# The role of the prostate in male fertility, health and disease

*Paolo Verze[1], Tommaso Cai[2] and Stefano Lorenzetti[3]*

Abstract | Ejaculation is a synchronized cascade of events that has the ultimate goal of activating sperm and enabling them to reach an egg for fertilization. The seminal plasma contains a complex mixture of fluids that is secreted from the testes, epididymis and male accessory glands. The prostate gland has a pivotal role in this process, as prostatic fluid enriched in $Zn^{2+}$, citrate and kallikreins is crucial for the molecular synchronization of the functional cascade triggered by ejaculatory stimuli. The prostate is the target of a number of common diseases that can affect male fertility at different ages. In both young and aged men, prostatic diseases or an unhealthy prostate can affect spermatozoa functioning and, therefore, male fertility. Consideration of prostate physiology emphasizes a number of points: the central role of $Zn^{2+}$ and citrate in the regulation of prostate epithelium homeostasis and in ejaculation; the influence of bacteria-related prostatic inflammation on male fertility; and the potential role of prostatic inflammation in promoting the development of prostatic hyperplastic growth and carcinogenesis.

Male fertility requires the cooperation of the different organs of the male urogenital system, each carrying out its assigned function. Through their interactions, the testes (which contain germ cells, Sertoli cells and Leydig cells), the epididymis, and the male accessory glands (prostate, seminal vesicles and bulbourethral glands) simultaneously contribute to the production of the human seminal plasma[1,2] (FIG. 1). The testes contribute by producing germ cells (spermatozoa), whereas the main contributions of the accessory glands include the secretion of proteins (for example, kallikreins (KLKs)and semenogelins by the prostate and seminal vesicles, respectively), growth factors (for example, testosterone and insulin-like 3 protein by Leydig cells), trace elements (for example, $Zn^{2+}$ by prostate epithelium) and other factors (for example, the metabolites citrate and spermine by the prostate epithelium and the glycoprotein mucin MG1 by the bulbourethral glands). 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[1].

Ejaculation, liquefaction and clotting make up a synchronized cascade that enables sperm to perform all the biological processes necessary in order to reach and fertilize the egg. Human seminal plasma is a very complex bodily fluid, the composition of which is strictly regulated by the hypothalamic–pituitary–adrenal (HPA) axis via the gonadotrophic or hypothalamic–pituitary–gonadal (HPG) axis. Seminal plasma composition is also affected by the metabolic and psychological state of the organism[1–3], and coordinates the action of all of the different 'players' involved in fertility within the human male reproductive system[4,5].

The prostate is the major male reproductive gland involved in male fertility. Indeed, male fertility intrinsically relies upon the content of the prostatic fluid secreted by the prostate epithelium. The key contribution of the prostatic fluid to male fertility is linked to its role as the trigger for each of the molecular pathways involved in ejaculation and, subsequently, in sperm activation and capacitation[1,2].

The prostate gland is essential to both bringing about the necessary conditions for male fertility and is also a direct target of a number of prevalent benign and malignant diseases (for example, prostatitis, benign prostatic hyperplasia (BPH) and prostate cancer) that are potentially linked with impaired fertility status.

Among all prostatic diseases, prostatitis has the greatest potential to affect fertility. Mounting evidence indicates that prostatic inflammation is directly linked with fertility alteration, a pertinent finding for men in their prime reproductive years[6]. Furthermore, recent data support the role of prostatic inflammation as a predisposing factor for development of BPH[7] and prostate cancer[8]. In addition, both medical and surgical treatments for BPH and prostate cancer can lead to the impairment of fertility status, which is of particular relevance to patients facing fertility issues at an advanced age.

[1]*Department of Urology, University of Naples Federico II, Via S. Pansini 5, 80131 Naples, Italy.*
[2]*Department of Urology, Santa Chiara Regional Hospital, Largo Medaglie d'Oro 9, 38123 Trento, Italy.*
[3]*Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità (ISS), Viale Regina Elena 299, 00161 Rome, Italy.*

*Correspondence to P.V.*
*paolo.verze@unina.it*

doi:10.1038/nrurol.2016.89
Published online 1 Jun 2016

**Key points**

- Male fertility is controlled by a $Zn^{2+}$-dependent short circuit of the Krebs cycle within prostate epithelial cells
- Homeostasis of the prostate epithelium is reliant on the intracellular androgen-dependent accumulation of $Zn^{2+}$ and citrate
- Sperm motility requires the coordinated action of the components of the two main fluids in the human seminal plasma: the prostatic fluid, which is enriched with $Zn^{2+}$, citrate and kallikreins, and the semenogelin-enriched seminal vesicle secretion
- The prostate is the direct target for a number of benign and malignant diseases that are potentially linked to impaired fertility status
- Prostatitis might be directly linked with changes in fertility

Of note, in both young and aged men, prostatic diseases or an unhealthy prostate gland can directly affect male fertility via alteration of both the gland's metabolic status and, either partially or principally, through inducing an inflammatory state. Bouts of prostate-based inflammation, caused by uropathogens and atypical bacteria, can alter spermatozoa functioning by either direct or immune-mediated damage[9].

Male ageing and cellular senescence of the prostate epithelium strongly influence male fertility and are associated with prostate diseases. This Review focuses on the reproductive function of the prostate gland, summarizing the physiological and molecular mechanisms that connect prostate homeostasis with male fertility and describing how these mechanisms are associated with prostatic diseases. We will highlight several areas: the central role of $Zn^{2+}$ and citrate in regulating activities of the prostate epithelium; the importance of the prostatic fluid and, in turn, the seminal plasma, as a critical source of potential predictive and/or diagnostic biomarkers for assessing age-dependent prostate health; the influence of bacteria-related prostate inflammation on male fertility; and the potential role of prostatic inflammation in promoting the development of prostatic hyperplastic growth and prostate carcinogenesis.

## Prostate structure and function

The prostate is the main accessory gland of the male reproductive system. It is composed of two main compartments, the stroma and the epithelium (FIG. 2), which influence each other reciprocally via different signalling pathways[5] to ensure normal prostatic development and homeostasis. Structurally, the completely differentiated human prostate gland is composed of ducts with an inner layer of epithelium surrounded by stroma. Anatomically, the human prostate gland is divided into three zones: the central zone, the transition zone and the peripheral zone (FIG. 2a). The transition zone surrounds the urethra proximal to the ejaculatory ducts. The central zone surrounds the ejaculatory ducts and projects under the bladder base. The peripheral zone constitutes the bulk of the apical, posterior and lateral aspects of the prostate. Clinically, the prostate is usually described as having two lateral lobes, separated by a central sulcus that is palpable on rectal examination, and a middle lobe, which might project into the bladder in older men. These lobes do not correspond to histologically defined structures in the normal prostate but are usually related to pathological enlargement of the transition zone laterally and the periurethral glands centrally.

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. Several lines of evidence also indicate that activated stroma might have a pivotal role in prostate inflammatory processes[5].

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[1–5]. The prostatic fluid, as with the other secretions of the male accessory glands, is an essential contributor to male fertility. Prostatic fluid contains a number of factors[1] that control the ejaculation process and regulate proteins (from the other accessory gland secretions) that activate sperm maturation; these factors are necessary for semen liquefaction, the clotting cycle and sperm motility.

The factors in prostatic fluid that provide these functions are as follows: KLKs, a specific subfamily of 15 serine proteases, which include PSA, encoded by *KLK3* (REF. 10); citrate, an intermediate metabolite of the Krebs cycle[11]; and $Zn^{2+}$, a trace element actively stored within the cytoplasm of the prostatic epithelial cells[12,13]. These main factors are linked, mechanistically and functionally, with each other by the metabolic peculiarities of the prostatic epithelial cells (FIG. 1). Prostatic epithelial cells are the only healthy human cells that produce energy by glycolysis (a hallmark of proliferating cancer cells) rather than the Krebs cycle. The normal human prostate accumulates the highest levels of $Zn^{2+}$ of any soft tissue in the human body[10,12,13]. This unique property relies on the fact that prostatic epithelial cells accumulate $Zn^{2+}$ by an androgen-dependent $Zn^{2+}$ cellular uptake and release cycle that is mediated by specific zinc transporters: the proteins ZIP1–4 for uptake and the proteins ZnT1–10 for release[12,13].

### Inhibitory functions of $Zn^{2+}$

The $Zn^{2+}$ accumulated within the prostatic epithelial cells (about 4% of the overall $Zn^{2+}$ content within the human male body) has a dual inhibitory function. Firstly, $Zn^{2+}$ blocks the initial step of the Krebs cycle — the oxidation of citrate in isocitrate, as performed by mitochondrial aconitase (mACON), a key enzyme for ATP production by respiration and terminal oxidation by mitochondria[13]. Hence, $Zn^{2+}$-dependent mACON inhibition causes short-circuitry of the Krebs cycle, leading to the accumulation of high levels of citrate. The free intra-cytoplasmatic $Zn^{2+}$ pool requires citrate as a substrate in order to specifically recognize mACON and not its cytoplasmic isoform[12]. Prostatic citrate acts mainly as an energy substrate for sperm, increasing its ATP production[11].

Secondly, $Zn^{2+}$ causes the temporary inactivity of prostatic tissue KLKs. Indeed, KLKs are expressed and secreted as zymogens or as pre-pro-KLKs, N-terminal

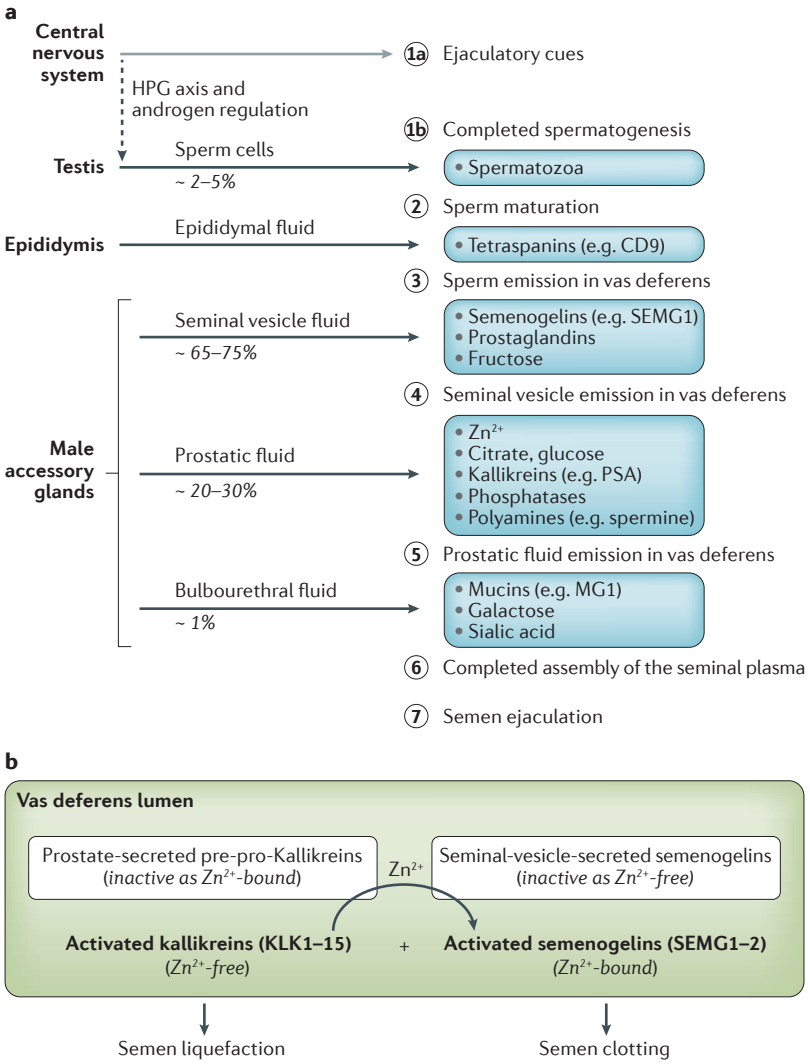


Figure 1 | **Main components of the human seminal plasma (whole ejaculate) and their functional relationship. a** | Composition of the seminal plasma and contribution of the different primary and accessory urogenital compartments. Each numbered event represents a sequential step of the ejaculatory cascade leading to the assembly of the whole ejaculate. **b** | Gland-secreted fluid ensures, among other functions, sperm motility upon ejaculation by the reciprocal activation of the $Zn^{2+}$-modulated proteins, kallikreins and semenogelins.

extended protein precursors connected in a network forming a highly regulated, sequentially activated proteolytic cascade pathway. Zymogens represent the completely inactive forms of such proteins and are activated by a triggering cue at the higher level of the proteolytic cascade pathway. Under physiological conditions within the prostate and prostatic fluid, KLKs are inactivated by allosteric reversible binding of $Zn^{2+}$. Thus, $Zn^{2+}$ represents the major inhibitor of the prostatic KLK proteolytic cascade, the triggering cues of which depend on ejaculatory stimuli driven by the central nervous system.

Once the ejaculation cue is triggered, the sperm-enriched epididymal fluid is mixed with both prostatic fluid enriched with $Zn^{2+}$, citrate and KLKs 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 KLKs, including PSA, start the semen liquefaction process that enables sperm to be released and move towards the Fallopian tube. Semen liquefaction after ejaculation depends on the activation of multiple KLKs. This process occurs by a semenogelin-dependent $Zn^{2+}$ redistribution owing to the fact that semenogelins have a higher affinity for $Zn^{2+}$ than do KLKs, leading to KLK proteolytic cascade activation generated by KLK5 autoactivation[10].

## Control functions of androgens

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 one of 48 human nuclear receptors, a subfamily of receptors that have a dual function, acting as both intracellularly located receptors and as ligand-activated transcription factors[14]. Indeed, 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). Hundreds of genes have been discovered to be regulated by DHT within the prostate epithelium[15–17], some of which are the key genes involved in prostate homeostasis.

Foremost is the *AR* gene itself, a master gene in prostate physiology, the DHT-dependent expression of which is essential for maintaining healthy epithelial homeostasis and minimizing the malignant progression in cases of prostate cancer. With ageing, circulating levels of testosterone and intraprostatic levels of DHT decrease progressively, impairing proper functioning of the gland and reducing its ability to maintain healthy tissue levels of intracellular $Zn^{2+}$, citrate and KLK-secreted proteins within the prostatic fluid. Consequently, the age-dependent decrease in prostatic tissue levels of DHT progressively impairs male fertility. At the same time, age-dependent impairment in $Zn^{2+}$ and citrate accumulation also leads to a metabolic change that, by weakening the ability to inhibit the Krebs cycle, favours the cancer-prone status of the prostatic epithelial cell — leading to full ATP production by respiration and terminal oxidation by mitochondria, a condition that facilitates prostate cancer manifestation and progression.

Secondly, Zn transporters essential for $Zn^{2+}$ uptake (ZIP1–4) and release (ZnT1–10), are also androgen regulated. In prostate epithelium, accumulated $Zn^{2+}$ (as mentioned, 4% of total human body content equivalent to 2–4 g) is bound to either citrate or to KLKs (zymogens and pre-pro-KLK forms) and, hence, is sequestered in intracellular vesicles ($Zn^{2+}$ concentration is 3–10-fold higher in these vesicles than in other human cells) with the free cytosolic $Zn^{2+}$ concentration kept within the pM to fM range to avoid cellular toxicity[15]. ZIP1 protein, which is expressed at the basolateral membrane of healthy prostate epithelia, is the major $Zn^{2+}$ uptake transporter from the blood. Its expression has been shown to be markedly decreased or absent in prostate cancer tissue compared with normal prostate or BPH tissue[18,19].

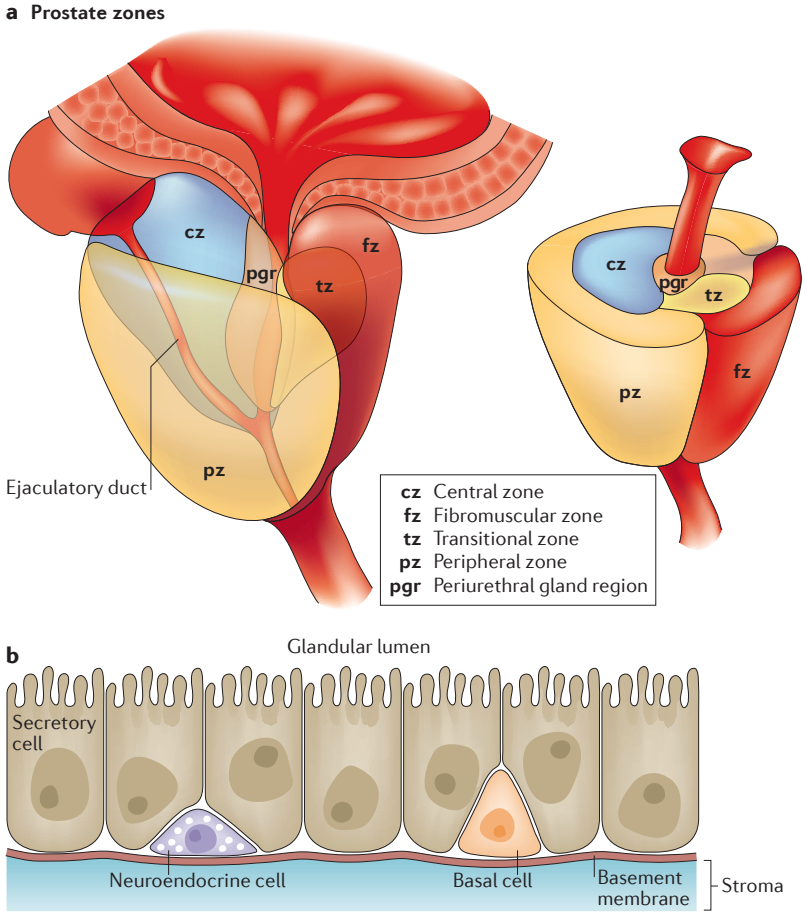


Figure 2 | **Gross and microscopic anatomy of the prostate gland. a** | General anatomical structure of the prostate. The human prostate gland is divided into three zones: the central zone, the transition zone and the peripheral zone. Normally, the transition zone accounts for 5–10% of the glandular tissue of the prostate. A fibromuscular band of tissue separates the transition zone from the remaining glandular compartments. The central zone constitutes 20–25% of the glandular tissue of the prostate and expands in a cone shape around the ejaculatory ducts to the base of the bladder. The peripheral zone represents the bulk of the prostatic glandular tissue (70%) and covers the posterior and lateral aspects of the gland. **b** | Microscopic structure of the prostate. The prostate consists of glandular epithelium embedded in a fibromuscular stroma. The epithelium is composed of two histologically distinct layers. The secretory luminal layer is made up of tall columnar cells that are responsible for the production of PSA, prostatic acid phosphatase and human kallikrein-2, which are secreted as part of the seminal fluid. This layer of cells is underpinned by a basal layer of cuboidal epithelial cells and neuroendocrine cells. This layer is in turn lined by a basement membrane consisting of extracellular matrix, which forms a divide between the basal cells and the stroma. Permission for part **a** obtained from Nature Publishing Group © De Marzo, A. M. *Nat. Rev. Cancer* **7**, 256–269 (2007).

In experimental animal models, both decreased ZIP1 expression and $Zn^{2+}$ depletion occurred at early stages of prostate cancer development[20]. ZIP1 has been hypothesized to be the key prostate cancer tumour suppressor gene[21]. ZIP2, ZIP3 and ZIP4 are expressed in healthy prostate tissue, but are localized at the apical membrane of prostate epithelia and, hence, are thought to maintain cellular $Zn^{2+}$ homeostasis via $Zn^{2+}$ reuptake from the prostatic fluid[22]. Interestingly, ZIP2, ZIP3 and ZIP4 are also downregulated in prostate cancer compared with levels in both normal prostate epithelial cells and in BPH tissue[21,22]. Zinc transporters ZnT1–10 are involved in $Zn^{2+}$ efflux, either exporting intracellular $Zn^{2+}$ or redistributing it in intracellular organelles, such as the mitochondria and secretory vesicles of the endosome or lysosomal compartment[22]. Clear evidence of their involvement in the establishment of prostate cancer is lacking, although in an experimental animal model of prostate cancer, loss of ZnT7 expression favoured prostate cancer formation[23].

Thirdly, the serine protease family of KLKs, essential for human reproduction and male fertility, are also regulated by androgens, as has been demonstrated for the two most studied proteases, kallikrein 2 and PSA (encoded by the genes *KLK2* and *KLK3*, respectively). Kallikrein 2 and PSA have the most organ-restricted expression profile of all KLKs, and are specifically and abundantly expressed in prostate epithelia. Their expression levels reflect the functional status and activity of the nuclear AR and its response to androgens. The unique combination of tissue specificity and the androgen-driven expression profile of kallikrein 2 and PSA provides a straightforward and biologically relevant endpoint to monitor the activity of the prostate epithelium in healthy and pathological conditions[24].

## Prostatitis and male fertility

Prostatitis syndrome is a common disease that can severely impact a man's quality of life. Its prevalence has been estimated at approximately 11–13% of adult men[6]. Overall, 5–10% of reported prostatitis cases are associated with microbiologically demonstrated bacterial strains[9]. Several andrological complications can arise from bacterial prostatitis and ultimately result in impaired male fertility[25].

## Bacterial prostatitis due to uropathogens

Acute bacterial prostatitis does not seem to be associated with the development of male infertility, but data are lacking. Chronic bacterial prostatitis (CBP) can be associated with development of male infertility, but further study is needed. The hypothesis is that bacterial infections of the male reproductive tract result in impairment of the secretory capacity of the prostate, which might in turn have a negative effect on all semen parameters, such as morphology and motility[26,27]. *Escherichia coli* infection has been reported to have a number of effects on human semen parameters: mitochondrial changes and membrane alterations in sperm cells[28]; a reduction in the percentage of sperm with an intact mitochondrial membrane potential[29]; spermatozoa immobilization and impaired acrosomal function[30]; and decreases in sperm viability and motility[31]. The mechanisms of reduced semen quality that occur in patients with chronic prostatitis can be divided into three categories: pathogens causing direct damage to spermatozoa, immune-mediated damage, and indirect sperm dysfunction owing to a reduction in the quality of prostate secretions.

***Direct damage to spermatozoa.*** Uropathogenic *E. coli* (UPEC) can secrete numerous virulence factors including haemolysin, a member of the repeats-in-toxin family of calcium binding, and pore-forming toxins, which directly

destroy genitourinary cells[32,33]. Moreover, UPEC can attenuate host inflammatory responses in testicular cells by suppressing cytokine production and can promote tissue damage[34]. *E. coli* can also adhere rapidly to human spermatozoa and result in their agglutination. This agglutination results in a profound decline in the motility of spermatozoa[35]. Moreover, spermatozoa agglutination is followed by severe alterations in sperm morphology[36]. However, although a direct relationship between *E. coli* infection and male infertility has been clearly demonstrated, it should be noted that in everyday clinical practice, even after eradication of the pathogen by antibiotic treatment, about 50% of men do not recover normal sperm counts[37]. This finding is probably the result of asymptomatic continuation of inflammation that can affect both testes, causing permanent impairment to fertility as a result of germ cell loss or duct obstruction[38,39].

***Immune-mediated damage.*** As mentioned, chronic inflammatory conditions of the male genitourinary tract can persist even after successful antibiotic therapy and can irreversibly alter sperm number and quality[37]. In 2008, Bhushan *et al.*[39] demonstrated that incubation of rat testicular cells with the pathogen or commensal *E. coli* did not result in the release of the proinflammatory cytokines IL-1α, IL-6, and TNF-α; these researchers say that, depending on the cell type infected, the lack of an inflammatory response in the host cells is probably the result of active suppression of the MYD88-dependent NF-κB pathway by the pathogen at various levels of the signalling cascade[39].

***Indirect sperm dysfunction caused by reduced prostate secretions.*** Indirect sperm dysfunction owing to a reduction in the quality of prostate secretions is another mechanism of reduced semen quality that might occur following CBP. Under normal conditions, the prostate secretes several factors (FIG. 1) that are crucial for sperm physiology[30]. Inflammation per se and secondary obstruction have been proposed as possible mechanisms through which different infectious agents might impair sperm function[40].

## Bacterial prostatitis due to C. trachomatis

*Chlamydia trachomatis* infections are the most prevalent bacterial sexually transmitted infections (STIs) worldwide and cause considerable morbidity and socioeconomic problems[41]. *C. trachomatis* infections are asymptomatic in about 50% of infected men and 70% of infected women, but there is a risk of reproductive tract consequences in both sexes[41]. In men, *C. trachomatis* initially infects the single-cell columnar layer of the urethral epithelium and then infects nearby epithelial cells leading to an ascending infection that can cause retrograde epididymitis and epididymo-orchitis[42]. Moreover, the ascending infection from the urethra can reach the prostate epithelium and cause infections in the prostate and seminal vesicles[42]. *C. trachomatis* infection of the prostate can cause inflammation of the prostatic tissue, impairing the normal functioning of the gland, which is responsible for secreting up to 30% of the volume of seminal plasma[43]. Eley and co-workers[44] have shown that *C. trachomatis* can also damage male sperm directly and, using electron microscopy, Erbengi[45] has shown the entry of the elementary body of *C. trachomatis* into the human spermatozoon head and demonstrated that *C. trachomatis* not only adhered to, but also penetrated, the spermatozoon tail structure.

Furthermore, the presence of immunoglobulin A (IgA) directed against *C. trachomatis* in semen from men with previous contact with *C. trachomatis* is associated with increased levels of γδ T lymphocytes, which also correlate with the presence of antisperm antibodies[46]. Men with infertility resulting from *C. trachomatis* infection develop high levels of mucosal antibodies against high-molecular-weight proteins such as MOMP2, HSP60 and HSP70 (REF. 47). Moreover, the prevalence of serum IgA antibodies to HSP60 is significantly higher in female partners of subfertile couples than in fertile controls[47], suggesting that an autoimmune response to HSP60 can develop following female genital tract infection with *C. trachomatis*, probably as a consequence of an immune response to an epitope of chlamydial HSP60 crossreacting with HSP60 epitopes in the host cells[48]. In 2010, Mazzoli *et al.*[49] showed significant correlations between mucosal IgA directed against *C. trachomatis* and sperm concentration ($P<0.001$) and percentage of sperm with abnormal morphology ($P<0.001$) in men with chronic prostatitis. Moreover, they found significant correlations between positivity to HSP60 or HSP70 and sperm concentration ($P<0.003$) and percentage of sperm with abnormal morphological forms ($P<0.001$)[49], highlighting that immune-mediated damage occurs to germinal cells as a result of *C. trachomatis* infection.

## Chronic abacterial prostatitis

In 1994, Leib and co-workers[50] evaluated a potential link between chronic abacterial prostatitis and infertility by comparing semen analysis findings in patients with chronic abacterial prostatitis and in a group of healthy volunteers. Several sperm motility parameters and morphology characteristics were found to be different in the two groups[50]. A correlation was also found between disease duration and the appearance of sperm morphological defects. The negative effect that prostatitis has on male fertility is probably the result of oxidative stress in semen or prostatic fluid in patients with prostatitis[51], with 30%–80% of male subfertility cases being thought to result from the damaging effects of oxidative stress on sperm[52].

## Prostatitis treatment and fertility

Treatment for prostatitis should be directed at removing the aetiopathogenic agent and improving semen quality. Eradication of the infection using antibiotic therapy does not always result in recovery of semen quality and other treatments might be needed. Whether prostatitis treatment can aid the recovery of male fertility and improve conception rate is still under debate[53]. Three different scenarios should be considered: CBP caused by common uropathogenic bacteria, CBP caused by *C. trachomatis*, and CBP caused by chronic nonbacterial prostatitis.

CBP caused by common uropathogenic bacteria has been shown to be associated with reductions in male fertility[36,37]; for example, the presence of sperm-agglutinating strain of *E. coli* in the vaginal tract has been reported as a cause of male fertility impairment[54]. However, the direct effect of eradication of the infection and recovery of male fertility has not been shown in a clinical scenario.

By contrast, eradication of *C. trachomatis* infection has been shown to be critical for the recovery of male fertility[55]. In 2012, a prospective, randomized, controlled study by Cai *et al.*[56] demonstrated that in patients with *C. trachomatis* genital infection and oligoasthenoteratozoospermia, administration of different antioxidative factors such as L-arginine, L-carnitine, acetyl-L-carnitine and ginseng extracts — together with the antibiotic prulifloxacin — was associated with improved semen parameters compared with treatment with prulifloxacin therapy alone. The improved quality of spermatozoa from an infertile status to a normal fertility index was demonstrated by two consecutive sperm analyses[56]. The anti-inflammatory and antioxidative effects of ginseng extracts improve the morphology and density of spermatozoa and L-arginine, L-carnitine and acetyl-L-carnitine enhance sperm motility, by stimulating the activity of endothelial nitric oxide synthase (eNOS)[56].

Oxidative stress is the major cause of the reduction in male fertility in patients with nonbacterial prostatitis[52], and it is thought that oral supplementation with antioxidants might improve sperm quality[52]. However, in a Cochrane systematic review published in 2014, Showell and co-workers suggested that further large, well-designed, randomized, placebo-controlled trials are needed to clarify the effectiveness and safety of oral supplementation with antioxidants for subfertile male partners in couples seeking fertility assistance[52]. The authors highlighted that oral supplementation with antioxidants might increase the clinical pregnancy rates, but as only low-quality evidence from just four small randomized controlled trials is available, large well-designed randomized placebo-controlled trials are needed to confirm the results[52].

## Prostatic inflammation and BPH

Several authors have stressed the role of prostatic inflammation in the genesis of BPH, highlighting that the prostate is an immunocompetent organ that is normally populated by a small number of inflammatory cells (that is, T and B lymphocytes, macrophages and mast cells)[57,58]. In the adult prostate, chronic inflammatory prostate infiltrates are different in BPH tissue compared with healthy tissue. The most common infiltrates in the prostate of patients with BPH are $CD4^+$ T lymphocytes, $CD19^+$ or $CD20^+$ B lymphocytes and macrophages. Regardless of the trigger, T lymphocytes, macrophages and B lymphocytes that are present in the adult prostate can cause damage to both epithelial and stromal cells, stimulate cytokine release and increase the concentration of growth factors that can promote an abnormal remodelling process characterized by fibromuscular growth[58]. Chronic prostatic inflammation seems to have a crucial role in the pathogenesis and progression of BPH. The remodelling of prostate tissue caused by an inflammatory injury might promote the structural changes that are commonly associated with benign disease[58].

Stimuli can cause an inflammatory response in the prostate in aged men: hormonal changes, infections (bacterial and viral), autoimmune responses, urinary reflux inside the prostate and systemic inflammation associated with metabolic syndrome[58]. Hormonal changes might promote an increased presence of inflammatory infiltrates in the prostate that could lead to tissue damage in both epithelial and stromal cells. Such tissue damage can initiate a chronic process of wound healing that might trigger prostate tissue remodelling and prostatic enlargement[58]. Interleukins and growth factors induce a self-stimulated mechanism characterized by the continuous activation of inflammatory cells, resulting in prostate enlargement through the secretion of growth factors[58]. Another key contributor to this pathway is the local hypoxia induced by prostate enlargement itself[58]. Consequently, reactive oxygen species are released and promote neovascularization and further release of growth factors. This mechanism promotes the establishment of a vicious cycle that leads to progressive increase in prostate volume[58].

## Prostate inflammation and carcinogenesis

Prostate inflammation might have a causative role in the complex process of prostate degeneration leading to prostate carcinogenesis. The remodelling of prostate tissue caused by an inflammatory injury might promote the structural changes that are commonly associated with both benign and malignant disease[7]. The prostate tissue collected at prostate biopsy or radical prostatectomy often shows the presence of an inflammatory infiltrate[8]. The presence of bacterial or viral DNA in prostate cancer cells suggests their potential activity as an inducer of oxidative stress, which, in turn, can lead to genotoxic activity[59]. Bacterial RNA gene sequences have been detected in radical prostatectomy specimens and in prostate tissue from patients with BPH. Concordance between inflammation and positive PCR results in simple and radical prostatectomy specimens suggests that bacteria might often have a role in histologically inflammatory prostatitis. Shah *et al.*[59] studied the distribution of proliferative inflammatory atrophy areas in radical prostatectomy specimens from men with clinically localized prostatic adenocarcinoma and found that proliferative inflammatory atrophy was significantly more common in the peripheral zone, next to areas of prostatic carcinoma. In 2007, Elkahwaji *et al.*[60] demonstrated that chronic inflammation induced by intraprostatic injection of *E. coli* in a mouse model is able to induce reactive hyperplasia associated with oxidative stress injury. This finding supports the proposed linkage between inflammation, oxidative DNA damage and prostate carcinogenesis.

The role of infections in prostate cancer pathogenesis and progression has been addressed in several studies. In particular, several animal models described reactive inflammatory changes and preinvasive mouse prostatic intraepithelial neoplasia in mice with chronic prostatitis[61]. Moreover, prostate inflammation has been shown

to accelerate prostate cancer progression in mice infected with human prostatic bacterial isolates, and acts by altering the prostate gland microenvironment by changes in the immune cell infiltrate and cytokine expression[61]. Although a relationship between prostatic inflammation and malignant disease has been demonstrated in *in vitro* and *in vivo* models, the clinical applicability of these findings need to be demonstrated.

## Prostate proliferative diseases and fertility

Ageing of the male reproductive system is characterized by changes in the endocrine system and proliferative disorders of the prostate gland. Theoretically, although age-related changes in the HPG axis occur, endocrine functions are generally sufficient to maintain fertility in elderly men. Progressive dysregulation at each level of the HPG axis and in local autocrine and paracrine interactions occurs with age, thereby inducing morphological changes in reproductive target organs such as the prostate, testis and penis[62]. In particular, age-related changes in levels and ratios of endocrine factors such as androgens, oestrogens, gonadotropins, and prolactin, and changes in the balance between autocrine and paracrine growth-stimulatory and growth-inhibitory factors such as insulin-like growth factors (IGFs), epidermal growth factor, nerve growth factor, IGF-binding proteins, and transforming growth factor β are considered to be responsible for abnormal prostatic growth[63]. However, to the best of our knowledge, no clinical data exist with regards to the assessment of the fertility status in patients with BPH and prostate cancer and whether the abnormal growth of the prostatic cell impairs its capacity to contribute to fertility. As infection and/or inflammation of the male genital tract can be involved in the induction of antisperm antibodies (ASAs) and as the prostate is an immunologically competent organ, a study was conducted to examine whether prostate diseases lead to the formation of ASAs[64]. The study findings showed that chronic prostatitis, BPH and prostate cancer do not induce the formation of antibodies to spermatozoa, sperm-specific antigens and seminal plasma components[64].

## Conclusions

Male fertility requires the cooperation of all of the different organs of the male urogenital system each carrying out its assigned function. The testes, epididymisand male accessory glands (prostate, seminal vesicles and bulbourethral glands) all contribute to the production of the human seminal plasma. Male fertility is controlled by a $Zn^{2+}$-dependent short circuit of the Krebs cycle within the prostate epithelial cells. Homeostasis and pathophysiological status of the prostate epithelium depends on the intracellular androgen-dependent accumulation of $Zn^{2+}$ and citrate. Furthermore, sperm motility relies on the coordinated action of the components of the two main fluids in human seminal plasma: the prostatic fluid enriched in $Zn^{2+}$, citrate and kallikreins, and the semenogelin-enriched seminal vesicle secretion. The prostate gland is the direct target for a number of benign and malignant diseases that are potentially linked with impaired fertility status. In particular, any impairments in the metabolic or inflammatory status of the prostate epithelium, affecting either accumulation of $Zn^{2+}$ and citrate or kallikrein secretory capability, might lead to alterations in fertility. For instance, prostatic inflammation caused by common bacteria or pathogens such as *C. trachomatis* might have a role in determining male fertility impairment, so that the presence of prostate infection should be carefully taken into account in the management of infertile patients. Further studies are needed to establish the role of treatment of prostatitis in the recovery of male fertility. Ageing of the male reproductive system is characterized by changes in the endocrine system and proliferative disorders of the prostate gland such as hyperplastic and cancerous growth; however, to date, no clinical data are available to show whether abnormal prostatic growth is linked with the impairment of fertility status. Owing to the increasing evidence supporting the peculiarity of the metabolism and function of the prostatic cell contributing to a fertile status, any condition predisposing to the alteration of the prostatic health (that is, inflammation or proliferative growth) are worth considering as potential factors predisposing to male infertility.


1. Gilany, K., Minai-Tehrani, A., Savadi-Shiraz, E., Rezadoost, H. & Lakpour, N. Exploring the human seminal plasma proteome: an unexplored gold mine of biomarker for male infertility and male reproduction disorder. *J. Reprod. Infertil.* **16**, 61–71 (2015).
2. Tena-Sempere, M. Ghrelin, the gonadal axis and the onset of puberty. *Endocr. Dev.* **25**, 69–82 (2013).
3. Nargund, V. H. Effects of psychological stress on male fertility. *Nat. Rev. Urol.* **12**, 373–382 (2015).
4. Rey, R. A. *et al.* Male hypogonadism: an extended classification based on a developmental, endocrine physiology-based approach. *Andrology* **1**, 3–16 (2013).
5. Nieto, C. M., Rider, L. C. & Cramer, S. D. Influence of stromal–epithelial interactions on androgen action. *Endocr. Relat. Cancer* **21**, T147–T160 (2014).
6. Wagenlehner, F. *et al.* Prostatitis and andrological implications. *Minerva Urol. Nefrol.* **65**, 117–123 (2013).
7. Ficarra, V. *et al.* The role of prostatic inflammation in low urinary tract symptoms (LUTS) due to benign prostatic hyperplasia (BPH) and its potential impact on medical therapy. *Curr. Urol. Rep.* **15**, 463–469 (2014).
8. Wagenlehner, F. M. E. *et al.* The role of inflammation and infection in the pathogenesis of prostate carcinoma. *BJU Int.* **100**, 733–737 (2007).
9. Roberts, R. O., Lieber, M. M., Bostwick, D. G. & Jacobsen, S. J. A review of clinical and pathological prostatitis syndromes. *Urology* **49**, 809–821 (1997).
10. Kalinska, M., Meyer-Hoffert, U., Kantyka, T., Potempa, J. Kallikreins. The melting pot of activity and function. *Biochimie* **122**, 270–282 (2016).
11. Medrano, A. *et al.* Utilization of citrate and lactate through a lactate dehydrogenase and ATP-regulated pathway in boar spermatozoa. *Mol. Reprod. Dev.* **73**, 369–378 (2006).
12. Franz, M. C. *et al.* Zinc transporters in prostate cancer. *Mol. Aspects Med.* **34**, 735–741 (2013).
13. Franklin, R. B., Milon, B., Feng, P. & Costello, L. C. Zinc and zinc transporters in normal prostate and the pathogenesis of prostate cancer. *Front. Biosci.* **10**, 2230–2239 (2005).
14. Lorenzetti, S. & Narciso, L. in *Computational Approaches To Nuclear Receptors* (eds Cozzini, P. & Kellogg, G. E.) 1–22 (RSC Publishing, 2012).
15. Takayama, K. & Inoue, S. Transcriptional network of androgen receptor in prostate cancer progression. *Int. J. Urol.* **20**, 756–768 (2013).
16. Horie-Inoue, K. & Inoue, S. Genome-wide integrated analyses of androgen receptor signaling in prostate cancer based on high-throughput technology. *Curr. Drug Targets* **14**, 472–480 (2013).
17. Lamont, K. R. & Tindall, D. J. Androgen regulation of gene expression. *Adv. Cancer Res.* **107**, 137–162 (2010).
18. Johnson, L. A., Kanak, M. A., Kajdacsy-Balla, A., Pestaner, J. P. & Bagasra, O. Differential zinc accumulation and expression of human zinc transporter 1 (*hZIP1*) in prostate glands. *Methods* **52**, 316–321 (2010).
19. Costello, L. C. & Franklin, R. B. The clinical relevance of the metabolism of prostate cancer; zinc and tumor suppression: connecting the dots. *Mol. Cancer* **5**, 17 (2006).
20. Franklin, R. B. *et al.* hZIP1 zinc uptake transporter down regulation and zinc depletion in prostate cancer. *Mol. Cancer* **4**, 32 (2005).
21. Desouki, M. M., Geradts, J., Milon, B., Franklin, R. B. & Costello, L. C. hZip2 and hZip3 zinc transporters are down regulated in human prostate adenocarcinomatous glands. *Mol. Cancer* **6**, 37 (2007).
22. Kolenko, V., Teper, E., Kutikov, A. & Uzzo, R. Zinc and zinc transporters in prostate carcinogenesis. *Nat. Rev. Urol.* **10**, 219–226 (2013).
23. Tepaamorndech, S., Huang, L. & Kirschke, C. P. A null-mutation in the *Znt7* gene accelerates prostate tumor formation in a transgenic adenocarcinoma mouse prostate model. *Cancer Lett.* **308**, 33–42 (2011).

24. Thorek, D. L., Evans, M. J., Carlsson, S. V., Ulmert, D. & Lilja, H. Prostate-specific kallikrein-related peptidases and their relation to prostate cancer biology and detection. *Thromb. Haemost.* **110**, 484–492 (2013).
25. Bartoletti, R. *et al.* Prevalence, incidence estimation, risk factors and characterization of chronic prostatitis/chronic pelvic pain syndrome in urological hospital outpatients in Italy: results of a multicenter case-control observational study. *J. Urol.* **178**, 2411–2415 (2007).
26. Domes, T. *et al.* The incidence and effect of bacteriospermia and elevated seminal leukocytes on semen parameters. *Fertil. Steril.* **97**, 1050–1055 (2012).
27. Mändar, R., Raukas, E., Türk, S., Korrovits, P. & Punab, M. Mycoplasmas in semen of chronic prostatitis patients. *Scand. J. Urol. Nephrol.* **39**, 479–482 (2005).
28. Fraczek, M. *et al.* Membrane stability and mitochondrial activity of human-ejaculated spermatozoa during *in vitro* experimental infection with *Escherichia coli*, *Staphylococcus haemolyticus* and *Bacteroides ureolyticus*. *Andrologia* **44**, 315–329 (2012).
29. Schulz, M., Sánchez, R., Soto, L., Risopatrón, J. & Villegas, J. Effect of *Escherichia coli* and its soluble factors on mitochondrial membrane potential, phosphatidylserine translocation, viability, and motility of human spermatozoa. *Fertil. Steril.* **94**, 619–623 (2010).
30. Diemer, T. *et al. Escherichia coli*-induced alterations of human spermatozoa. An electron microscopy analysis. *Int. J. Androl.* **23**, 178–186 (2000).
31. Marconi, M., Pilatz, A., Wagenlehner, F., Diemer, T. & Weidner, W. Impact of infection on the secretory capacity of the male accessory glands. *Int. Braz. J. Urol.* **35**, 299–309 (2000).
32. Dobrindt, U., Hochhut, B., Hentschel, U. & Hacker, J. Genomic islands in pathogenic and environmental microorganisms. *Nat. Rev. Microbiol.* **2**, 414–424 (2004).
33. Marrs, C. F., Zhang, L. & Foxman, B. *Escherichia coli* mediated urinary tract infections: are there distinct uropathogenic *E. coli* (UPEC) pathotypes? *FEMS Microbiol. Lett.* **252**, 183–190 (2005).
34. Dhakal, B. K. & Mulvey, M. A. The UPEC pore-forming toxin α-hemolysin triggers proteolysis of host proteins to disrupt cell adhesion, inflammatory, and survival pathways. *Cell Host Microbe* **11**, 58–69 (2012).
35. Diemer, T., Ludwig, M., Huwe, P., Hales, B. & Weidner, W. Influence of urogenital infection on sperm function. *Curr. Opin. Urol.* **10**, 39–44 (2000).
36. Kaur, K. & Prabha, V. Impairment by sperm agglutinating factor isolated from *Escherichia coli*: receptor specific interactions. *Biomed. Res. Int.* **54**, 84–97 (2013).
37. Ludwig, M. *et al.* Experimental *Escherichia coli* epididymitis in rats: a model to assess the outcome of antibiotic treatment. *BJU Int.* **90**, 933–938 (2002).
38. Lu, Y. *et al.* Necrosis is the dominant cell death pathway in uropathogenic *Escherichia coli* elicited epididymo-orchitis and is responsible for damage of rat testis. *PLoS ONE* **8**, e52919 (2013).
39. Bhushan, S. *et al.* Uropathogenic *Escherichia coli* block MyD88-dependent and activate MyD88-independent signaling pathways in rat testicular cells. *J. Immunol.* **180**, 5537–5547 (2008).
40. Dohle, G. R. Inflammatory-associated obstructions of the male reproductive tract. *Andrologia* **35**, 321–324 (2003).
41. Cai, T., Mazzoli, S., Mondaini, N., Malossini, G. & Bartoletti, R. *Chlamydia trachomatis* infection: a challenge for the urologist. *Microbiol. Res.* **2**, e14 (2011).
42. Krishnan, R. & Heal, M. R. Study of the seminal vesicles in acute epididymitis. *Br. J. Urol.* **67**, 632–637 (1991).
43. Alwaal, A., Breyer, B. N. & Lue, T. F. Normal male sexual function: emphasis on orgasm and ejaculation. *Fertil. Steril.* **104**, 1051–1060 (2015).
44. Eley, A., Pacey, A. A., Galdiero, M., Galdiero, M. & Galdiero, F. Can *Chlamydia trachomatis* directly damage your sperm? *Lancet Infect. Dis.* **5**, 53–57 (2005).
45. Erbengi, T. Ultrastructural observations on the entry of *Chlamydia trachomatis* into human spermatozoa. *Hum. Reprod.* **8**, 416–421 (1993).
46. Munoz, G., Posnett, D. N. & Witkin, S. S. Enrichment of γδ T lymphocytes in human semen: relation between γδ T cell concentration and antisperm antibody status. *J. Reprod. Immunol.* **22**, 47–57 (1992).
47. Karinen, L. *et al.* Antibodies to *Chlamydia trachomatis* heat shock proteins Hsp60 and Hsp10 and subfertility in general population at age 31. *Am. J. Reprod. Immunol.* **52**, 291–297 (2004).
48. Domeika, M., Domeika, K., Paavonen, J., Mardh, P. A. & Witkin, S. S. Humoral immune response to conserved epitopes of *Chlamydia trachomatis* and human 60-kDa heat-shock protein in women with pelvic inflammatory disease. *J. Infect. Dis.* **177**, 714–719 (1998).
49. Mazzoli, S. *Chlamydia trachomatis* infection is related to poor semen quality in young prostatitis patients. *Eur. Urol.* **57**, 708–714 (2010).
50. Leib, Z., Bartoov, B., Eltes, F. & Servadio, C. Reduced semen quality caused by chronic abacterial prostatitis: an enigma or reality? *Fertil. Steril.* **61**, 1109–1116 (1994).
51. Potts, J. M. & Pasqualotto, F. F. Seminal oxidative stress in patients with chronic prostatitis. *Andrologia* **35**, 304–308 (2003).
52. Showell, M. G. *et al.* Antioxidants for male subfertility. *Cochrane Database Syst. Rev.* **12**, CD007411(2014).
53. Hochreiter, W. W., Duncan, J. L. & Schaeffer, A. J. Evaluation of the bacterial flora of the prostate using a 16S rRNA gene based polymerase chain reaction. *J. Urol.* **163**, 127–130 (2000).
54. Kaur, K. & Prabha, V. Spermagglutinating *Escherichia coli* and its role in infertility: *in vivo* study. *Microb. Pathog.* **69–70**, 33–38 (2014).
55. Ochsendorf, F. R. *et al. Chlamydia trachomatis* and male infertility: *Chlamydia*-IgA antibodies in seminal plasma are *C. trachomatis* specific and associated with an inflammatory response. *J. Eur. Acad. Dermatol. Venereol.* **12**, 143–152 (1999).
56. Cai, T. *et al.* Semen quality in patients with *Chlamydia trachomatis* genital infection treated concurrently with prulifloxacin and a phytotherapeutic agent. *J. Androl.* **33**, 615–623 (2012).
57. Steiner, G. *et al.* Phenotype and function of peripheral and prostatic lymphocytes in patients with benign prostatic hyperplasia. *J. Urol.* **151**, 480–484 (1994).
58. Gandaglia, G. *et al.* The role of chronic prostatic inflammation in the pathogenesis and progression of benign prostatic hyperplasia (BPH). *BJU Int.* **112**, 432–441 (2013).
59. Shah, R., Mucci, N. R., Amin, A., Macoska, J. A. & Rubin, M. A. Postatrophic hyperplasia of the prostate gland: neoplastic precursor or innocent bystander? *Am. J. Pathol.* **158**, 1767–1773 (2001).
60. Elkahwaji, J. E., Zhong, W., Hopkins, W. J. & Bushman, W. Chronic bacterial infection and inflammation incite reactive hyperplasia in a mouse model of chronic prostatitis. *Prostate* **67**, 14–21 (2007).
61. Simons, B. W. *et al.* A human prostatic bacterial isolate alters the prostatic microenvironment and accelerates prostate cancer progression. *J. Pathol.* **235**, 478–489 (2015).
62. Sampson, N. *et al.* The ageing male reproductive tract. *J. Pathol.* **211**, 206–218 (2007).
63. Untergasser, G. *et al.* Proliferative disorders of the aging human prostate: involvement of protein hormones and their receptors. *Exp. Gerontol.* **34**, 275–287 (1999).
64. Hoover, P. & Naz, R. K. Do men with prostate abnormalities (prostatitis/benign prostatic hyperplasia/prostate cancer) develop immunity to spermatozoa or seminal plasma? *Int. J. Androl.* **35**, 608–615 (2012).


### Author contributions

P.V., T.C. and S.L. equally contributed to researching data for the article, discussion of content, writing and reviewing/editing of the manuscript under the coordination of the corresponding author.

### Competing interests statement

The authors declare no competing interests.