of Andrology, 24*(4), 317–321.
477. Yiwen, C., Yueyue, W., Lianmei, Q., Cuiming, Z., & Xiaoxing, Y. (2021). Infection strategies of mycoplasmas: Unraveling the panoply of virulence factors. *Virulence, 12*(1), 788–817. doi:https://doi.org/10.1080/21505594.2021.1889813
478. Yoshida, S., Harada, T., Iwabe, T., Taniguchi, F., Mitsunari, M., Yamauchi, N., Deura, I., Horie, S., & Terakawa, N. (2004). A combination of interleukin-6 and its soluble receptor impairs sperm motility: implications in infertility associated with endometriosis. *Human Reproduction, 19*(8), 1821–1825. doi:https://doi.org/10.1093/humrep/deh324
479. Yoshida, T., Deguchi, T., Meda, S., Kubota, Y., Tamaki, M., Yokoi, S., Yasuda, M., & Ishiko, H. (2007). Quantitative detection of Ureaplasma parvum (biovar 1) and Ureaplasma urealyticum (biovar 2) in urine specimens from men with and without urethritis by real-time polymerase chain reaction. *Sexually Transmitted Diseases, 34*(6), 416–419. doi:https://doi.org/10.1097/01.olq.0000243621.89212.40
480. Yoshida, T., Ishiko, H., Yasuda, M., Takahashi, Y., Nomura, Y., Kubota, Y., Tamaki, M., Maeda, S., & Deguchi, T. (2005). Polymerase chain reaction-based subtyping of ureaplasma parvum and ureaplasma urealyticum in first-pass urine samples from men with or without urethritis. *Sexually Transmitted Diseases, 32*(7), 454–457. doi:https://doi.org/10.1097/01.olq.0000158932.78183.95

481. Yu, Y., Sikorski, P., Bowman-Gholston, C., Cacciabeve, N., Nelson, K. E., & Pieper, R. (2015). Diagnosing inflammation and infection in the urinary system via proteomics. *Journal of Translational Medicine, 13*, 111. doi:https://doi.org/10.1186/s12967-015-0475-3
482. Zalata, A., Hafez, T., Van Hoecke, M. J., & Comhaire, F. (1995). Evaluation of beta-endorphin and interleukin-6 in seminal plasma of patients with certain andrological diseases. *Human Reproductio*n, *10*(12), 3161–3165. doi:https://doi.org/10.1093/oxfordjournals.humrep.a135879

SUMMARY IN ESTONIAN

Mycoplasma genitalium'i ja teiste uretriiti tekitavate seksuaalsel teel levivate infektsioonide levimus ning mõju mehe reproduktiivtrakti tervisele

1. Sissejuhatus

Ureetra ehk kusetoru ülesanne on osaleda seemnevedeliku ja uriini transpordis. Samal ajal võib ureetra muutuda sissepääsuks uretriiti tekitavatele sugulisel teel levivatele mikroobidele, nagu *Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium* ja *Trichomonas vaginalis*. Uretriidi ravimata jätmisel võivad tekkida erinevad tüsistused, nagu epididümo-orhiit, prostatiit ja reaktiivne artriit. Lisaks võivad uretriidiga seotud seksuaalsel teel levivad infektsioonid (edaspidi: STLId) kahjustada meeste reproduktiivfunktsiooni (Unemo, jt., 2020; Greenberg, 1979; Lanjouw, jt., 2016; Jensen, jt., 2022; Van Gerwen, jt., 2021). Kuigi nende mõju meeste reproduktiivtervisele on uuritud mitme aastakümne jooksul, on tõenduspõhine info STLIde kohta üsna ebaühtlane: suurem osa publitseeritud teadustöödest on keskendunud *C. trachomatis*'e ning vähesel määral ka *N. gonorrhoeae* ja *T. vaginalis*'e uurimisele (Fode, jt., 2016). Info suhteliselt uue patogeeni *M. genitalium*'i kohta on pigem kesine (Huang, jt., 2015; Farahani, jt., 2021). Isegi kliinilises igapäevapraktikas pööratakse sellele haigustekitajale üsna vähe tähelepanu ja tema rolli uretriidi tekkes on seni tihtipeale ignoreeritud.

Uretriidiga seotud STLIde epidemioloogia varieerub ja sõltub erinevatest faktoritest, nagu näiteks geograafiline piirkond, uuringu populatsioon, diagnostilised meetodid ja kriteeriumid (Ness, jt., 1997; Ochsendorf, 2008). Eestis puudus seni selge ülevaade meeste uretriidi etioloogia kohta. Peale selle puudus ülevaade uretriidiga seotud STLIde levimusest Eesti viljatute paaride meespartnerite seas.

Traditsiooniliselt on uretriidi diagnoos põhinenud haiguse füüsilistel tunnustel, sümptomitel ja põletikumarkerite määramisel traditsiooniliste meetoditega (Horner, jt., 2016). Viimasel ajal on lisandunud uus laboratoorne meetod: uriini voolutsütomeetriline analüüs (Ito, jt., 2016). Selle meetodi praktikasse rakendamine vajab aga lisauuringuid, mis on rahvusvaheliste referentsväärtuste kokkulepete eeldus.

2. Töö eesmärgid

Uurimistöö peamine eesmärk oli hinnata uretriidi esinemissagedust erinevates Eesti populatsioonides ja selle mõju meeste urogenitaalsüsteemi tervisele, samuti selgitada uute diagnostiliste meetodite rakendatavust uretriidiga patsientide seas.

Selleks püstitati järgmised uurimisülesanded.

1. Kirjeldada uretriidiga seotud STLIde tekitajate (*N. gonorrhoeae, C. trachomatis, M. genitalium* ja *T. vaginalis*) esinemissagedust erinevates Eesti populatsioonides, nagu kõrge seksuaalse riskikäitumisega heteroseksuaalsed mehed, viljatute paaride meespartnerid ja rasedate naiste meespartnerid.
2. Võrrelda uretriidiga meeste kliinilist pilti erinevate STLIde tekitajate korral.
3. Kirjeldada ja võrrelda põletikulist reaktsiooni meeste reproduktiivtraktis erinevate STLIde tekitajate korral.
4. Hinnata diagnostiliste algoritmide ja kriteeriumite kasutatavust/funktsionaalsust STLIde tekitajate ennustamiseks meeste puhul.
5. Tuvastada STLIde tekitajate mõju seemnevedeliku kvaliteedile ja vere PSA tasemele.

3. Uuritavad ja meetodid

Uurimistöösse kaasati järgmised uurimisrühmad: kõrge seksuaalse riskikäitumisega heteroseksuaalsed mehed (n = 825), laboratoorselt kinnitatud uretriidiga mehed (n = 306) ja nende kontrollrühmaks olevad uretriidita ja urogenitaaltrakti kaebusteta mehed (n = 192), viljatute paaride meespartnerid (n = 2000) ning rasedate naiste meespartnerid (n = 248).

Kõrge seksuaalse riskikäitumisega meestele tehti ureetrasekreedist PCR-analüüsid nelja STLI tekitaja määramiseks, samuti analüüsiti nende kaebusi ja kliinilist pilti ning lokaalset põletikureaktsiooni.

STLIde tekitajate levimust hinnati ka viljatuse kaebusega paaride meespartneritel, samuti rasedate naiste meespartneritel, keda saab käsitleda kui viljakate meeste võrdlusrühma. Samadel meestel hinnati ka STLIde mõju sperma parameetritele ja vere PSA tasemele.

Uretriidiga meeste rühm ja nende kontrollrühm osalesid uuringus, kus hinnati voolutsütomeetria kasutatavust uretriidi diagnostikas, et töötada välja referentsväärtused selle meetodi kasutamiseks rutiinses töös.

Statistilised analüüsid tehti Microsoft Office Word 2010 ja Excel 2010 tarkvara (Microsoft Corporation, Washington, USA), SigmaStati programmi (Systat Software, Chicago, IL, USA) ja RStudio tarkvara (R versioon 3.6.1. (2019-07-05), RStudio Inc., Massachusetts, USA) kasutades. Rühmadevaheliste erinevuste hindamiseks kasutati Fisheri täpset testi, hii-ruut-testi, t-testi, Wilcoxoni testi ja Kruskal-Wallise ning Mann-Whitney testi koos Bonferroni korrektsiooniga. Erinevate parameetrite vahelisi seoseid hinnati Spearmani korrelatsioonanalüüsiga. Seemnevedeliku leukotsüütide kontsentratsiooni ja seminaalplasma IL-6 kontsentratsiooni parima lävendi leidmiseks ja infektsioonide olemasolu ennustamiseks konstrueeriti ROC-kõverad.

Uuringutes osalemine oli vabatahtlik. Uuringud kiitis heaks Tartu Ülikooli inimuuringute eetika komitee (224/T-18 [25.03.2013], 228/M-32 [26.08.2013], 254/M-17 [21.12.2015], 152/4 [18.09.2006], 288/M-13 [17.12.2018] ja 188/M-16 [14.12.2009]). Uuringud korraldati kooskõlas Helsingi deklaratsiooni põhiprintsiipidega. Igalt patsiendilt võeti kirjalik nõusolek uuringus osalemiseks.

4. Tulemused

4.1. STLIde esinemissagedus Eesti meestel

Kõrge seksuaalse riskikäitumisega heteroseksuaalsete meeste seas oli STLIde tekitajate esinemissagedus järgmine: *C. trachomatis* 14,8%, *M. genitalium* 4,2%, *N. gonorrhoeae* 2,5%, *T. vaginalis* 0,7% ja kombineeritud STLIde juhtumid 1,5% (kaks *C. trachomatis*'e ja *M. genitalium*'i juhtumit, kaks *N. gonorrhoeae* ja *M. genitalium*'i juhtumit, kuus *C. trachomatis*'e ja *N. gonorrhoeae* juhtumit).

STLIde tekitajate esinemissagedus viljatute paaride meeste seas oli väga väike: *M. genitalium*'i leiti 1,1% ning *C. trachomatis*'t 1,2% meestest. Nendel meestel ei leitud ühtegi *N. gonorrhoeae*, *T. vaginalis*'e või kombineeritud STLIde juhtumit. See tulemus on enam-vähem kooskõlas arenenud riikide STLIde tekitajate esinemissageduse mustriga, kus *C. trachomatis* ja *M. genitalium* on sagedasemad uretriidiga seotud patogeenid kui *T. vaginalis* ja *N. gonorrhoeae*.

STLIde tekitajate esinemissagedus rasedate naiste meespartnerite seas oli samuti väike: 1,6% (4 patsienti 248-st). Kõigil neljal patsiendil tuvastati *C. trachomatis*'e monoinfektsioon, ülejäänud kolme tekitajat ei leitud.

4.2. Kliiniline leid uretriidiga patsientidel

Kõrge seksuaalse riskikäitumisega heteroseksuaalsete meeste STLIde uuringus osutusid suurima kaebuste arvuga seotud patogeenideks *N. gonorrhoeae* (edaspidi ka: NG) ja *T. vaginalis* (edaspidi ka: TV). Gonorröa korral olid põhilised kaebused püsiv hägune eritis kusitist (72,7%) ning ebamugavustunne kusitis urineerimise ajal (63,6%), *T. vaginalis*'e korral aga püsiv ebamugavustunne kusitis (50%) ja kusitiava punetus (50%). Suuremal osal nendest patsientidest oli vähemalt üks kaebus (95,5% gonorröa korral ning 50% TV korral), kuid 22,7% NG ja 33,3% TV juhtudest olid kergete sümptomitega ning 4,5% NG ja 50% TV juhtudest kaebused puudusid üldse. Seetõttu ei saa konkreetset STLI tekitajat välja selgitada vaid patsiendi kaebuste profiili põhjal.

M. genitalium'i (edaspidi ka: MG) ja *C. trachomatis*'e (edaspidi ka: CT) korral olid kaebused üsna sarnased ning leebemad võrreldes gonorröa juhtumitega. CT ja MG korral kliiniline pilt samuti varieerus ning mõned patsiendid (18,6% CT ja 22,2% MG juhtumitest) olid asümptomaatilised. Vähene kaebusteta patsientide osakaal CT ja MG korral on kooskõlas mõne varasema uuringu tulemustega (Taylor-Robinson D., jt., 2009; Bowden, jt., 1998; Bradshaw, jt., 2006; Falk, jt., 2004; Gottesman, jt., 2017; Högdahl & Kihlström, 2007), kuid erineb teiste uuringute tulemustest (Carne, jt., 2013; Detels, jt., 2011; Coble, jt., 2006; Sonnenberg, jt., 2015; Zheng, jt., 2014).

Uretriidi makroskoopilised tunnused olid erinevate STLIde patogeenide korral samuti mittespetsiifilised. Sarnast leidu on kirjeldatud mõningates varasemates töödes (Jordan, jt., 2020), kuid mitte teistes (Wetmore, jt., 2011; Ito, jt., 2016).

Meie uuritavate hulgas olid ka mõned STLI leiuta patsiendid, kellel esinesid erinevad kliinilised leiud ja sümptomid ja/või põletikuline reaktsioon esmasjoaja/või keskjoauriini proovis. On võimalik, et nendel juhtudel võis tegemist olla teatud harvade põhjustega, nagu mehaanilised faktorid (Péc, jt., 1992) või teised mikrobioloogilised tekitajad (Dan, jt., 2012; Bachmann, jt., 2015; Horner, jt., 2016), mida kõnealuse uurimistöö raames ei analüüsitud.

4.3. Põletikuline reaktsioon uretriidiga patsientidel: uriin

Uurimistöös näitasime, et NG kutsus esile tugevaima põletikulise reaktsiooni esmasjoauriinis, mõõdetuna nii ribatesti kui ka voolutsütomeetria abil. CT ja MG korral oli põletikureaktsioon mõõdukas ja TV korral nõrk. Meie uurimistöös puudus põletikureaktsioon 7,5% voolutsütomeetriaga mõõdetud juhtudel ja 26,4% ribatestiga mõõdetud juhtudel. See tähendab, et uretriidiga seotud STLIde diagnoosimisel ei tohi tugineda vaid esmasjoauriini põletikulisele reaktsioonile, kuna mõnel juhul STLIde tekitajad ei kutsu esile piisavat põletikku. Praegusel ajal pole voolutsütomeetria uretriidi diagnostikas laialt levinud ning meie uurimistöö on üks esimestest, mis hindas süstemaatiliselt põletikulise reaktsiooni esinemist ja tugevust erinevate STLIde tekitajate korral. Uuringute vähesus takistab rahvusvahelise konsensuse saavutamist selle laboratoorse meetodi universaalsetes läviväärtustes (Grosso, jt., 2012; Ito, jt., 2014; Ito, jt., 2016; Pond, jt., 2015).

4.4. Põletikuline reaktsioon uretriidiga patsientidel: seemnevedelik

Me leidsime, et STLIde tekitajate reastamine põletikulise reaktsiooni tugevuse järgi seemnevedelikus oli mõnevõrra erinev võrreldes esmasjoauriiniga. Nimelt olid *M. genitalium*'i korral seemnevedeliku põletikumarkerid (IL-6 ja leukotsüütide kontsentratsioon) kõrgemad võrreldes klamüüdiaga, kuigi see erinevus ei olnud statistiliselt oluline. Kõnealune erinevus võib viidata erinevatele patomehhanismidele erinevate STLIde tekitajate korral. Teised uuringud on samuti näidanud erinevate STLIde patogeenide kliiniliste ja laboratoorsete näitajate erinevat jaotust ureetra preparaatides (Shahmanesh, 1989; Pate, jt., 2001; Falk, jt., 2004), uriinis (Wiggins, jt., 2006) ja seemnevedelikus (Al-Sweih, jt., 2012).

Me leidsime, et STLI positiivsetel patsientidel oli seemnevedeliku leukotsüütide kontsentratsioon kõrgem võrreldes STLI negatiivsete patsientidega. See erinevus tulenes põhiliselt *M. genitalium*'i positiivsete juhtude arvelt. Meie uurimistöö tulemused, mis näitasid positiivset seost *M. genitalium*'i ja leukotsütospermia vahel, erinevad Kjaergaard jt. (1997) uuringu tulemustest, kus sellist seost ei leitud. Pole välistatud, et niisugune tulemuste erinevus on põhjustatud osalisest metodoloogilisest erinevusest.

Meie ja mõne teise uurija (Kjaergaard, jt., 1997; de Barbeyrac, jt., 2006) tulemused ei toeta klamüüdia seost leukotsütospermiaga. Meie uurimistöös tuvastati suurem neutrofiilide protsentuaalne osakaal seemnevedelikus klamüüdia-positiiv-

setel viljatute rühma patsientidel, kuid mitte seemnevedeliku neutrofiilide kontsentratsiooni tõus. Neutrofiilide protsentuaalne osakaal seemnevedelikus on suhtarv ja selle jaoks puudub aktsepteeritud referentsväärtus, et seemnevedeliku põletikulist protsessi hinnata. Seetõttu ei saa seda parameetrit kasutada usaldusväärse markerina seemnevedeliku põletiku uurimisel. Mõned uurimused on siiski leidnud seose leukotsütospermia ja *C. trachomatis*'e infektsiooni vahel (Hosseinzadeh, jt., 2004). Üks põhjus, miks klamüüdia seos püospermiaga ei leia kõikides uuringutes kinnitust, võib olla teadusuuringute võimetus eristada värsket ja vana klamüüdiainfektsiooni, kuna suur osa *C. trachomatis*-positiivsetest patsientidest on asümptomaatilised. Süsteemsete seroloogiliste testide kasutamine diagnostikas ei ole mõistlik, kuna klamüüdia tekitatud antikehad jäävad verre ringlema pikemaks ajaks ning positiivne antikehade test ei erista varasemat ja hetkel põetavat infektsiooni (Schuppe, jt., 2017).

Tuginedes meie uurimistöö tulemustele, pakume *M. genitalium*'i ja/või *C. trachomatis*'e infektsiooni eristamise optimaalseks lõikepiiriks 0,28 miljonit leukotsüüti ühes seemnevedeliku milliliitris. See piir on madalam kui Maailma Tervishoiu Organisatsiooni (edaspidi ka: MTO) pakutud 1 mln/ml (World Health Organization, 2010). Soovitus langetada leukotsütospermia diagnoosimiseks praegu kehtivat piiri on antud ka mõnes teises töös (Punab, jt., 2003; Gdoura, jt., 2008b).

4.5. STLIde mõju mehe reproduktiivtervisele

Kui me hindasime MG ja CT positiivseid juhtusid koos, siis leidsime nendel patsientidel oluliselt väiksema spermatosoidide üldarvu ja väiksema progressiivse liikuvusega spermatosoidide arvu kui STLI-negatiivsetel meestel. Nimetatud kahe patogeeni eraldi käsitlemisel ei ületanud nende mõju statistilise olulisuse piiri, kuna juhtude arv katserühmas oli väike. Tasub mainida, et tõenduspõhine informatsioon *M. genitalium*'i kohta on tänapäeval veel lünklik. Mõned uurijad ei ole leidnud selle bakteri mõju seemnevedeliku parameetritele (Kjaergaard, jt., 1997; Gimenes, jt., 2014a), kuid Yan jt. (2018) leidsid *M. genitalium*'i negatiivse mõju spermide progressiivsele liikuvusele. *In vitro* uuringud on kinnitanud klamüüdia mõju spermidele, kuid *in vivo* uuringute tulemused selle patogeeni kohta on vastuolulised (Redgrove & McLaughlin, 2014).

Ülaltoodud info annab põhjust arvata, et MG ja CT võivad mõjutada spermatosoide ning mängida rolli mehe viljatuses. Nende bakterite mõju viljatusele vajab edasisi uuringuid suurtes kohortides või teistes uuritavate rühmades, kus STLI esinemissagedus on suurem.

5. Uurimistöö järeldused

1. *C. trachomatis* on suurima esinemissagedusega seksuaalse riskikäitumisega Eesti meeste seas, kuid *M. genitalium* on olulisel teisel kohal. Neile järgnevad *N. gonorrhoeae* ja *T. vaginalis*. Kombineeritud STLI tekitajate esinemissagedus on väike. Uretriidiga seotud STLIde tekitajate esinemissagedus

rasedate naiste meeste ja viljatute paaride meeste seas on väike. Seega peab kõige uuem STLI tekitaja *M. genitalium* kindlasti kuuluma meeste uretriidi standardsesse diagnostikapaneeli.

2. *N. gonorrhoeae* on seotud suurima kaebuste arvuga meespatsientidel, kuid pool *T. vaginalis*'e ja ligi viiendik *M. genitalium*'i ning *C. trachomatis*'e juhtudest on asümptomaatilised. Ükski uretriidi makroskoopiline tunnus ei ole patognoomiline konkreetse haiguse või STLI tekitaja suhtes. Seetõttu ei välista sümptomite ja haigustunnuste puudumine uretriidiga seotud STLI võimalust. Nii patsientide seksuaalse käitumise kui ka sümptomite hindamine on oluline, kui võetakse vastu otsus, kas teha põletiku- ja STLI-analüüs.
3. Gonokokil on suurim võime tekitada kusetorus põletikku, sellele järgnevad klamüüdia ja *M. genitalium*, kuid trihhomoonase puhul ureetra põletikuline reaktsioon praktiliselt puudub. *M. genitalium* kutsub esile põletikulist reaktsiooni ka seemnevedelikus, kuid lävend on enamasti palju madalam MTO pakutust. Viimane tähendab, et kasutusel olevad seemnevedeliku põletikulise protsessi parameetrite referentsväärtused ei luba kindlalt välistada nakkuse võimalust ja võivad viia patsiendi kliinilise väärkäsitluseni.
4. Meie uurimistöö põhjal võib anda kaks soovitust uretriidi diagnoosimise parandamiseks:
   a) esmasjoauriini voolutsütomeetriline analüüs on kiire ja objektiivne meetod nakkuskahtlusega meeste uretriidi skriinimiseks;
   b) hetkel kehtivat seemnevedeliku põletiku diagnoosimise läviväärtust on vaja langetada, et leida üles rohkem patsiente, kes vajavad ravi.
5. *M. genitalium* ja *C. trachomatis* avaldavad negatiivset mõju seemnevedeliku parameetritele. Seetõttu ei saa välistada nende atüüpiliste bakterite potentsiaalset mõju meeste viljakusele. *N. gonorrhoeae* ja *T. vaginalis*'e mõju seemnevedeliku parameetritele ei olnud võimalik meie uuringus hinnata nende patogeenide puudumise tõttu rasedate naiste meeste ja viljatute paaride meeste katserühmades. Uurimistöös ei tuvastatud STLI mõju PSA tasemele. Nende mikroobide põhjustatud infektsiooni täpsema mõju väljaselgitamine nõuab edasisi uuringuid.

ACKNOWLEDGEMENTS

This study was conducted at the Centre of Andrology at Tartu University Hospital, Estonia.

I would like to thank and express my deepest and sincere gratitude to the patients and to a number of people without whom this research could not have been accomplished. Especially, I would like to thank:

My supervisor, Professor Margus Punab, for introducing me to the field of andrology as the teacher, for being a reliable colleague at work, for his kind support and advice, for initially designing the research, for administrative management of the work, and for sharing his knowledge about the science and the art of scientific writing.

My co-supervisor, Professor Reet Mändar, for patiently guiding me through these years of research work, for administrative management of the work, for sharing her knowledge about the science and the art of scientific writing, for her enthusiasm in keeping me motivated, and for her kind support and advice.

Professor Külli Kingo and Associate Professor Martti Laan for reviewing the dissertation and for their valuable comments.

Heti Pisarev for her valuable statistical advice.

All the employees of Centre of Andrology at Tartu University Hospital for their everyday help in overcoming any kind of problems in everyday practice.

All the colleagues and friends for their support and understanding.

My relatives, my parents, my brother, my sister and Bonifacius for their support, love and understanding during these years of scientific work.

This study was supported by Estonian Research Council (Grants No. IUT34-19 and PUT181), Estonian Ministry of Education and Research (Grant No. KOGU-HUMB) and Enterprise Estonia (Grant No. EU48695). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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2. Tjagur, S., Mändar, R., & Punab, M. (2020). Profile of sexually transmitted infections causing urethritis and a related inflammatory reaction in urine among heterosexual males: A flow-cytometry study. *PloS ONE*, *15*(12), e0242227. https://doi.org/10.1371/journal.pone.0242227
3. Tjagur, S., Mändar, R., Poolamets, O., Pomm, K., & Punab, M. (2021). Mycoplasma Genitalium Provokes Seminal Inflammation among Infertile Males. *International Journal of Molecular Sciences*, 22(24), 13467. https://doi.org/10.3390/ijms222413467
4. Kikas, T., Punab, A. M., Kasak, L., Poolamets, O., Vihljajev, V., Pomm, K., Reiman, M., Tjagur, S., Korrovits, P., Punab, M., & Laan, M. (2023). Micro-deletions and microduplications linked to severe congenital disorders in

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seksuaalsel teel levivad infektsioonid,
meeste reproduktiivne süsteem,
eesnäärme patoloogiad

Publikatsioonide loetelu:

1. Tjagur, S., Mändar, R., & Punab, M. (2018). Prevalence of Mycoplasma genitalium and other sexually transmitted infections causing urethritis among high-risk heterosexual male patients in Estonia. *Infectious Diseases*, *50*(2), 133–139. https://doi.org/10.1080/23744235.2017.1366044
2. Tjagur, S., Mändar, R., & Punab, M. (2020). Profile of sexually transmitted infections causing urethritis and a related inflammatory reaction in urine among heterosexual males: A flow-cytometry study. *PloS ONE*, *15*(12), e0242227. https://doi.org/10.1371/journal.pone.0242227
3. Tjagur, S., Mändar, R., Poolamets, O., Pomm, K., & Punab, M. (2021). *Mycoplasma genitalium* Provokes Seminal Inflammation among Infertile Males. *International Journal of Molecular Sciences, 22*(24), 13467. https://doi.org/10.3390/ijms222413467
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Liikmelisus kutseorganisatsioonides:
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