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Title:

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Date:

2022-05

Citation:

Burke, N. D., Nixon, B., Roman, S. D., Schjenken, J. E., Walters, J. L. H., Aitken, R. J. & Bromfield, E. G. (2022). Male infertility and somatic health — insights into lipid damage as a mechanistic link. *Nature Reviews Urology*, 19 (12), pp.727-750. <https://doi.org/10.1038/s41585-022-00640-y>.

Persistent Link:

<https://hdl.handle.net/11343/356415>

Male infertility and somatic health — insights into lipid damage as a mechanistic link

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Abstract | Over the past decade, mounting evidence has shown an alarming association between male subfertility and poor somatic health, with substantial evidence supporting the increased incidence of oncological disease, cardiovascular disease, metabolic disorders and autoimmune diseases in men who have previously received a subfertility diagnosis. This paradigm is concerning, but might also provide a novel window for a crucial health reform in which the infertile phenotype could serve as an indication of potential pathological conditions. One of the major limiting factors in this association is the poor understanding of the molecular features that link infertility with comorbidities across the life course. Enzymes involved in the lipid oxidation process might provide novel clues to reconcile the mechanistic basis of infertility with incident pathological conditions. Building research capacity in this area is essential to enhance the early detection of disease states and provide crucial information about the disease risk of offspring conceived through assisted reproduction.

Infertility is defined as the inability to conceive after 12 months of unprotected, regular intercourse¹. Infertility affects as many as 15–20% of couples worldwide and, contrary to long-held beliefs, up to 50% of infertility instances involve a male factor, making defective sperm function the largest, single defined cause of human infertility^{2,3}. Poor male fertility can arise from a number of genetic and developmental perturbations, including Klinefelter syndrome⁴, DNA-damage repair gene mutations⁵, cryptorchidism and hypospadias⁶. Overall, 4–9.2% of infertile men are now assigned a genetic cause^{7–10}. Genetic origins of male infertility include mutations of known infertility associated genes such as *CFTR*, *NR5A1* and *TEX11* (REFS.^{7,10}), Y-chromosome microdeletions^{8,9} and gross autosomal and sex chromosome aberrations^{8,9}. However, owing to the distinct lack of diagnostically relevant genes, a majority of infertile men do not receive a diagnosis and are, therefore, classified as having ‘unexplained infertility’^{7,11}. Additionally, male infertility

is also influenced by the paternal condition at the time of conception, with stressors such as obesity, smoking, chemotherapy and exposure to known environmental toxicants being crucial deleterious factors that compromise fertility^{12,13}. These contributors are important, but account for <50% of all male infertility instances¹⁴; indeed, unexplained and idiopathic infertility, with men presenting with no abnormal seminal parameters or unexplained reduction in semen quality, respectively, account for over half of all male infertility diagnoses^{12,15}.

Fertility and somatic health share a multifaceted and dynamic relationship¹². In the past several years, efforts have been made to identify evidence^{16–20} supporting associations between poor semen parameters, male infertility and medical conditions including cardiovascular, autoimmune, oncological and other chronic diseases. Concerning data showed an association between male infertility and increased hospitalization and mortality attributed to these chronic medical conditions^{18,19,21–23}. During an assessment

of the upcoming priorities for infertility research developed by the Priority Setting Partnership for Infertility, a diverse panel of fertility-field stakeholders including health-care funders, providers, professionals, researchers and people with infertility problems, comorbidities and risk factors for male infertility were considered amongst the highest priorities²⁴. Risk factors and common features linking infertility with poor somatic health have been identified, but the aetiology of the bidirectional relationship between reproductive health and associated comorbidities is unknown, contributing to a lag in the development of mechanistic models for a detailed investigation of these associations^{12,25–29}. Understanding the relationship between male infertility and somatic health is undeniably complex, especially considering the lack of therapeutic means to ameliorate male infertility^{30–32}.

In this Perspective, we focus on the male infertile phenotype as a proxy and predictor of potential incident pathological conditions and comorbidities, considering a comorbidity as the existence of a chronic condition in addition to a primary diagnosis. We discuss the relationship between male infertility and subsequent somatic health deterioration, and postulate about the common molecular aetiologies, such as lipid peroxidation and oxidative cellular damage, that could be explored as a potential link between these pathological conditions. We consider comorbidities as physical, non-communicable chronic diseases, such as cardiovascular disease and cancer. This classification excludes potential comorbidities that are infectious in nature (HIV and hepatitis C) or chronic mental health conditions, such as mood disorders, which have been linked to infertility^{33,34}. The burden of an infertility diagnosis is known to affect psychological health^{35,36}, and infection is a known cause of declining semen parameters and male infertility^{37,38}; however, these diseases were excluded from the surveyed literature to focus on investigating the utility of men’s fertility status in predicting future physical chronic illness. In studies in which semen parameters were used to discriminate fertile and infertile men, parameters were derived from the fifth edition of the WHO (World

Health Organization) laboratory manual for the examination and processing of human semen or equivalent^{39,40}. Childlessness was not considered a suitable surrogate for infertility, as childless men might choose not to have children. Risk factors for male infertility, including obesity and smoking, are also risk factors for common infertility comorbidities^{12,41–46}; thus, epidemiological studies in which the effect of age, weight and smoking status on statistical analysis was assessed were considered.

Comorbidities of male infertility with known genetic origin

For a number of pathological conditions of male infertility, such as azoospermia, definitive progress has been made in elucidating the underlying causes, leading to the identification of a genetic origin for several conditions. A record of all known genetic alterations associated with infertility resulted in a list of 104 genes confidently linked to 120 human male infertility or genitourinary development phenotypes⁷. Some of the common genetic aetiologies of male infertility are also known to have a causative role in somatic comorbidities.

Klinefelter syndrome. Klinefelter syndrome (47 XXY, 48 XXYY, 48 XXXY and other karyotypes) is the most common genetic abnormality of sex chromosomes, with a prevalence of >1 in 500 births⁴⁷. The hallmark of Klinefelter syndrome is male infertility^{4,48}. Symptoms characterizing the Klinefelter syndrome disease phenotype include hypergonadotropic hypogonadism, reduced seminiferous tubule diameter and spermatogenic function, and azoospermia and cryptozoospermia⁴⁹. Hospitalization in men with Klinefelter syndrome is higher than in the general population, and hospital admissions are increased for patients from 15 of 16 diagnosis groups, including patients receiving diagnoses of circulatory, blood and endocrine disorders⁵⁰. Klinefelter syndrome increases the prevalence of comorbidities, including diabetes mellitus, autoimmune diseases, and oncological diseases⁵⁰. Additionally, mortality is increased in men with Klinefelter syndrome, with a reported 40% increased mortality risk and a reduced median survival of 2.1 years compared with healthy individuals from an age-matched cohort⁵¹. Similarly, in a study including data from cytogenetics centres in Britain, a 1.5 times higher mortality was reported in men with Klinefelter syndrome (up to 85 years of age) than the national average (461 deaths in 100,000 men versus 303.4 deaths in 100,000 men)⁵². Specifically,

increased mortality caused by cardiovascular, neurological and pulmonary conditions was reported in men with Klinefelter syndrome^{51,52}. In summary, men with Klinefelter syndrome experience infertility concomitant with increased hospitalization, diagnosis of comorbidities and mortality.

DNA repair gene mutations. Successful spermatogenesis relies on efficient DNA repair mechanisms for progression of the germline through mitosis and meiosis, meiotic recombination events and spermiogenesis⁵. The integrity of sperm cell DNA is of particular importance for pregnancy outcomes and offspring health^{53–55}. DNA repair gene mutations linked to male infertility are abundant and include mutations in members of the nucleotide excision repair (NER), DNA mismatch repair (MMR) and DNA-damage response signalling pathways^{5,56}. For example, the MMR protein mutL homologue 1 (*MLH1*) facilitates chiasmata separation and recombination in spermatocytes⁵⁷. Ablation of *MLH1* in mice results in incomplete, premature chiasmata separation and germ cell arrest in meiosis I⁵⁷. In humans, *MLH1* mutations are associated with an increased risk of azoospermia, oligospermia and increased sperm DNA damage^{58,59}. *MLH1* gene mutations are also responsible for hereditary non-polyposis colorectal cancer syndrome (Lynch syndrome), which is associated with an increased risk of colorectal cancer, among other cancers⁶⁰. Additionally, in mice, depletion of the NER protein excision repair cross-complementation group 1 (*Ercc1*) increases apoptotic germ cells, and mutations in *Ercc1* and the MMR gene mutS homologue 2 (*Msh2*) lead to the development of Sertoli-cell-only seminiferous tubules⁶¹. *ERCC1* and *MSH2* mutations are established risk factors for cancer, including liver, bladder and colon cancers^{61–65}. Together, these results highlight that mutations in the DNA repair genes *MHL1*, *ERCC1* and *MSH2* lead to infertility and a concomitant increased risk of oncological comorbidities.

CFTR mutations. Cystic fibrosis, a recessive disorder characterized by abnormal lung function and insufficient pancreatic secretion⁶⁶ is caused by mutations of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene^{67–69}. *CFTR* is a transmembrane channel protein responsible for ion (chloride (Cl⁻) and bicarbonate (HCO₃⁻)) transfer in cells and tissues^{67–69}. In addition to the many debilitating symptoms

of cystic fibrosis, male patients also suffer from congenital bilateral absence of the vas deferens (98% of patients), which is the main cause of infertility in men with cystic fibrosis⁷⁰. Notably, the *CFTR*-related infertility phenotype can also develop independently of the pulmonary condition. This phenomenon can be ascribed to the heterozygous nature of some *CFTR* mutations, as a single mutant allele is sufficient to disrupt the reproductive function, whereas the clinical manifestation of cystic fibrosis requires homozygous *CFTR* mutations^{66,71}. *Cftr* gene mutations have been shown to affect several stages of mouse germ cell development. In pachytene spermatocytes, mutations in *Cftr* disrupt cell volume regulation, whereas *Cftr* mutations in mature sperm cells inhibit capacitation, a crucial post-differentiation process in which sperm cells gain the functional competence to fertilize an oocyte⁷¹. These phenotypes are likely to involve the role of *CFTR* in the conduction of HCO₃⁻ and Cl⁻, as observed in other systems including the pancreas⁷². This role of *CFTR* in the reproductive system contributes to an infertility phenotype characterized by reduced sperm number, quality, function and poor in vitro fertilization (IVF) outcomes in men with *CFTR* mutations and cystic fibrosis^{71,73,74}.

Overall, genetic aetiologies of male factor infertility such as Klinefelter syndrome, mutations in DNA-damage repair genes and *CFTR* mutations, among others^{75–79}, are associated with decreased systemic health and increased rates of comorbidities and mortality. Many of these genetic mutations, disorders and syndromes are accompanied by specific phenotypic characteristics or symptom clusters, which prompt genetic counselling and diagnosis⁸⁰. However, the majority of infertility diagnoses are not linked to a genetic cause.

Comorbidities of male infertility with non-genetic origin

Overall, >90% of men with an infertility diagnosis lack an identifiable genetic cause. Half of these men suffer unexplained and idiopathic infertility, and exposure to numerous environmental factors is known to precipitate male infertility^{81,82}. Environmental factors, including heavy metals, industrial and agricultural chemicals (often pesticides and herbicides⁸³), and synthetic polymers, exert pathogenicity as endocrine disruptors acting on the pituitary–hypothalamic–gonadal axis, interrupt spermatogenesis through cytotoxicity, or interrupt sperm cell function, culminating in infertility in a dose- and duration-dependent manner⁸⁴.

Treatment with ionizing radiation also has an established role in loss of fertility^{85,86}, although emerging evidence additionally implicates chronic exposure to non-ionizing radiation (radiofrequency electromagnetic radiation) in declining semen parameters⁸⁷. Further studies are required to establish a connection between radiofrequency electromagnetic radiation and male infertility⁸⁴.

Lifestyle factors associated with male infertility include tobacco smoking, which is considered a known cause of infertility⁸⁸ and other morbidities⁸⁹, recreational drug use⁸⁴ and obesity^{90–93}. An inverse relationship between BMI or waist circumference and sperm concentration as well as sperm count was identified in several studies^{92,94,95}. This trend was not confirmed in other studies^{96,97}; however, results from a meta-analysis⁹¹ concluded that overweight men (BMI 25–29.9 kg/m²) were at a higher risk of oligospermia than men of normal weight (BMI 18.5–24.9 kg/m²), and obese men (BMI >30 kg/m²) had a significantly increased risk of oligospermia (odds ratio (OR) 1.42, 95% CI 1.12–1.79) and azospermia (OR 1.81, 95% CI 1.23–2.66). Obesity is a risk factor for several morbidities and mortality⁹⁸, and forms a conduit between the onset of infertility and concomitant chronic illnesses. Weight loss, among other interventions, including improved diet, exercise and androgen therapy, often reverses pathological conditions of male infertility associated with obesity; for example, weight loss can reverse hypogonadotropic hypogonadism and increase testosterone levels, total sperm count and semen volume^{90,99}. Interestingly, in a pubertal obesity model in which mice were fed a high-fat diet (HFD) until puberty and then resumed a normal diet during ageing (pubertal obese mice), the effects of obesity on the reproductive tract were not entirely reversible¹⁰⁰. For example, n-6 polyunsaturated fatty acids (PUFAs) were preferentially accumulated in the testes of pubertal obese mice and promoted a pro-inflammatory state in these organs; this effect reflects an intermediate testicular lipid profile in which, upon reversal to a normal diet, testicular lipid composition of monounsaturated fatty acids and PUFAs returned to intermediate levels in HFD mice and control mice. In the pubertal obesity model, following HFD reversal to a normal diet, mice were protected from the development of metabolic dysfunctions observed in HFD mice, despite the intermediate testicular lipid profile. However, pubertal obese mice developed

long-term sperm defects persisting >4.5 months after diet reversal, including an increase in morphological defects and a decrease in viability and motility¹⁰⁰. These results highlight the sensitivity of spermatogenesis to disruption, and the lifelong risks of overall health dysfunction on fertility status.

Despite the known contribution of genetic, environmental and lifestyle factors to male infertility, >50% of male infertility diagnoses remain unexplained or idiopathic^{15,101}; in these instances, the molecular links between infertility and infertility-related comorbidities remain unknown^{12,25–29}. In the past decade, prevalent and incident comorbidities of infertility, and often mortality, have been considered together as a singular entity defining men's health^{12,27}. During the ageing process, morbidities accumulate and eventually lead to death. Moreover, medical interventions including surgery and ongoing medications also increase with ageing as a result of increased morbidities. The accumulation of morbidities and the decreased quality of life experienced during debilitating chronic illness can lead to the development of subsequent comorbidities and overall health deterioration, with no discernible mechanisms linking each morbidity to the others. In retrospective analyses of men's health after infertility diagnosis, comorbidities can be classified into two distinct groups^{17,34,102–104}, 'prevalent' (if the disease was present before or within 1 year of the infertility diagnosis, TABLE 1); and 'incident' (if a comorbidity was diagnosed ≥12 months after the infertility diagnosis, TABLE 2). With this classification, the onset of incident comorbidities, which is subsequent to male infertility diagnosis, remains unadulterated by medical intervention and other confounding factors; thus, infertile men for whom infertility is the first medical diagnosis might be a unique population for understanding the causes of primary infertility and for providing insights into the mechanisms that link infertility diagnosis to the future onset of chronic illnesses and comorbidities.

Prevalent comorbidities. Morbidities are chronic and additive in nature, and, therefore, the number of comorbidities (co-occurring diseases) typically increases with age¹⁰⁵. Young individuals of reproductive age should, therefore, be healthier than individuals of advanced age. However, in general, the presence of chronic illnesses in infertile men at the time of diagnosis is higher than the average population^{16–20}.

The prevalence of comorbidities (considered as chronic illnesses diagnosed before or at the time of infertility diagnosis) in infertile men and age-matched groups might be considered to investigate the increased rate of concomitant chronic illnesses among infertile men. Several metrics to help describe the relationship between prevalent comorbidities and male infertility are available. The Charlson Comorbidity Index (CCI) provides a weighted score to each comorbidity, and a sum of all the weighted scores provides an insight into the number and severity of chronic medical comorbidities^{16,17,33}. However, the CCI does not provide any evidence of the prevalence of single diseases (which disease in particular is a medical comorbidity in these men) and includes communicable diseases such as HIV (which are independent risk factors for decreased semen parameters and increased comorbidity risk). Moreover, disease weighting potentially inflates the score in men with few but particularly severe comorbidities, which are assigned a high CCI value. These limitations hamper the utility of the CCI in predicting the likelihood of specific chronic illnesses. However, the CCI has been used to predict future overall health and mortality in patients with type 2 diabetic nephropathy¹⁰⁶, and the change in CCI over time in these patients showed prognostic potential in instances of multimorbidity (presence of multiple medical conditions)¹⁰⁷. Other statistical analyses such as odds ratios and the Chi-squared goodness of fit test can provide insights into the prevalence of comorbidities on a disease-by-disease basis, offering an improved understanding of the rate of diagnosis of specific chronic comorbidities in infertile men. Using disease prevalence as a prognostic marker of future health has some limitations, such as the effect that the treatment of comorbidities has on overall health and infertility diagnosis; however, an increase in different prevalent comorbidities has been reported in infertile men (TABLE 1).

Almost half (8 of 19) of the prevalent comorbidities reported in infertile men are cardiovascular in nature, including hyperlipidaemia and hyperuricaemia¹⁰⁸, hypertensive disease^{17,103,108,109}, non-ischaemic heart disease, peripheral vascular disease and cerebrovascular disease¹⁷. Moreover, an increased prevalence of multiple sclerosis is reported in men with an infertility diagnosis¹¹⁰. The prevalence of general metabolic disorders (including endocrine disease) was also increased in men with subfertile semen parameters¹⁷. Other comorbidities with increased prevalence

Table 1 | Male infertility and associated prevalent comorbidities (comorbidities diagnosed before male infertility diagnosis)

Disease type	Comorbidity	Study information	Outcomes	Results
Autoimmune	Multiple sclerosis	Infertile men ($n=24,011$) versus normospermic or sterilized men ^a ($n=27,052$) from the Danish National IVF register (1994–2015)	Prevalence of multiple sclerosis	OR 1.61 (95% CI 1.04–2.51) ¹¹⁰
Cardiovascular	Hyperlipidaemia	Infertile men ($n=3,328$) versus men with normal semen analyses ($n=452$) consecutively assessed at the Department of Urology, Yamaguchi University Hospital (Japan, 1995–2017)	Prevalence of hyperlipidaemia	5.9% versus 2.7%, $P<0.01$ (one-way ANOVA) ¹⁰⁸
Cardiovascular	Hypertensive disease	Normotensive and hypertensive men who underwent fertility analysis at the Stanford Reproductive Endocrinology and Infertility Centre (1994–2011, cohort size = NR)	Inverse correlation of (i) semen volume, (ii) sperm motility and (iii) sperm count with prevalence of hypertensive disease	(i) $P<0.001$; (ii) $P=0.008$; (iii) $P=0.005$ (mixed effect linear model) ¹⁰³
Cardiovascular	Hypertensive disease	Infertile men ($n=3,328$) versus men with normal semen parameters ($n=452$) consecutively assessed at the Department of Urology, Yamaguchi University Hospital (Japan, 1995–2017)	Prevalence of hypertensive disease	17.8% versus 7.1%, $P<0.0001$ (one-way ANOVA) ¹⁰⁸
Cardiovascular	Hypertensive disease	Hypertensive men ($n=25$) versus normotensive men ($n=25$) attending the seminology laboratory sperm bank of La Sapienza University (Italy)	(i) Sperm forward progressive motility and (ii) sperm viability	(i) 41.2% versus 51.2%, $P<0.01$; (ii) 68.7% versus 80.8%, $P<0.01$ (Student's <i>t</i> test) ¹⁰⁹
Cardiovascular	Hypertensive disease	Men assessed for infertility ($n=9,387$) at the Stanford Reproductive Endocrinology and Infertility Clinic (1994–2011)	Positive correlation between prevalence of hypertensive disease and the number of abnormal semen parameters (0, 1 or >2 abnormal parameters)	$P=0.02$ (Chi-squared test) ¹⁷
Cardiovascular	Hyperuricaemia	Infertile men ($n=3,328$) versus men with normal semen analyses ($n=452$) consecutively assessed at the Department of Urology, Yamaguchi University Hospital (Japan, 1995–2017)	Prevalence of hyperuricaemia	5.2% versus 2.0%, $P<0.01$ (one-way ANOVA) ¹⁰⁸
Cardiovascular	Non-ischaemic heart disease	Men assessed for infertility ($n=9,387$) at the Stanford Reproductive Endocrinology and Infertility Clinic (1994–2011)	Positive correlation between prevalence of non-ischaemic heart disease and the number of abnormal semen parameters (0, 1 or >2 abnormal parameters)	$P=0.04$ (Chi-squared test) ¹⁷
Cardiovascular	Peripheral vascular disease + cerebrovascular disease	Men assessed for infertility ($n=9,387$) at the Stanford Reproductive Endocrinology and Infertility Clinic (1994–2011)	Positive correlation between prevalence of peripheral vascular disease and cerebrovascular disease and the number of abnormal semen parameters (0, 1 or >2 abnormal parameters)	$P=0.05$ (Chi-squared test) ¹⁷
Metabolic	Diabetes mellitus	Men with diabetes ($n=225$) and men without diabetes ($n=632$) from the Qatar Hamad General Hospital outpatient clinics (2008)	(i) Primary infertility and (ii) secondary infertility	(i) 16% versus 9.3% (ii) 19.1% versus 14.4%, $P=0.003$ (Student's <i>t</i> test) ¹¹⁷
Metabolic	Endocrine, nutritional, metabolic and immunity disorders	Men assessed for infertility ($n=9,387$) at the Stanford Reproductive Endocrinology and Infertility Clinic (1994–2011)	Positive correlation between prevalence of endocrine, nutritional, metabolic and immunity disorders and the number of abnormal semen parameters (0, 1 or >2 abnormal parameters)	$P=0.02$ (Chi-squared test) ¹⁷
Metabolic	Hypogonadism	Men with <39 million sperm per ejaculate ($n=2,583$) versus men with >39 million sperm per ejaculate ($n=2,594$) referred for fertility assessment at a tertiary university hospital (Italy, 2013–2016)	Prevalence of hypogonadism	OR 12.2 (95% CI 10.2–14.6) ¹¹²
Metabolic	Hypogonadism	Men with <10 million sperm per ejaculate (severe oligospermia and azospermia; $n=1,210$) versus men with >39 million sperm per ejaculate ($n=2,594$) referred for fertility assessment at a tertiary university hospital (Italy, 2013–2016)	Prevalence of hypogonadism	OR 33.1 (95% CI 27.0–40.6) ¹¹²

Table 1 (cont.) | Male infertility and associated prevalent comorbidities (comorbidities diagnosed before male infertility diagnosis)

Disease type	Comorbidity	Study information	Outcomes	Results
Metabolic	PDM	Men with primary infertility with PDM ($n = 114$) versus men with primary infertility without PDM ($n = 630$) assessed at a single academic centre for primary couple's infertility (Italy, 2015–2017)	Idiopathic non-obstructive azoospermia	33% versus 12.9%, $P < 0.001$ (Chi-squared test) ¹¹⁴
Oncological	Testicular cancer	Patients with testicular cancer versus patients cryopreserving sperm for future use (including pre-vasectomy patients ^a , patients deployed for military service and patients undergoing gender reassignment) at the New England Cryogenic Centre (Boston, USA, 2004–2017)	Sperm concentration	28 million/ml (95% CI 9.8–54.0) versus 65 million/ml (95% CI 38.6–94.0), $P < 0.001$ (Mann–Whitney U test) ¹¹¹
Oncological	Testicular cancer	Patients with testicular cancer versus patients cryopreserving sperm for future use (including pre-vasectomy patients ^a , patients deployed for military service and patients undergoing gender reassignment) at the New England Cryogenic Centre (Boston, USA, 2004–2017)	Total motile sperm count	63 million (95% CI 25.7–131.5) versus 156.8 million (95% CI 79.8–239.7), $P < 0.001$ (Mann–Whitney U test) ¹¹¹
Connective tissue	Skin disease (mainly atopic dermatitis; 86 of 104 patients)	Infertile men ($n = 3,328$) versus men with normal semen analyses ($n = 452$) consecutively assessed at the Department of Urology, Yamaguchi University Hospital (Japan, 1995–2017)	Prevalence of skin disease	3.0% versus 1.1%, $P < 0.05$ (one-way ANOVA) ¹⁰⁸
Connective tissue	Diseases of skin and subcutaneous tissue	Men assessed for infertility ($n = 9,387$) at the Stanford Reproductive Endocrinology and Infertility Clinic (1994–2011)	Positive correlation between prevalence of diseases of skin subcutaneous tissue and the number of abnormal semen parameters (0, 1 or > 2 abnormal parameters)	$P = 0.01$ (Chi-squared test) ¹⁷
Urogenital	Diseases of the genitourinary system	Men assessed for infertility ($n = 9,387$) at the Stanford Reproductive Endocrinology and Infertility Clinic (1994–2011)	Positive correlation between prevalence of disease of the genitourinary system and the number of abnormal semen parameters (0, 1 or > 2 abnormal parameters)	$P < 0.01$ (Chi-squared test) ¹⁷

ANOVA, analysis of variance test; CI, confidence interval, NR, not reported; OR, odds ratio; PDM, prediabetes mellitus. ^aVasectomized men are assumed to be fertile.

in men subsequently diagnosed as infertile include testicular cancer¹¹¹, skin disease^{17,108}, genitourinary diseases¹⁷ and hypogonadism¹¹².

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus recognizes that individuals can fall into an intermediate disease state between healthy (normoglycaemic) and diabetic, referred to as prediabetes, which indicates a high risk of future development of diabetes and cardiovascular disease¹¹³. Prediabetes is diagnosable in individuals with a fasting plasma glucose concentration of 5.6–6.9 mmol/l, among other diagnostic criteria^{113,114}. Men with a fasting plasma glucose concentration > 7 mmol/l (REF.¹¹⁰) or who are currently taking diabetic medications are considered diabetic^{115,116}. Among men with primary infertility (men who never conceived before the infertility diagnosis), men with prediabetes had significantly higher rates of idiopathic non-obstructive azoospermia than men without prediabetes (33% versus 12.9%, $P < 0.001$)¹¹⁴, indicating an increase in the severity of infertility pathology in men with

a concomitant diagnosis of prediabetes mellitus¹¹⁴. An increased prevalence of type 2 diabetes mellitus was also observed in men with both primary and secondary infertility (men who had fathered a child before infertility diagnosis) compared with fertile individuals¹¹⁷. Interestingly, incident diagnoses of prediabetes have not been reported among infertile men, despite the increased prevalence of prediabetes among infertile men with idiopathic non-obstructive azoospermia. Possible reasons for this evidence might include symptom progression to diabetes, which is a plausible hypothesis considering the increased incidence of diabetes mellitus among infertile men^{18,118} (TABLE 2), or lack of consideration for the prediabetic disease state, which is often regarded as a risk factor rather than a clinical entity¹¹³.

With regard to prevalent comorbidities, an essential aspect to consider is that comorbidity treatments, such as treatment of cancer with alkylating chemotherapeutics¹¹⁹, can influence fertility status in some individuals, and, therefore, might obfuscate

the links between prevalent comorbidities and subsequent infertility.

Incident comorbidities. The link between prevalent comorbidities and infertility might be explained by several factors, including instances in which medical interventions rather than comorbidities induce infertility; conversely, an incident diagnosis of comorbidities rules out medical interventions as a cause of infertility, and suggests that infertility or underpinning origins might be the underlying causes of concomitant diseases (incident comorbidities). In men diagnosed with male infertility, an increased incidence of the diagnosis of five disease types (autoimmune, cardiovascular, metabolic, oncological and urogenital diseases) has been reported^{18,34,102,120} (TABLE 2).

Similar to what had been observed for prevalent comorbidities, an increased incidence of general immune disorders (including systemic lupus erythematosus) was also observed (TABLE 2) among infertile men compared with vasectomized men

Table 2 | Rate of incident comorbidity diagnosis (comorbidities diagnosed ≥ 12 months after diagnosis of male infertility) in infertile men

Disease type	Comorbidity	Study information	Outcomes	Results
Autoimmune	General immune disorders (including systemic lupus erythematosus)	Infertile men ($n = 33,077$) versus (i) vasectomized men ^a ($n = 77,693$) and (ii) men with no fertility claims ($n = 330,770$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of general immune disorders (including systemic lupus erythematosus)	(i) HR 3.11 (95% CI 2.00–4.86); (ii) HR 2.12 (95% CI 1.52–2.96) ¹⁰²
Autoimmune	Graves' disease	Infertile men ($n = 33,077$) versus vasectomized men ^a ($n = 77,693$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of Graves' disease	HR 1.46 (95% CI 1.10–1.92) ¹⁰²
Autoimmune	Hashimoto thyroiditis	Infertile men ($n = 33,077$) versus vasectomized men ^a ($n = 77,693$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of Hashimoto thyroiditis	HR 1.60 (95% CI 1.02–2.52) ¹⁰²
Autoimmune	Multiple sclerosis	Men with testicular hypofunction ^b ($n = 5,049$) versus a reference cohort including men with minor hospitalizations ^c ($n = 3,400,000$) from the English national Hospital Episode Statistics (HES) database (1999–2011)	Incidence of multiple sclerosis	RR 4.62 (95% CI 2.3–8.24), $P < 0.0001$ (Chi-squared test) ¹²¹
Autoimmune	Multiple sclerosis	Infertile men ($n = 33,077$) versus vasectomized men ^a ($n = 77,693$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of multiple sclerosis	HR 1.91 (95% CI 1.10–3.31) ¹⁰²
Autoimmune	Psoriasis	Infertile men ($n = 33,077$) versus (i) vasectomized men ^a ($n = 77,693$) and (ii) men with no fertility claims ($n = 330,770$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of psoriasis	(i) HR 1.28 (95% CI 1.09–1.50); (ii) HR 1.20 (95% CI 1.04–1.37) ¹⁰²
Autoimmune	Rheumatoid arthritis	Infertile men ($n = 33,077$) versus (i) vasectomized men ^a ($n = 77,693$) and (ii) men with no fertility claims ($n = 330,770$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of rheumatoid arthritis	(i) HR 1.56 (95% CI 1.19–2.05); (ii) HR 1.29 (95% CI 1.02–1.62) ¹⁰²
Cardiovascular	Cardiovascular disease	Men hospitalized for incident cardiovascular disease ($n = 961$); semen analyses conducted at Frederiksberg Hospital (Copenhagen, 1977–2010), incident hospitalizations derived from the Danish National Patient Registry (1977–2015)	Rate of severe oligospermia (sperm concentration < 15 million/ml; $n = 146$) compared with normospermia (sperm count > 40 million/ml; $n = 549$)	HR 1.4 (95% CI 1.2–1.6) ¹⁸
Cardiovascular	Heart disease	Infertile ^d men ($n = 4,459$) versus (i) men who underwent infertility testing ^e ($n = 21,840$) and (ii) vasectomized men ^a ($n = 78,226$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of heart disease	(i) HR 1.22 (95% CI 1.03–1.45); (ii) HR 1.38 (95% CI 1.16–1.63) ¹²⁰
Cardiovascular	Hyperlipidaemia	Infertile ^d men ($n = 4,459$) versus vasectomized men ^a ($n = 78,226$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of hyperlipidaemia	HR 1.17 (95% CI 1.04–1.32) ¹²⁰
Cardiovascular	Hyperlipidaemia	Infertile men ($n = 13,027$) versus vasectomized men ^a ($n = 79,099$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of hyperlipidaemia	HR 1.4 (95% CI 1.07–1.22) ³⁴
Cardiovascular	Hypertensive disease	Infertile men ($n = 13,027$) versus vasectomized men ^a ($n = 79,099$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of hypertensive disease	HR 1.09 (95% CI 1.02–1.17) ³⁴
Cardiovascular	Ischaemic heart disease	Infertile men ($n = 13,027$) versus (i) men who underwent fertility assessment ($n = 23,860$) and (ii) vasectomized men ^a ($n = 79,099$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of ischaemic heart disease	(i) HR 1.48 (95% CI 1.19–1.84); (ii) HR 1.41 (95% CI 1.19–1.67) ³⁴
Cardiovascular	Other heart disease	Infertile men ($n = 13,027$) versus (i) men who underwent fertility assessment ($n = 23,860$) and (ii) vasectomized men ^a ($n = 79,099$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of other heart disease	(i) HR 1.14 (95% CI 1.0–1.30); (ii) HR 1.16 (95% CI 1.04–1.29) ³⁴

Table 2 (cont.) | **Rate of incident comorbidity diagnosis (comorbidities diagnosed ≥ 12 months after diagnosis of male infertility) in infertile men**

Disease type	Comorbidity	Study information	Outcomes	Results
Cardiovascular	Peripheral vascular disease	Infertile men ($n = 13,027$) versus vasectomized men ^a ($n = 79,099$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of peripheral vascular disease	HR 1.52 (95% CI 1.12–2.07) ³⁴
Metabolic	Diabetes	Infertile ^d men ($n = 4,459$) versus vasectomized men ^a ($n = 78,226$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of diabetes	HR 1.91 (95% CI 1.49–2.46) ¹²⁰
Metabolic	Diabetes	Infertile men ($n = 13,027$) versus (i) men who underwent fertility assessment ($n = 23,860$) and (ii) vasectomized men ^a ($n = 79,099$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of diabetes	(i) HR 1.3 (95% CI 1.1–1.53); (ii) HR 1.81 (95% CI 1.57–2.08) ³⁴
Metabolic	Diabetes mellitus (type 1 and type 2)	Men hospitalized for incident diabetes mellitus (type 1 and type 2; $n = 168$); semen analyses conducted at Frederiksberg Hospital (Copenhagen, 1977–2010), incident hospitalizations derived from the Danish National Patient Registry (1977–2015)	Rate of (i) severe oligospermia (sperm concentration < 15 million/ml; $n = 16$) and (ii) azoospermia ($n = 21$) compared with normospermia (sperm concentration > 40 million/ml; $n = 99$)	(i) HR 1.4 (95% CI 1.1–1.8); (ii) HR 1.9 (95% CI 1.4–2.5) ¹⁸
Metabolic	Diabetes mellitus	Infertile men (including oligospermia, azoospermia, aspermia and unspecified cause; $n = 12,857$) versus normospermic or sterilized men ^a ($n = 12,376$) in the Danish National IVF register (1994–2012)	Incidence of diabetes mellitus	HR 1.45 (95% CI 1.06–1.97) ¹¹⁸
Metabolic	Diabetes mellitus	Oligospermic (infertile) men ($n = 8,538$) versus normospermic or sterilized men ^a ($n = 12,376$) in the Danish National IVF register (1994–2012)	Incidence of diabetes mellitus	HR 1.44 (95% CI 1.01–2.06) ¹¹⁸
Metabolic	Diabetes mellitus	Azoospermic (infertile) men ($n = 1,275$) versus normospermic or sterilized men ^a ($n = 12,376$) in the Danish National IVF register (1994–2012)	Incidence of diabetes mellitus	HR 2.1 (95% CI 1.25–3.56) ¹¹⁸
Metabolic	Diabetes mellitus	Aspermic (infertile) men ($n = 137$) versus normospermic or sterilized men ^a ($n = 12,376$) in the Danish National IVF register (1994–2012)	Incidence of diabetes mellitus	HR 3.2 (95% CI 1.0–10.31) ¹¹⁸
Metabolic	Liver disease	Infertile men ($n = 13,027$) versus vasectomized men ^a ($n = 79,099$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of liver disease	HR 1.53 (95% CI 1.31–1.80) ³⁴
Oncological	All malignancies (ICD-9-CM 140–209) ^f	Infertile men ($n = 76,083$) versus (i) fertile, non-vasectomized men ($n = 760,830$) and (ii) vasectomized men ^a ($n = 112,655$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of all malignancies (ICD-9-CM 140–209)	(i) HR 1.49 (95% CI 1.37–1.63); (ii) HR 1.19 (95% CI 1.07–1.32) ¹³⁰
Oncological	Other cancers	(i) Infertile men (all infertility diagnoses including azoospermia; $n = 2,238$) and (ii) azoospermic men ($n = 1,787$) versus the general Texas population; data from the Baylor College of Medicine Special Procedures Laboratory, Scott Department of Urology Andrology Database linked to the Texas Cancer Registry ^g (USA; 1989–2009)	Incidence of other cancer diagnoses	(i) SIR 1.7 (95% CI 1.2–2.5); (ii) SIR 2.9 (95% CI 1.4–5.4) ¹³¹
Oncological	High-grade prostate cancer	Infertile men ($n = 4,549$) versus men without male factor infertility ($n = 14,557$); data from 15 Californian fertility registries (1967–1998) linked to the Californian Cancer Registry ^g (USA, 1988–2004)	Incidence of high-grade prostate cancer	HR 2.6 (95% CI 1.4–4.8) ¹²⁹
Oncological	Prostate cancer	Infertile men ($n = 76,083$) versus fertile, non-vasectomized men ($n = 760,830$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of prostate cancer	HR 1.78 (95% CI 1.41–2.25) ¹³⁰
Oncological	Prostate cancer	Infertile men who conceived a first born through (i) ICSI ($n = 14,882$) or (ii) IVF ($n = 20,618$) versus fertile men who conceived spontaneously ($n = 1,145,990$), based on all children born alive in Sweden (1994–2014); paternity data from the Swedish Multi-generation Register, and cancer data from the national Swedish Cancer Registry (1994–2014)	Incidence of prostate cancer	(i) HR 1.64 (95% CI 1.25–2.15); (ii) HR 1.33 (95% CI 1.06–1.66) ¹²⁸

Table 2 (cont.) | **Rate of incident comorbidity diagnosis (comorbidities diagnosed ≥ 12 months after diagnosis of male infertility) in infertile men**

Disease type	Comorbidity	Study information	Outcomes	Results
Oncological	Prostate cancer	Men hospitalized for incident prostate cancer ($n=63$); semen analyses conducted at Frederiksberg Hospital (Copenhagen, 1977–2010), incident hospitalizations derived from the Danish National Patient Registry (1977–2015)	Rate of (i) severe oligospermia (sperm concentration <15 million/ml; $n=2$) and (ii) azoospermia ($n=9$) compared with normospermia (sperm concentration >40 million/ml; $n=39$)	HR 1.5 (95% CI 1.1–2.1); (ii) HR 2.1 (95% CI 1.5–2.9) ¹⁸
Oncological	Testicular cancer	Infertile men ($n=76,083$) versus (i) fertile, non-vasectomized men ($n=760,830$) and (ii) vasectomized men ^a ($n=112,655$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of testicular cancer	(i) HR 1.99 (95% CI 1.47–2.70); (ii) HR 1.50 (95% CI 1.01–2.22) ¹³⁰
Oncological	Testicular cancer	(i) Men who performed clinical semen analyses (unspecified fertility ^c ; $n=32,442$) and (ii) men with oligospermia (sperm concentration <20 million/ml; $n=10,509$) versus men in the general Danish population; analyses performed at the Sperm Analysis Laboratory (Copenhagen, 1963–1995), cancer data from the Danish Cancer Registry (1943–1995)	Incidence of testicular cancer	(i) SIR 1.6 (95% CI 1.3–1.9); (ii) SIR 2.3 (95% CI 1.6–3.2) ¹²⁴
Oncological	Testicular cancer	(i) Men who performed clinical semen analyses (unspecified fertility ^c ; $n=20,433$) and (ii) men with oligospermia (sperm concentration <15 million/ml) versus fertile men ($n=20,433$); patients presented for infertility at the University of Utah Andrology Clinic (1996–2001) or data retrieved from the Intermountain Healthcare Analytic Health Repository for semen analyses (2002–2011); cancer data from the Utah State Cancer Registry ^d (USA, 1996–2012)	Incidence of testicular cancer	(i) HR 3.3 (95% CI 1.6–6.9); (ii) HR 11.9 (95% CI 4.9–28.8) ¹²³
Oncological	Testicular cancer	Men hospitalized for incident testicular cancer ($n=67$); semen analyses conducted at Frederiksberg Hospital (Copenhagen, 1977–2010), incident hospitalizations derived from the Danish National Patient Registry (1977–2015)	Rate of (i) severe oligospermia (sperm concentration <15 million/ml; $n=9$) and (ii) azoospermia ($n=9$) compared with normospermia (sperm concentration >40 million/ml; $n=35$)	(i) HR 1.6 (95% CI 1.2–2.2); (ii) HR 2.0 (95% CI 1.4–2.7) ¹⁸
Oncological	Testicular cancer	Infertile men (with at least one abnormal semen parameter; $n=3,847$) versus men from an age-matched and race-matched subpopulation from the SEER database ^e ; cancer rates derived from the SEER database ^e (USA)	Incidence of testicular cancer	SIR 18.3 (95% CI 18.0–18.8) ^{h 125}
Oncological	Testicular cancer	Infertile men ($n=4,549$) versus men without male factor infertility ($n=14,557$); data from 15 Californian fertility registries (1967–1998) linked to the Californian Cancer Registry ^g (USA, 1988–2004)	Incidence of testicular cancer	HR 2.8 (95% CI 1.3–6.0) ¹⁰⁴
Urogenital	Renal disease	Infertile men ($n=13,027$) versus vasectomized men ^a ($n=79,099$) from the Truven Health MarketScan Commercial Claims and Encounters database (USA, 2001–2009)	Incidence of renal disease	HR 1.60 (95% CI 1.14–2.25) ³⁴

CI, confidence interval; HR, hazard ratio; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; RR, rate ratio; SIR, standardized incidence ratio.

^aVasectomized men are assumed to be fertile. ^bTesticular hypofunction is considered a proxy for infertility. ^cHospitalizations include inguinal hernia, internal derangement of knee, haemorrhoids, deflected nasal septum, nasal polyp, cataract, gall bladder disease, varicose veins, hip replacement, knee replacement, tonsillectomy, adenoidectomy, ingrown toenail and other nail diseases, otitis externa, otitis media, sebaceous cyst and bunion (not associated with multiple sclerosis incidence when studied independently). ^dSymptomatic varicocele is a risk factor for infertility and is considered a proxy for infertility. ^eMen who performed semen analyses are assumed to be a highly infertile population. ^fAll malignancies include all invasive cancers from ICD-9-CM 140–209 (stomach, colorectal, liver and gallbladder, pancreas, lung, melanoma, breast, prostate, testis, bladder, kidney, brain and nervous system, thyroid and others) excluding skin squamous cell cancer, skin basal cell cancer and non-invasive cancers. ^gSurveillance, Epidemiology and End Results (SEER) database, including states' sub-databases. ^hMen with comorbidity-associated risk factors (such as cryptorchidism) removed from the analysis.

(for whom vasectomy is considered a proxy to fertility) or men randomly selected from a population of individuals enrolled in insurance policies without an infertility diagnosis¹⁰². An increased incidence of multiple sclerosis was also reported in infertile men compared with men without an infertility diagnosis¹⁰², and in men with

testicular hypofunction (which is considered a proxy for infertility)¹²¹ compared with men who experienced hospitalizations for minor conditions (excluding testicular hypofunction) in the study period (January 1999–December 2011)¹²¹. Other autoimmune disorders with increased incidence among infertile men compared

with vasectomized men or men without an infertility diagnosis included Graves' disease, psoriasis, rheumatoid arthritis and thyroiditis¹⁰² (TABLE 2).

Results from three retrospective cohort studies^{18,34,120} (TABLE 2) showed that the incidence of cardiovascular diseases was increased among infertile men

compared with fertile or vasectomized men. Cardiovascular comorbidities with increased incidence reported in these studies included general cardiovascular disease¹⁸, heart disease¹²⁰, hyperlipidaemia^{34,120}, hypertensive disease³⁴, ischaemic heart disease³⁴, peripheral vascular disease³⁴ and other heart diseases³⁴. Considering prevalent and incident comorbidities of infertility (TABLE 1 and TABLE 2), the prevalence and incidence of cardiovascular diseases are clearly increased in infertile men, with similar diseases observed in men with both prevalent and incident comorbidities.

With regard to a higher incidence of metabolic disease in infertile men than in fertile individuals, results from four independent studies^{18,34,118,120} showed an increased occurrence of diabetes mellitus in infertile men following the infertility diagnosis. In two studies^{18,118}, a reduction in sperm count was shown to be associated with an increased risk of incident diabetes mellitus. Last, infertility was also shown to be associated with an increased risk of incident liver disease, a serious, chronic illness³⁴.

Spermatogenic disruptions that lead to infertility, including cryptorchidism and various other aetiologies of non-obstructive azoospermia, might result in cell-cycle aberrations that can also precipitate into oncological disease¹²². In over half (9 of 14) of the studies in which incident comorbidities in infertile men have been assessed, increased incidence of cancer among infertile men was reported, with the most frequent cancer being testicular cancer^{18,104,123–125}. Stratification of infertility by severity (based on sperm count) repeatedly showed that among infertile men, oligospermic individuals are at the highest risk of developing testicular cancer^{18,123,124}. Results from a retrospective study showed that infertile men (3,847 patients) had a >18-fold higher risk of developing testicular cancer after the infertility diagnosis than age-matched and demographic-matched men from the Surveillance, Epidemiology and End Results database (representing 26% of the USA population; standardized incidence ratio (SIR) 18.3, 95% CI 18.0–18.8)¹²⁵.

In a clinical setting, the severity of male infertility positively correlates with an increased probability of a patient being referred for intracytoplasmic sperm injection (ICSI) instead of IVF owing to a decreased fertilization capacity in increasingly severe pathological conditions of male infertility^{126,127}. ICSI is a more complex fertilization technique than IVF, which involves direct injection of a single sperm cell into the oocyte and was originally

developed for men with extremely low sperm counts and other severe semen aberrations¹²⁷. Men who fathered children with the assistance of either IVF or ICSI were shown to have an increased risk of subsequent prostate cancer diagnosis compared with men who spontaneously fathered children, with men referred for ICSI being at the highest risk¹²⁸. Similarly, men with a sperm concentration <15 million/ml (severe oligospermia) had a higher incidence of prostate cancer than men with a sperm concentration >40 million/ml (REF. 18). Results from a study in which men seeking fertility assessment were stratified based on infertility diagnosis (male-factor negative or male-factor positive) and histological grade of cancer (Gleason score) showed that men with male factor infertility (male-factor positive) were 2.6 times more likely to develop high-grade prostate cancer (Gleason score 8–10) than men without male factor infertility (male factor-negative) (HR 2.6, 95% CI 1.4–4.8)¹²⁹. In addition to testicular and prostate cancers, the number of other cancer diagnoses in infertile men was 1.2–1.7 times higher than fertile men^{130,131}. Overall, men diagnosed with male factor infertility and men with increasingly severe pathological conditions of infertility, such as oligospermia, sperm count <14 million/ml, or referral for ICSI over IVF, are at an increased risk of subsequently developing cancer, in particular, cancers of the reproductive tract and prostate cancer.

In summary, men with an infertility diagnosis are at a higher risk of receiving a subsequent diagnosis of comorbidities (TABLE 2 and FIG. 1). Stratification of infertile men based on the severity of semen abnormalities or infertility showed that men with worse fertility outlooks were at the highest risk of comorbidity diagnosis. However, the mechanistic pathways linking infertility with potential comorbid diseases, as well as the molecular conductivity (specific aetiological metabolites or stress molecules that propagate disease pathogenesis) that might underpin infertility-related poor health are not fully understood.

The role of reactive oxygen species in male infertility

Many risk factors have been proposed as underlying causes of male infertility, but one common, recurrent factor is the association between increased reactive oxygen species (ROS) or oxidative stress and poor sperm function¹⁰¹. Indeed, increased levels of cellular oxidative stress have been observed in sperm samples from >40% of infertile men^{101,132} and underpin male

reproductive pathological conditions such as varicocele and azoospermia, as well as many instances of unexplained infertility¹³³. Specifically, the state of cellular oxidative stress in spermatozoa measured through the production of ROS and the overproduction of lipid peroxidation products and metabolites has been repeatedly linked to male infertility^{133–138}. Results from *in vitro* studies have identified causality between the induction of cellular oxidative stress as well as lipid peroxidation and a range of adverse pathological conditions of spermatozoa, including deficits in sperm motility, capacitation (post-ejaculatory sperm maturation in the female reproductive tract), acrosome reaction rates (a process that precedes sperm–egg fusion) and the successful interaction of sperm with the zona pellucida surrounding ovulated oocytes^{138,139}.

Reactive oxygen species and sperm function.

Cellular ROS in spermatozoa often arise as superoxide radicals (O_2^-), typically of mitochondrial origin¹⁴⁰; nitric oxide synthase (NOS) can then use the reduced cofactor nicotinamide adenine dinucleotide phosphate oxidase (NADPH) to generate nitric oxide (NO) from endogenous O_2^- (REF. 141). When the production of intracellular ROS or reactive metabolites such as peroxynitrite ($ONOO^-$) and hydrogen peroxide (H_2O_2) saturates the cell's antioxidant defences, oxidative stress occurs; ultimately, this pathological state of redox imbalance can also cause the oxidation of membrane lipids, organelle degradation, DNA and protein damage and, eventually, cell death^{135,142}. Overproduction of ROS can lead to cellular decline and poor fertility outcomes, but basal levels of ROS are required for promoting crucial stages of sperm development. For example, ROS have an active role in metabolic pathways involved in capacitation and in the subsequent induction of acrosomal exocytosis^{143–146}.

ROS produced within sperm cells were shown to contribute to protein tyrosine phosphorylation, a prerequisite step for sperm–egg interaction¹⁴⁷. Moreover, in boars and mice, bicarbonate-induced radical signalling facilitates cholesterol efflux from the sperm plasma membrane to increase membrane fluidity through oxysterol formation¹⁴⁸. These reactive pathways within sperm cells are highly intricate and involve not only ROS but also reactive nitrogen and potentially reactive sulfur species, as well as non-radical species, which are less well characterized^{149,150}. In a study in which

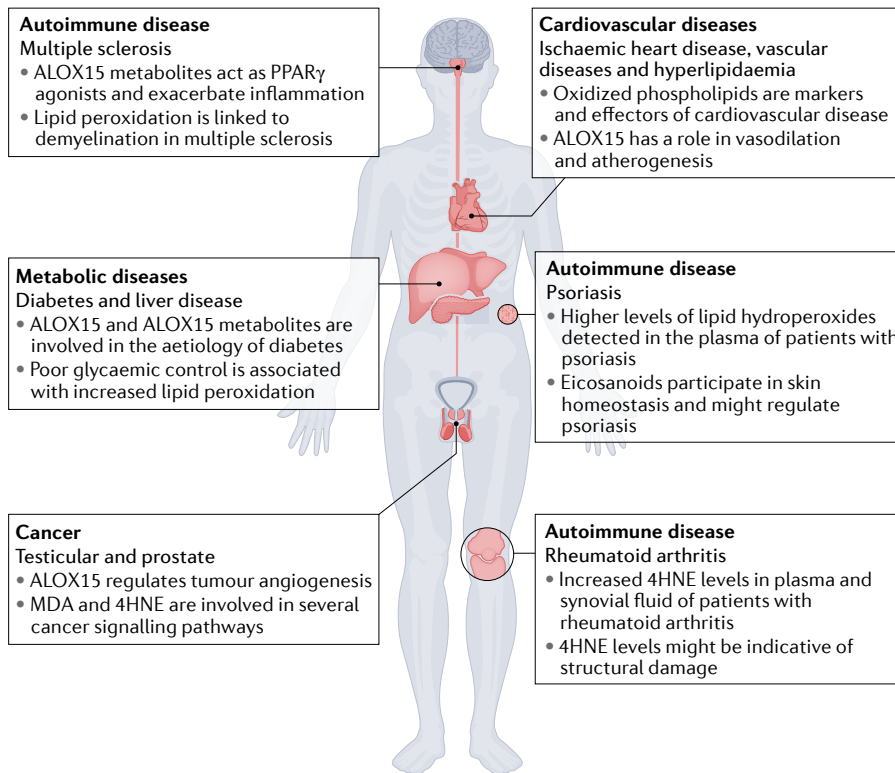


Fig. 1 | Incident comorbidities of male infertility and the role of lipid oxidation. Comorbidities diagnosed ≥ 12 months after a diagnosis of male infertility are considered 'incident comorbidities'. Known incident comorbidities of male infertility that are induced or exacerbated by lipid peroxidation and oxidative cellular damage can be grouped into four distinct categories (autoimmune, metabolic, oncological and cardiovascular). The lipoxygenase enzyme arachidonate 15-lipoxygenase (ALOX15) has a role in the molecular aetiology of many of these diseases through lipid oxidation and the generation of inflammatory mediators such as eicosanoids. 4HNE, 4-hydroxynonenal; MDA, malondialdehyde; PPAR γ , peroxisome proliferator-activated receptor- γ .

human sperm samples were analysed, mitochondrial ROS production was shown to be essential to sustaining ATP production at levels that permit extensive protein phosphorylation events necessary for sperm capacitation, as well as hyperactivated sperm motility¹⁵¹. The authors also showed that human sperm mitochondria actively produce ROS even in the absence of glucose or other energy substrates¹⁵¹. This evidence suggests the existence of a clear synergy between glycolysis and oxidative phosphorylation in human sperm cells to support post-testicular sperm maturation processes through ROS production. During epididymal transit and storage, sperm cells are also exposed to a pro-oxidant environment^{152,153} that induces further compaction of the sperm nucleus through the oxidation of protamine thiol groups and subsequent formation of disulfide bonds between cysteine residues, which stabilize the chromatin structure. This process helps to protect paternal chromosomes and assists in the 'locking' of the sperm nucleus in a condensed state¹⁵². Interestingly, this highly

compacted state offers little protection to the sperm mitochondrial genome, which is often more vulnerable to oxidative damage than the nuclear genome in the mature sperm cell^{152,154}. Damage to the mitochondrial DNA by ROS can affect ATP production, resulting in a rapid decline in sperm motility¹⁵⁵. At high concentrations, ROS can also disrupt mitochondrial membrane potential leading to lipid peroxidation, caspase activation and initiation of apoptosis^{155–157}. The functional consequences of this disruption of mitochondrial function are disorganization of the axoneme required for sperm motility¹⁵⁸, which can lead to asthenozoospermia¹⁵⁹.

In addition to the effects of ROS on sperm mitochondria, secondary redox signalling can lead to several deleterious protein modifications such as s-nitrosylation and protein carbonylation and/or modification by reactive carbonyl species (RCS)^{160–162}. S-nitrosylation is known to affect the enzymes involved in ATP production in sperm cells, and, specifically, tyrosine nitration alters the

activity of enzymes involved in glycolysis and the Krebs cycle, resulting in impaired sperm motility¹⁶². Sperm mitochondria require a dedicated folding machinery to correct unfolded and misfolded proteins, the presence of which increases under stress conditions¹⁶³. This folding machinery has also been found to be dysregulated in male infertility¹⁶³. The relationship between mitochondrial protein homeostasis and infertility remains largely unexplored; however, in the past years, important synergies between reproductive and somatic health have been revealed by analysing the effects of mitochondrial dysfunction mitigation on infertility phenotypes. Notably, transgenic mice lacking inner mitochondrial membrane peptidase 2-like (*Immp2l*) showed late-onset spermatogenesis impairment (from 13 months of age) coupled with an age-dependent degeneration of cerebellar granule neurons (16 months onwards) leading to impaired fertility and to the development of ataxia and cerebellar hypoplasia^{164,165}. The development of these conditions was caused by an increase in mitochondrial superoxide production, increased levels of protein carbonyl content and 4-hydroxynonenal (4HNE, a marker of lipid peroxidation), as well as increased nitrotyrosine protein modification in *Immp2l*^{-/-} mice compared with control animals¹⁶⁴, amongst other signs of premature ageing^{166,167}. Remarkably, the use of a mitochondria-targeted antioxidant (SKQ1) that contains both a plastoquinol residue (antioxidant) and decyltriphenylphosphonium (a lipophilic, membrane-penetrating cation) improved spermatogenesis, reduced apoptosis of germ cells and increased expression of the antioxidant glutathione peroxidase 4 (GPX4) in *Immp2l*^{-/-} animals¹⁶⁸. Moreover, SKQ1 treatment was able to protect cerebellar granule neurons from degeneration¹⁶⁹, suggesting that mitochondrial damage and oxidative stress have an underlying role in fertility loss and neuronal degradation in these animals. Mechanistically, SKQ compounds have been found to prevent both cardiolipin oxidation and fatty acid cycling, both of which might contribute to SKQ antioxidant capacity¹⁷⁰. Additionally, SKQ1, as well as some mitophagy-promoting compounds, have received considerable attention for their ability to extend health span and, in many instances, lifespan^{171,172} in rodents, fish, plants, invertebrates and fungi by attenuating senescence pathways^{173–175}. Thus, understanding the relationship and mechanistic overlap between mitochondrial dysfunctions in reproductive and somatic

health conditions holds considerable promise in elucidating mechanisms to prevent age-related reproductive and somatic diseases.

The cytosol of somatic cells contains an abundance of antioxidant defence mechanisms to protect cells from the detrimental effects of ROS¹⁷⁶. During differentiation, germ cells experience substantial nuclear reduction and cytoplasmic remodelling events, which result in a considerable loss of cytoplasmic space and a consequent reduction in the antioxidant defences in the mature sperm cell^{177,178}. Consequently, spermatozoa are at an increased risk of oxidative attack. This risk is exacerbated by the high level of PUFAs contained in the sperm plasma membrane that are susceptible to oxidative attack¹³⁵. Despite the limited cytoplasmic space, sperm cells do possess antioxidant enzymes to counteract ROS at physiological levels, including thioredoxin 1 (TRX1), copper, zinc superoxide dismutase (CuZn SOD) and TRX reductase (TR)¹⁷⁹. Additionally, sperm cell mitochondria contain crucial antioxidants such as GPX4, peroxiredoxin 2–6 (PRDX2–6), manganese superoxide dismutase (MnSOD) and thioredoxin 2 (TRX2)¹⁷⁹. In addition to the presence of antioxidants in spermatozoa, seminal plasma also contains the antioxidants catalase (CAT), SOD, GPX and peroxiredoxins, as well as free radical scavengers¹⁷⁹. A well-reported negative relationship exists between seminal plasma antioxidant levels and male infertility^{179,180}.

Lipid peroxidation in reproductive and somatic health. The peroxidation of PUFA-containing phospholipids is the first step in the oxidative damage of cell membranes¹⁸¹. Lipid peroxidation occurs through the oxidation of carbon–carbon double bonds in unsaturated fatty acids, and the most common oxidants are ROS produced by either intracellular metabolic changes or extracellular sources such as ionizing radiations or thermal stress¹⁸¹. Lipid peroxidation results in a cascade of biochemical changes within cells that can lead to cell death, senescence or cellular dysfunction^{182,183}; moreover, lipid oxidation has been shown to induce changes to the mechanical properties of lipid membranes¹⁸⁴. Importantly, the site of oxidation is now thought to be a crucial determinant of the nature of the biophysical changes occurring in lipid membranes. For example, results from a study in which the systematic oxidation of phospholipids was evaluated¹⁸⁴ showed that, although the lipid membrane

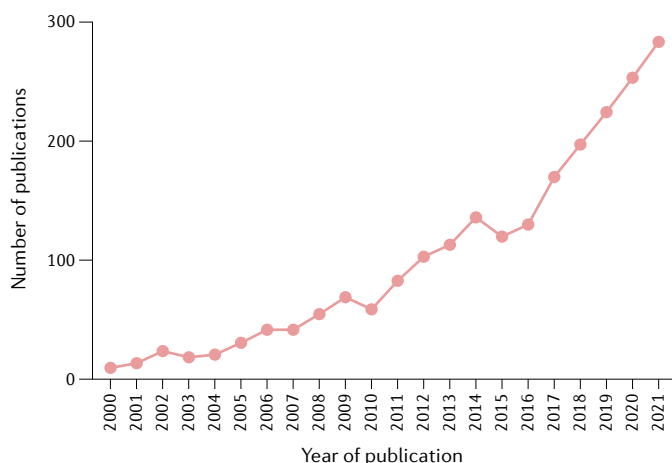
commonly expands following oxidation at all sites, peroxidation at sites in the inner part of the bilayer resulted in the destabilization and softening of the membrane. Conversely, peroxidation at sites close to the membrane–water interface led to increased membrane rigidity and ordered packing¹⁸⁴. This new understanding of structural effects of lipid peroxidation might explain the diverse contribution of lipid oxidation events to the onset and pathogenesis of an extremely large proportion of human diseases. Indeed, lipid peroxidation-induced tissue damage has been observed within almost all human tissues and underpins and/or exacerbates a large number of disease classes including, but not limited to atherosclerosis, heart disease, Alzheimer disease and Parkinson disease, immune disorders, diabetes, pre-eclampsia, arthritis, asthma and chronic obstructive pulmonary disease, hypertension, hepatic disease and infertility (male and female factor)^{169,185–196} (FIG. 1). With regard to male infertility, lipid peroxidation and increased cellular ROS have been proposed as crucial processes that contribute to the high number of unexplained infertility diagnoses in men^{134,197–199}. Indeed, the number of studies in which oxidative stress and lipid peroxidation have been assessed in relation to male infertility has substantially increased in the past two decades (FIG. 2a,b), mainly owing to new data from clinical cohorts, development of novel quantification methods for ROS or oxidative DNA damage, and emerging mass spectrometry-based techniques to assess lipid oxidation events^{182,200–202}.

Considering the diversity of diseases involving an increase in lipid peroxidation and end products (such as RCS), enzyme catalysts involved in the lipid peroxidation process in cell membranes might provide insights into the development of many disease states, and could potentially act as molecular links between somatic disease and reproductive disorders. The limitation in verifying this hypothesis is the lack of molecular knowledge surrounding enzyme-driven lipid peroxidation in the male germline, especially in humans¹⁸². The concept of systemic oxidative stress that first presents in the testis and then develops in other organs has emerged as a pressing area of interest for many reproductive biologists; however, more than a decade ago, results from an important study including 38 normozoospermic infertile patients and 17 fertile volunteers showed that high levels of lipid peroxidation might be related to abnormal fatty acid composition reflected in both the seminal plasma and the blood

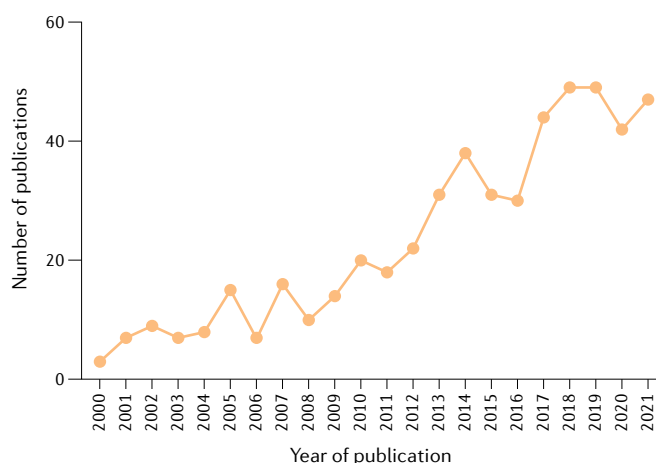
plasma of these patients¹³³. Specifically, higher arachidonic acid:docosahexaenoic acid (AA:DHA) ratios were found in infertile patients than in the fertile volunteer counterparts, and these ratios correlated with increased levels of lipid peroxidation. This evidence supports similar results from previous studies^{203,204}. In another study²⁰⁵, an increased content of free unsaturated, unesterified fatty acids, which positively correlated with increased ROS generation, was reported in human spermatozoa. Moreover, the direct exposure of human spermatozoa to free unsaturated fatty acids was shown to induce oxidative stress and loss of motility, enforcing the hypothesis that lipidic imbalances might affect fertility²⁰⁵. Imbalances in the AA:DHA ratio have also been frequently observed in many systemic inflammatory disorders including cystic fibrosis, osteoporosis and some neurological disorders^{133,206,207}, indicating that AA:DHA imbalance might be a conserved phenomenon between reproductive and somatic disorders.

Efforts to understand the links between infertility, systemic ROS and lipid peroxidation have been focused on assessing antioxidant levels and the antioxidant capacity of biological fluids (blood and seminal plasma) in relation to lipid aldehyde accumulation in these fluids. Lipid aldehyde analysis is important, as these lipid peroxidation products can damage DNA and proteins leading to cell death and widespread tissue damage¹⁹⁵. In one study including 29 patients with grade III varicocele, lipid aldehyde levels were measured in the seminal fluid, peripheral blood and spermatic vein blood of these patients before and after varicocelectomy. Patients presented with high malondialdehyde (MDA) levels in all samples but, following varicocelectomy, blood and seminal plasma MDA levels were significantly reduced compared with preoperative levels ($P < 0.05$ in seminal plasma; $P < 0.001$ in peripheral and spermatic plasma) and this reduction positively correlated with increased sperm concentration²⁰⁸. Decreased levels of NO in seminal plasma and improved semen parameters (sperm motility and morphology) were also reported in patients after varicocelectomy, indicating that both seminal and systemic ROS levels are linked to improved sperm function following varicocelectomy. In a comparative analysis of blood and seminal plasma samples from fertile and infertile men, antioxidant levels (measured through total antioxidant capacity (TAC) and vitamin E concentration) were significantly

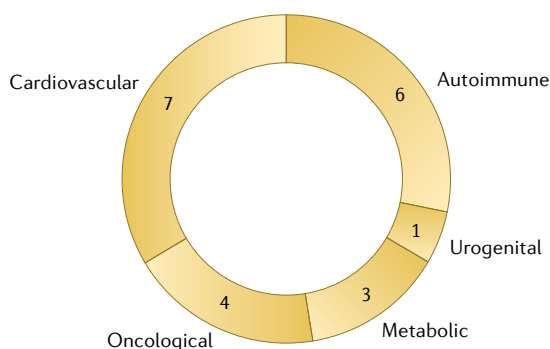
a Male infertility and oxidative stress



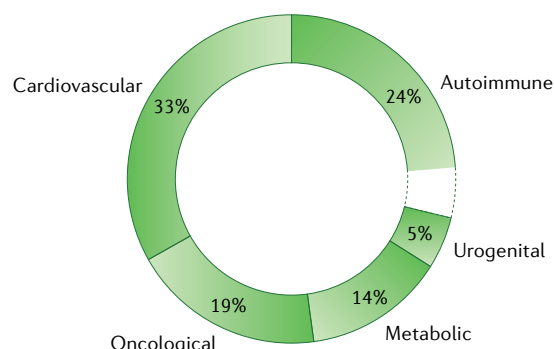
b Male infertility and lipid peroxidation



c Incident comorbidities associated with male infertility



d Lipid peroxidation is associated with 95% of all incident comorbidities



e ALOX15 is associated with 85% of all incident comorbidities of male infertility

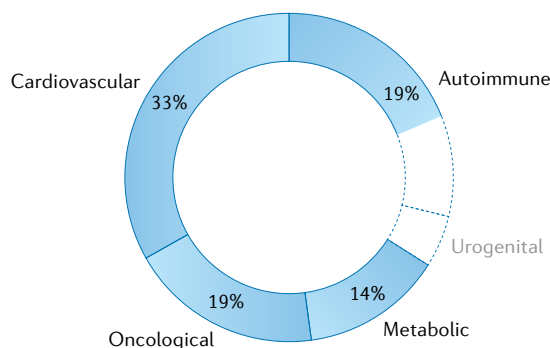


Fig. 2 | Male infertility, incident comorbidities and lipid oxidation. The number of publications including the terms ‘male infertility’ and ‘oxidative stress’ (**a**) and ‘male infertility and lipid peroxidation’ (**b**) substantially increased over the past two decades (source: PubMed NCBI; date retrieved: 4 July 2022). Incident comorbidities associated with male infertility (TABLE 2) were divided into disease categories, and the number of diseases linked to male infertility in each category is shown (part c). Overall, 95% of the 21 incident comorbidities (TABLE 2) of male infertility have been associated

with lipid peroxidation and the overabundance of reactive carbonyl species such as 4-hydroxynonenal and malondialdehyde^{169,185–194} (part d). Using the online database DisGeNET (version 7.0), and through the examination of relevant literature, lipoxygenase enzyme arachidonate 15-lipoxygenase (ALOX15) was found to be consistently associated with 85% of all known incident comorbidities of male infertility, which include all diseases in the oncological, metabolic and cardiovascular categories, as well as several autoimmune diseases^{248,250–254} (part e).

decreased in the seminal plasma of infertile men ($P < 0.05$)²⁰⁹, and TAC ($P = 0.037$), carotenoids ($P = 0.032$) and vitamin E concentrations ($P = 0.035$) were all

significantly lower in the blood of infertile men than in fertile individuals, with TAC and carotenoid levels positively correlating with sperm motility, morphology and

concentration, and TAC and vitamin E levels positively correlating with seminal plasma antioxidant values²⁰⁹. These results highlight the importance of regulating oxidation in

blood and seminal plasma in the context of male infertility. Similarly, in another study, a significant positive correlation was reported between semen parameters (sperm count and progressive motility) and antioxidant levels in blood serum (SOD and glutathione, $P=0.036$ and $P=0.018$, respectively) in infertile men²¹⁰. In these studies^{209,210}, lipid aldehyde accumulation (measured through MDA) was also significantly increased in the seminal plasma of infertile men ($P=0.032$)²⁰⁹, and the levels of MDA in seminal plasma negatively correlated with sperm motility ($P=0.039$) and morphology ($P=0.029$)^{209,210}. Results from these studies indicate that low antioxidant levels and high MDA levels in blood serum might indicate poor reproductive capacity in men. Results from a study in which a potentiometric quantification system was used to simultaneously assess both antioxidant and oxidant activity within biological fluids showed that men with severe pathological conditions of infertility (teratospermia, asthenoteratospermia and oligoasthenoteratospermia) had substantially lower seminal plasma antioxidant activity than fertile men²¹¹.

Collectively, these results indicate that low circulating blood and seminal plasma antioxidant levels frequently reflect declining semen parameters and a state of oxidative stress within the reproductive tract. Moreover, examination of lipid peroxidation products and antioxidant levels in blood and seminal plasma might offer diagnostic utility in assessing fertility status^{209–211} and other disease states²¹¹; this approach might also have an important role in determining the paradigmatic and mechanistic links between male infertility and somatic health.

With regard to the potential relationship between lipid peroxidation and the incident comorbidities of male infertility, 21 distinctive diseases have been identified as incident comorbidities of male infertility (as the diagnosis occurred ≥ 12 months after a diagnosis of infertility); these diseases fit into five individual categories: cardiovascular, autoimmune, urogenic, metabolic and oncological (FIG. 2c and TABLE 2). Overall, 95.2% of these incident comorbidities of male infertility (TABLE 2) have been shown to be either induced or exacerbated by oxidative tissue damage following the propagation of lipid peroxidation products^{169,185–194} (FIG. 2d). Considering the remarkable conservation of the lipid peroxidation process across tissues and disease states, the characterization of enzymatic and non-enzymatic causes of lipid peroxidation in cells is receiving extensive attention.

In somatic cells, several enzymes are known to catalyse the lipid oxygenation and hydrogen abstraction processes that lead to the production of RCS including the following: lipoxygenase enzymes (LOXs), which are involved in the oxidation of several PUFA (and sometimes sterol) substrates including AA to produce lipoxins, leukotrienes and hydroperoxyeicosatetraenoic acids (HPETEs); cyclooxygenases (COXs) that often metabolize AA to produce prostaglandins; and cytochrome P450 enzymes (CYPs) that generate epoxyeicosatrienoic acids, leukotoxins and prostacyclin among other products^{139,183,212}. The molecular mechanisms of lipid substrate oxidation by each of these enzymes have been extensively described²¹³; however, many of these enzymes have dioxygenase activity and/or peroxidase activity, and have often structurally diverse isoforms, which provide broad substrate specificity and diverse modes of catalysis¹⁸³. In somatic cells, PUFA oxygenation is usually initiated by COXs, LOXs and, to a lesser extent, CYPs; moreover, a crossover often occurs among COX, LOX and CYP enzymes to produce specialized pro-resolving mediators such as lipoxins and E-resolvins²¹³. In addition to the production of numerous inflammatory mediators, the products of lipid oxidation reactions can then be further metabolized to produce several classes of highly reactive molecules including RCS, often referred to as lipid aldehydes. The most frequently studied RCS are 4HNE, MDA²⁰¹ and acrolein¹⁶⁰. In addition to these pathways of lipid oxidation, non-enzymatic mechanisms, such as the Fenton reaction, can also contribute to the production of RCS^{175,203}.

Many of the same enzymes involved in lipid peroxidation in somatic cells, including COX1 and COX2, are known to be expressed in the male germline too^{214,215}, although the role of these enzymes in germ cells is not well understood. However, enzymes such as COX1 and COX2 have been regularly suggested as biomarkers of varicocele and related pathological conditions of infertility and, therefore, might be explored as catalysts of lipid peroxidation in the male germline²¹⁴. Similarly, CYP enzymes are essential for spermatogenesis, and some CYPs have been shown to have a role in the metabolism of reproductive toxicants such as acrylamide²¹⁶, and in hormone metabolism²¹⁷; however, the potential relationship of CYPs with lipid peroxidation events in the testis has not been well established. Conversely, over the past decade, the mechanistic role of LOXs in the male germline has started to be evaluated²¹¹;

results from a pivotal study in the field²¹¹ showed that the knockout of the LOX family member 15-lipoxygenase-1 (*Alox15*) in mice heterozygous for a catalytically inactive form of GPX4 could rescue the severe subfertility phenotype observed in these mice²¹⁸. This evidence indicates that an antagonist relationship exists between ALOX15 and GPX4 in the metabolism of hydroperoxyl lipids in the male germline. Additionally, pharmacological inhibition of ALOX15 can prevent RCS production in the male germline in both humans and mice, providing protection for spermatozoa during oxidative stress^{139,199,219} and indicating that LOXs are important regulators of RCS production.

RCS are highly cytotoxic and reactive products, and can cause widespread cellular damage through the formation of covalent DNA and protein adducts²²⁰. With regard to proteins, RCS can post-translationally modify cysteine, lysine and histidine amino acid residues²²⁰, leading to diverse protein fates including conformational changes and misfolding, degradation or even aggregation²²¹. In mature human spermatozoa, exposure to exogenous 4HNE at physiological levels (6.25–50 μM)²²² induces a loss of progressive motility in a dose-dependent and time-dependent manner^{138,160}, which has been ascribed to the targeting of numerous protein substrates for covalent modification by 4HNE in the male germline, including protein kinase A (PKA), dynein heavy chain 17 axonemal (DNAH17)²²³, A kinase anchoring protein 4 (AKAP4)²²⁴ and succinate dehydrogenase (SDHA)^{101,160}. In human sperm cells, under conditions of oxidative stress, 4HNE is now known to adduct SDHA, which constitutes the catalytic subunit of complex II of the mitochondrial electron transport chain; this adduction leads to exacerbated electron leakage from the mitochondria resulting in the propagation of mitochondrial ROS^{160,201}. Subsequently, electron leakage from the damaged mitochondria leads to further lipid peroxidation, accentuating the production of RCS and generating a deleterious cycle of cellular damage^{160,199,225–229}. Moreover, not only spermatozoa but also mammalian oocytes exposed to 4HNE are vulnerable to SDHA adduction by 4HNE, with deleterious consequences for oocyte quality²³⁰. In addition to targeting SDHA, elevated levels of 4HNE cause further damage to sperm cells via the adduction of heat shock protein A2 (HSPA2), a molecular chaperone required for cell surface remodelling events that permit human sperm–egg interaction and fertilization²³¹. The selective

vulnerability of HSPA2 to 4HNE-mediated adduction has been shown in both human spermatozoa¹³⁸ and mouse germ cells²³², with HSPA2 adduction by 4HNE in mouse germ cells resulting in the aberrant proteolytic degradation of this protein by disrupting the complex formed under physiological conditions by HSPA2 and the protective co-chaperone BCL-2-associated athanogene 6 (BAG6)²³². The unexpected degradation of HSPA2 under oxidative conditions also recapitulates a relatively common phenotype of unexplained male infertility, characterized by the inability of patients' spermatozoa to interact with the oocyte zona pellucida during IVF owing to a marked reduction in the levels of HSPA2 (REF.¹³⁸).

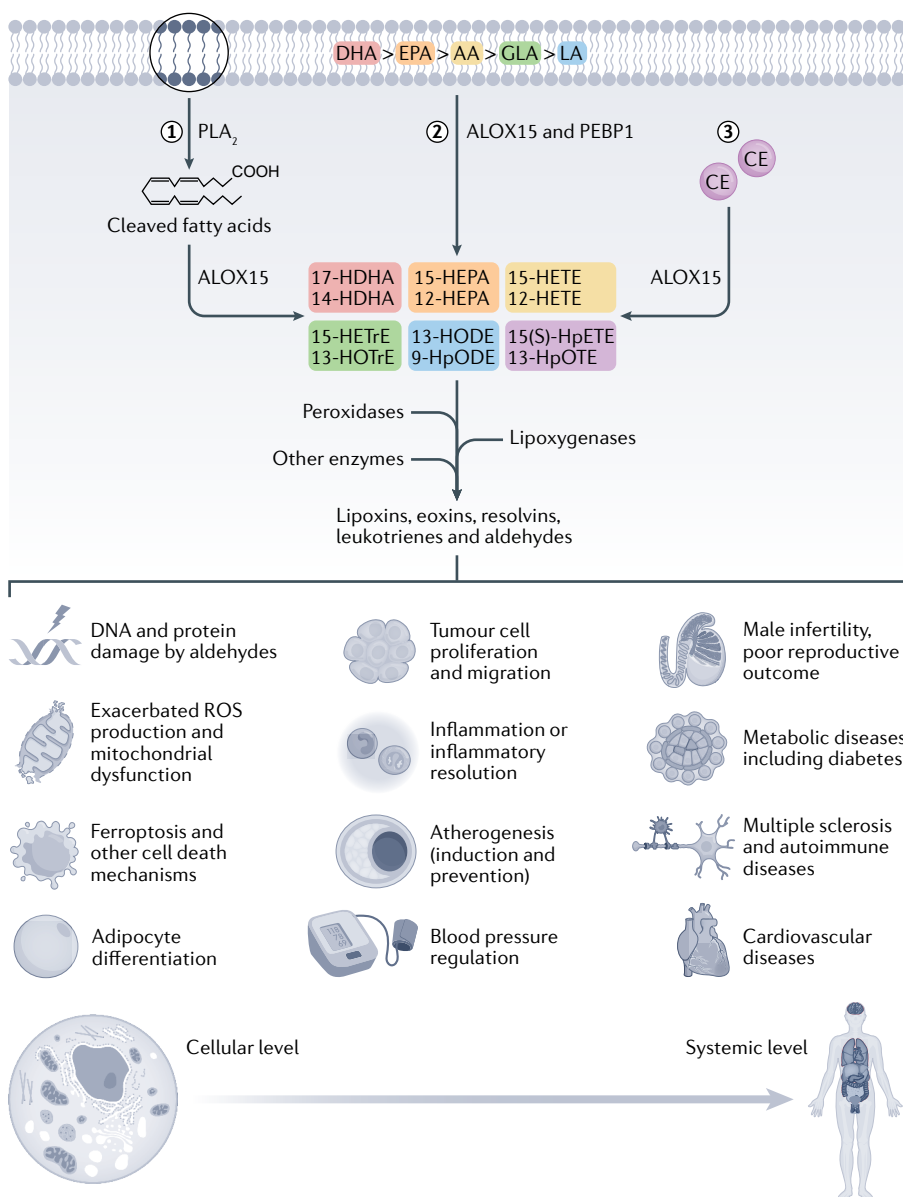
RCS have also been shown to contribute to protein misfolding events leading to widespread protein aggregation in the male germline²²¹ and also in neurons, in which RCS can induce structural changes that promote protofibril formation of amyloid β and mitochondrial creatine kinase²³³. RCS and the enzymes involved in RCS generation, such as LOXs and COXs, have also been characterized as crucial regulators of ferroptosis, an iron-regulated and caspase-independent cell death modality²³⁴. Ferroptosis primarily starts through either inhibition of GPX4 activity or reduced glutathione levels, which lead to the toxic accumulation of lipid hydroperoxides within cells^{235,236}. These lipid hydroperoxides are then degraded to form either hydroxy fatty acids and/or RCS that subsequently propagate lipid peroxidation and promote toxicity²³⁵. This lipid peroxidation-induced cell death has received considerable attention, as the pharmacological modulation of this process holds promise in the treatment of drug-resistant cancers, ischaemic organ injuries and some degenerative diseases linked to lipid peroxidation^{234,237}.

Lipid metabolism processes underpinning ferroptosis are undeniably complex and involve enzymatic and non-enzymatic lipid oxidation events, but ALOX15 and the lipid-metabolizing enzymes acyl-CoA synthetase long chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) generate an important pathway leading to the production of lipid hydroperoxides that contribute to ferroptosis^{234,238,239}. In this pathway, ACSL4 is thought to dictate the sensitivity of cells to ferroptosis through enriching membranes in long-chain PUFAs, which are crucial substrates for oxidation. Specifically, the PUFAs currently known to be oxidized in ferroptosis are AA and adrenic acid (AdA)²⁴⁰. AA and AdA

acyl-CoA derivatives are produced through ACSL4 catalysis, and subsequently esterified by LPCAT3 into phosphatidylethanolamines (AA-PE and AdA-PE)^{239,240}. ALOX15 has been shown to directly oxidize AA-PE and AdA-PE into lipid hydroperoxides, which act as signals of ferroptotic cell death^{240,241}. In a subsequent study, 12-LOX (also known as ALOX12 or ALOX12/15) has also been implicated in ferroptosis through another pathway involving the activation of 12-LOX by TP53 (REF.²⁴²).

In the male germline, little is known regarding the link between ferroptosis and infertility. However, round spermatids have been shown to have a unique sensitivity to ferroptosis compared with spermatocytes and mature spermatozoa, which can be induced by GPX4 inhibition and is mediated by the increased expression

and activity of ALOX15 and ACSL4 (REF.¹⁹⁵). Moreover, ferroptosis in round spermatids can be directly stimulated by exogenous 4HNE¹⁹⁵ and is attenuated by iron chelation and ALOX15 inhibition, indicating an important role for ALOX15 and RCS in germ cell death. Subsequently, ferroptosis has been detected in rodent testis in response to several insults including busulfan²⁴³, arsenite²⁴⁴ and cigarette smoke²⁴⁵, indicating that this process might hold relevance in the response of the germline to environmental stress. In addition to germ cells, ferroptosis is induced in Sertoli cells following oxygen-glucose deprivation and reoxygenation related to testicular ischaemia-reperfusion²⁴⁶. This process might suggest a role of ferroptotic cell death in the impairment of spermatogenesis associated with male infertility²⁴⁶.



Despite these new findings, the knowledge of lipid metabolism in male germ cells is still emerging and, therefore, the ability to compare pathways associated with germline oxidative stress with those associated with somatic oxidative stress is still limited. However, the inhibition of lipid peroxidation pathways such as pathways regulated by LOXs and COXs can clearly be a novel tool for reducing the production of harmful RCS, supporting the action of existing antioxidants and exogenous antioxidant therapies. Novel evidence of the contribution of these mechanisms to lipid peroxidation (and ferroptosis) in the male germline offers a unique opportunity to provide a mechanistic link between lipid peroxidation-induced cell damage in male infertility and incident comorbidities of infertility, and could provide options for the development of lipid-targeted therapeutics.

Lipoxygenases and lipid peroxidation. LOXs catalyse a highly controlled form of lipid peroxidation through dioxygenase activity and non-haem iron; first, hydrogen atom removal occurs through ferric iron (FeIII) and assistance from proton-coupled electron

transfer, producing a lipid alkyl radical and ferrous iron (FeII)^{213,247}. Rearrangement of the radical then takes place to form the stable conjugated diene; dioxygen is then usually inserted at the opposite end from the site of hydrogen removal to generate the lipid peroxy radical, which is reduced by FeII and protonated, forming a lipid hydroperoxide^{213,247,248}. This reaction also results in the reformation of FeIII to undergo further cycles of catalysis. In addition to this dioxygenase activity, LOXs can catalyse other processes involving free radicals such as hydrogen abstraction and radical rearrangement, and 5-LOXs and ALOX15 possess leukotriene-A₄-synthase activity to produce epoxides from hydroperoxides²⁴⁸.

In humans, six LOX genes exist with diverse tissue expression: *ALOX12*, *ALOX12B*, *ALOX15*, *ALOX15B*, *ALOX5* and *ALOXE3* (REF.²¹³). With regard to potential links among LOX family proteins, male infertility and medical comorbidities, analysis of data from the DisGeNET knowledge platform for disease genomics²⁴⁹ showed that a putative role for ALOX15 alone can be associated with 85% of the incident comorbidities of male infertility^{248,250–254} (FIG. 2e).

The substrate preferences of LOXs are under constant investigation; in humans, the known substrate preference for 15-LOXs (from highest to lowest) is: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), AA, γ -linolenic acid (GLA), linoleic acid (LA)²⁵⁵ (FIG. 3). Typically, LOXs are constitutively expressed²¹³; however, LOX expression, for example *ALOX15B*, can increase in the presence of cytokines, hypoxia and lipopolysaccharide²¹³. ALOX5 requires interaction with partner proteins to be activated²¹³, whereas 15-LOXs do not require accessory proteins to carry out oxygenation reactions and can modulate substrate specificity by interacting with phosphatidylethanolamine-binding protein 1 (PEBP1 (REF.²⁴¹); FIG. 3). Differently from ALOX15, for which a lot of information is available, little is known regarding the regulation of ALOXE3 and the 12-LOXs, as complete crystal structures of ALOX12, ALOX12B and ALOXE3 are still unavailable.

The diverse substrate specificity of LOX enzymes enables LOXs to catalyse the production of a wide range of lipid mediators involved in both physiological and pathological processes (FIG. 3), generally involving modulation of platelet aggregation, modulation of MAP kinase pathways, tumour suppression, cell-cycle regulation, and modulation of inflammatory mediators and epidermal barrier acquisition, amongst other functions²⁵⁶. Thus, LOXs are implicated in an extensive range of diseases (FIG. 3), including neurodegenerative disorders, asthma, cardiovascular disease and atherosclerosis^{182,256,257}.

ALOX15 has been identified as responsible for the catalysis of a majority of sperm lipid peroxidation events through the oxidation of PUFA substrates that are enriched in the male germline^{139,199,248,258,259}. PUFA targets of ALOX15 in the male germline include ω -6 fatty acids such as AA and LA and the ω -3 fatty acid DHA²⁵⁰. ALOX15-dependent oxygenation of PUFAs produces a number of important but short-lived lipid mediators, including 12-hydroperoxyeicosatetraenoic acid (12-HpETE) and 15-HpETE as well as 12-hydroxyeicosatetraenoic acid (12-HETE) and 15 HETE²⁵⁸. These products are crucial intermediaries for the production of inflammatory mediators such as eoxins and resolvins (FIG. 3).

ALOX15 is known to have a putative role in the morphogenesis of spermatozoa through facilitating the shedding of residual cell cytoplasm (known as cytoplasmic droplet), in both boar²⁶⁰ and mouse²⁶¹ spermatozoa during sperm maturation.

◀ **Fig. 3 | Biosynthesis of oxidized phospholipids by ALOX15 and consequences for human health and fertility.** Human lipoxygenase (LOX) enzymes show preference towards certain polyunsaturated fatty acid (PUFA) substrates over others. 15-lipoxygenase-1 (ALOX15) known substrate preference (from highest to lowest) is docosahexaenoic acid (DHA) > eicosapentaenoic acid (EPA) > arachidonic acid (AA) > γ -linolenic acid (GLA) > linoleic acid (LA). ALOX15 can oxygenate lipid substrates through three main pathways. (1) The best characterized pathway involves the initial release of sn-2 fatty acids from membrane phospholipids through the action of phospholipase enzymes such as phospholipase A₂ (PLA₂). LOXs (as well as cyclooxygenases and cytochrome P450s) subsequently oxygenate these lipids. The oxygenated lipids are then re-esterified (not shown) and can also be esterified with plasmalogen lysophospholipids. (2) ALOX15 can also act directly on membrane phospholipids without the action of phospholipases; the substrate specificity of ALOX15 can be directed by interaction with phosphatidylethanolamine-binding protein 1 (PEBP1). (3) Finally, ALOX15 can oxygenate the PUFA moiety of cholesterol esters (CEs); the oxygenated PUFAs are then hydrolysed from CE and can be esterified to lysophospholipids. These pathways lead to the generation of lipid hydroperoxides and a pool of diverse reaction intermediates and final products. These products include (but are not limited to) the following: 15-hydroxyeicosatetraenoic acid (15-HETE) and 12-hydroxyeicosatetraenoic acid (12-HETE); 15-hydroxyeicosapentaenoic acid (15-HEPA) and 12-hydroxyeicosapentaenoic acid (12-HEPA); 17-hydroxy docosahexaenoic acid (17-HDHA) and 14-hydroxy docosahexaenoic acid (14-HDHA); 13-hydroxyoctadecadienoic acid (13-HODE); 9-hydroperoxy-octadecadienoic acid (9-HpODE); 15-hydroxyeicosatrienoic acid (15-HETrE) and 13-hydroxyeicosatrienoic acid (13-HOTrE); 13(S)-hydroperoxy-9Z, 11E-octadecadienoic acid (13(S)-HpODE); 15-hydroperoxyicoso-5,8,11,13-tetraenoic acid (15(S)-HpETE) and 13-OH-9Z,11E,15Z-octadecatrienoic acid (13-HpOTE). Products have been colour coded with the respective substrates when possible. The products of ALOX15-driven lipid oxygenation are subsequently further metabolized by other LOXs, peroxidases or independent pathways to produce lipoxins, eoxins, resolvins, leukotrienes and lipid aldehydes (also referred to as reactive carbonyl species) such as 4-hydroxynonenal, malondialdehyde, acrolein and other, less abundant, products. Alternatively, enzymes such as glutathione peroxidase 4 (GPX4) and other antioxidants can metabolize these products to maintain cellular homeostasis, and enable these metabolites to mediate other physiological processes (not shown). A wide range of products originate from LOX-mediated lipid oxygenation, which have extensive consequences for cells, tissues and organisms. Mechanisms of these effects involve, for example, modification of proteins by aldehydes, mitochondrial dysfunction and ferroptosis. Accumulation of some LOX metabolites can lead to widespread effects on tumour metastasis, inflammation and even blood pressure. At the systemic level, many of these metabolic and inflammatory changes can promote disease states such as infertility, metabolic diseases, autoimmune disease, cardiovascular disease and cancer. ROS, reactive oxygen species.

Results from a study in which the acrosome reaction was suppressed in bull spermatozoa after the inhibition of ALOX15 using two pharmacological LOX inhibitors also showed an involvement of ALOX15 in promoting the acrosome reaction in mammalian spermatozoa²⁶². Coherently with these results, mice lacking *Alox15* are subfertile and produce spermatozoa in which cytoplasmic droplets are retained²⁶².

Considering the substantial evidence of the detrimental effect of oxidative stress on sperm function, the therapeutic use of antioxidant supplements has been the subject of numerous clinical trials, many of which are ongoing^{263–265}. However, to date, these antioxidant-based interventions to intercept ROS and improve spermatozoa quality have failed to meet expectations, often owing to a lack of patient selection on the basis of oxidative stress in spermatozoa or semen^{30–32,266}. However, the upstream role of ALOX15 in the propagation of lipid peroxidation in human spermatozoa in response to increased exogenous ROS has been characterized. Notably, the selective inhibition of ALOX15 activity with 6,11-dihydro[1]benzothiopyrano[4,3-b]indole (PD146176) resulted in an attenuation of mitochondrial and total cellular ROS, membrane lipid peroxidation, and 4HNE production after challenging sperm cells with an oxidative insult¹³⁹. Moreover, PD146176-mediated inhibition of ALOX15 also resulted in the recovery of sperm motility, and prevented the loss of sperm binding to zona pellucida, which is usually observed after exposure to oxidants¹³⁹. These results also have clinical relevance, considering that increased levels of ALOX15 have been observed in spermatozoa of patients with unexplained male infertility, which might explain the overproduction of 4HNE in the sperm cells of these individuals¹⁹⁹.

ALOX15-dependent lipid peroxidation in the male germline has been more extensively characterized than other lipid metabolism pathways, and ALOX15 was shown to be involved in the majority of known comorbidities of infertility (TABLE 2), suggesting that ALOX15 might act as a putative enzymatic bridge between oxidative stress-induced male infertility and incident comorbidities. However, ALOX15 is just one example of the many lipid-modulating enzymes that could propagate lipid peroxidation resulting in reproductive and somatic health problems, and is very unlikely to be the only mediator of these processes. Conversely, numerous pre-disposing factors linking somatic

and reproductive diseases might exist. Additionally, COXs, CYPs and important lipid-modulating enzymes such as ACSL4 and LPCAT3 are also likely to contribute to the reproductive-somatic health paradigm. However, further research on the roles of these enzymes in reproduction are needed before any link between alterations of these candidates across disease states and male fertility can be critically assessed.

ALOX15 in chronic medical conditions

ALOX15 is implicated in ROS production and lipid peroxidation in human spermatozoa, and also in the progression of several chronic medical conditions in which oxidative stress and the accumulation of ALOX15 metabolites are leading causative agents^{248,250,252} (FIG. 1 and FIG. 2).

Inflammation. Inflammation is symptomatic of tissue distress or damage and the presence of numerous diseases²⁵², and ALOX15 metabolites have been shown to have a role in inflammation. ALOX15 metabolites have both pro-inflammatory and anti-inflammatory properties²⁵²; the pro-inflammatory ALOX15 metabolite 12(S)-HETE is a potent chemoattractant for neutrophils and leukocytes²⁶⁷. In rabbits, intradermal injection of 15(S)-HpETE elicited plasma exudation among other inflammatory symptoms²⁶⁸. Moreover, results from several studies support these findings and showed pro-inflammatory roles for both 12(S)-HETE and 15(S)-HETE^{268–270}; for example, direct 12(S)-HETE and 12(S)-HpETE addition to adipocytes (3T3-L1 adipocytes) induced a significant increase in the transcription and secretion of inflammatory cytokines TNF ($P < 0.05$) and IL-6 ($P < 0.05$), among others^{268–271}. In bronchoalveolar lavage (lung) fluid samples from patients with asthma and allergic airway inflammation, ALOX15 metabolite 15(S)-HETE stimulates pro-inflammatory cytokine expression and airway eosinophilia (characteristic of inflammation)^{262,272,273}. Moreover, results from an in vitro permeability assay in human endothelial cells monolayers (human umbilical vein endothelial cell (HUVEC)) showed that pro-inflammatory eoxins derived from 15(S)-HpETE, the product of PUFA oxidation by ALOX15 in eosinophils and epithelial airway cells, cause endothelial cell dysfunction through enhanced vascular permeability²⁷⁴.

Anti-inflammatory properties of ALOX15 metabolites have also been reported^{275–277}. Indeed, in addition to pro-inflammatory roles, 12(S)-HETE and 15(S)-HETE also exert anti-inflammatory

functions in other cellular contexts. In a rat model of surgically induced transient focal cerebral ischaemia, ALOX15 metabolites were shown to selectively induce transcription and cytoplasmic–nuclear translocation of peroxisome proliferation-activated receptor- γ (PPAR γ), which is required for PPAR γ activation^{278,279}. PPAR γ is a modulator of inflammation that inhibits the expression of pro-inflammatory mediators in diverse conditions including brain ischaemia and peritonitis^{278,279}. Additionally, 15(S)-HpETE, the ALOX15 metabolite of AA, can serve as a major source of substrate for the production of lipoxins, a family of anti-inflammatory mediators produced from PUFAs, following two sequential enzymatic attacks involving ALOX5 and epoxide hydrolase, respectively^{280,281}. Consistent with an anti-inflammatory role of ALOX15, *Alox15* deletion in two models of inflammatory arthritis (humanized *Tnf* transgenic mice or K/BxN serum transfer arthritis mice) induced uncontrolled inflammation and exacerbated joint destruction, enhanced inflammatory gene expression and decreased levels of lipoxin A₄ compared with the *Alox15* wild-type counterpart in both arthritic models²⁸². Other anti-inflammatory and pro-resolving roles for ALOX15 metabolites include the synthesis of lipoxin B₅, resolvins, protectins and 13(S)-hydroxy-9Z,11E-octadecadienoic acid (13(S)-HODE)²⁷⁵. The anti-inflammatory roles of these metabolites have been observed in different models including mouse models of induced brain ischaemia, in which treatment with protectin (10,17S-docosatriene) reduced the activation of the inflammatory transcription factor–DNA binding nuclear factor- κ B (NF- κ B) and decreased mRNA expression of pro-inflammatory enzymes (*Cox1* and *Cox2*)²⁸³; moreover, topical application of lipoxins in mouse ears inhibited hallmarks of inflammation including neutrophil infiltration and vascular permeability²⁸⁴. The contrasting pro-inflammatory and anti-inflammatory roles of ALOX15 metabolites are highlighted in different studies; however, the actions of ALOX15 are increasingly shown to be cell and tissue specific and could, therefore, be linked to the differential abundance of substrates, metabolites and enzymes required for subsequent synthesis reactions.

Metabolic disease. The free fatty acid profile, including PUFA, reflects a person's age, sex and lifestyle; in mouse models, dysregulated fatty acid profiles following

dietary intervention (HFDs) result in a deterioration of the health status, including deterioration of heart function (contractility)²⁸⁵ and permanent damage to sperm quality¹⁰⁰. Unsurprisingly, altered fatty acid composition was shown to have a pathogenic role in type 2 diabetes mellitus and metabolic disease; the exact mechanisms remain unelucidated, but omega-3 and omega-6 free fatty acid competition for desaturation enzymes have been hypothesized to favour pro-inflammatory states, leading to type 2 diabetes mellitus when an unbalanced omega-6:omega-3 ratio in favour of omega-6 fatty acids occurs^{286,287}. ALOX15 is abundantly expressed in the tissues involved in metabolic homeostasis, such as pancreatic islets, adipose tissue and leukocytes^{252,259}. Apolipoprotein E (*ApoE*)-deficient mice spontaneously develop non-alcoholic fatty liver disease (NAFLD) secondary to hyperlipidaemia, and are used as a model for this disease. In a study in which the *ApoE*^{-/-} model was used, *Alox15* was markedly upregulated in the liver of these mice compared with control animals²⁵¹. Moreover, results from a study in which *ApoE*^{-/-} mice were used showed a twofold increase in SOD activity ($P < 0.05$) and increased 4HNE production ($P < 0.05$), collectively considered as increased hepatic oxidative stress, in hypercholesterolaemic *ApoE*^{-/-} mice ($n = 5$) compared with wild-type animals ($n = 5$)²⁸⁸. *Alox15/ApoE* double knockout mice had decreased hepatic steatosis, inflammation and macrophage infiltration, among other features, compared with single knockout *ApoE*^{-/-} mice, indicating attenuated NAFLD²⁵¹. Conversely, increased expression of *Alox15* in adipocytes and adipose tissue was observed in obese Zucker rats, an autosomal-recessive genetic model of metabolic syndrome²⁸⁹, and *Alox15* expression²⁹⁰ and activity²⁹¹ were also upregulated following diet-induced obesity in rodents²⁹¹. The modulation of *Alox15* expression in these contexts might occur through either PPAR γ activity, which is increased under HFD conditions and might induce a damaging feedforward mechanism²⁷⁸, or T_H2 cytokines^{292,293}, although the exact mechanistic details are yet to be elucidated. Overall, ALOX15 and ALOX15 metabolites exacerbate disease states (including NAFLD and inflammation) in NAFLD and obesity preclinical models, which is consistent with observations in biopsy samples from obese patients, in which increased ALOX15 expression and increased downstream cytokine (IL-6 and IL-12a) and chemokine (CXCL10) expression (induced by 12-lipoxygenase and

15-lipoxygenase activity) was observed in visceral fat compared with subcutaneous fat²⁹⁴. Additionally, *Alox15* expression is upregulated in both cell culture and in vivo animal models of diabetes²⁵⁰. Thus, decreased insulin secretion has been observed following treatment of human islet cells with nanomolar amounts of the ALOX15 metabolites 12(S)-HpETE and 12(S)-HETE^{295,296}. *Pdx1-Cre;Alox15* knockout mice, in which *Alox15* expression was specifically ablated in pancreatic islets, were completely protected from dysglycaemia after being fed an HFD ($P = 0.05$), and showed better insulin secretion than littermate control animals ($P < 0.05$)²⁹⁷. These results indicate that *Alox15* expression in pancreatic islets mediates high-fat diet-induced pancreatic dysfunction. Similarly, inactivation of *Alox15* in NOD mice, a spontaneous mouse model of diabetes, reduced the development rate of spontaneous diabetes (2.5%) compared with control animals (>60%) ($n = 40$, $P < 0.0001$)²⁹⁸. Results from other studies also showed that ALOX15 might be a causative agent of diabetic comorbidities including diabetic retinopathy, diabetic peripheral neuropathy and diabetic nephropathy²⁵². Together, this evidence suggests that ALOX15 expression and the abundance of ALOX15 metabolites are closely involved in the aetiology of diabetes^{252,298}, although the exact role of ALOX15 in the onset and progression of diabetes mellitus requires further investigation.

Cardiovascular disease. Lipid radicals have been shown to have a role in the molecular aetiology of cardiovascular disease²⁹⁹, for example, driving endothelial cell dysfunction (considered the initial step in atherosclerotic plaque formation) and exacerbating ischaemia-reperfusion damage²⁹⁹. Specifically, ALOX15 has a role in many conditions of the cardiovascular system, including vasodilation, atherogenesis and the vascularization of tumours (angiogenesis)^{300–305}. The ALOX15 metabolites 11,14,15-trihydroxyeicosatrienoic acids and 11,12,15-trihydroxyeicosatrienoic acids have been reported to elicit vasoactive effects³⁰², inducing artery relaxation in bovines³⁰⁶ and rabbits³⁰¹, in rabbits, artery relaxation induced by AA was inhibited by nordihydroguaiaretic acid, a potent antioxidant and lipoxygenase inhibitor, but not by the CYP450 inhibitor metyrapone, indicating that, in rabbit aorta, AA-induced relaxation is possibly dependent on a lipoxygenase metabolite³⁰¹. In another study, *Alox15* knockout mice were shown to be protected from hypertension

(induced by N^G-nitro-L-arginine-methyl ester (L-NAME) and deoxycorticosterone acetate/high-salt) compared with wild-type animals ($P < 0.01$)³⁰⁷. Interestingly, this effect was not mediated by the synthesis of vasoactive ALOX15 metabolites in mouse arteries (as no ALOX15 expression was detected in mouse aorta), but peritoneal macrophages seem to be the source of ALOX15 in this process. The protective effects of *Alox15* deficiency were lost when mice were injected with wild-type macrophages ($n = 5$, $P < 0.01$); moreover, wild-type mice acquired resistance to L-NAME-induced hypertension after macrophage depletion through clodronate injection ($n = 6–8$, $P < 0.01$)³⁰⁷. Thus, in this study, ALOX15 vasoactive properties are independent of vascular expression of ALOX15, and are conferred from other sources (macrophages and epithelial cells)^{307–309}.

Multiple sclerosis and autoimmune disease.

Multiple sclerosis is an autoimmune, demyelinating disease of the central nervous system resulting in motor and sensory deficits; ROS have been shown to have different roles in the immune system³¹⁰ and, consequently, in the pathogenesis of autoimmune diseases including multiple sclerosis, through saturation of the cell antioxidant capacity and generation of novel autoantigens^{311–313}. In patients with multiple sclerosis ($n = 492$), altered plasma lipid profiles and dyslipidaemia, including high total cholesterol and low-density lipoprotein levels, were shown to be significantly associated with increased disease severity (measured through expanded disability status score and multiple sclerosis status score; $P = 0.001$ and $P = 0.006$, respectively), suggesting a crucial role for lipids in the pathogenesis of this disease²⁵³. In a study in which an experimental mouse model of multiple sclerosis was used, the treatment with baicalein (a lipoxygenase inhibitor acting on ALOX15), attenuated clinical severity (measured through paralysis progression), suppressed phagocytosis and decreased production of pro-inflammatory cytokines and chemokines in the central nervous system³¹⁴, suggesting that the pharmacological inhibition of ALOX15 might have a therapeutic application in human multiple sclerosis³¹⁴. Conversely, *Alox15*-deficient mice experienced a significantly more severe manifestation of multiple sclerosis (paralysis progression) than wild-type animals following induction of experimental allergic encephalomyelitis ($P = 0.007$)³¹⁵. Mechanistically, this response

is hypothesized to be driven by ALOX15 metabolites, which are agonists of PPAR γ and induce PPAR γ anti-inflammatory activity; thus, the absence of these metabolites exacerbates inflammation. However, in the absence of mechanistic follow-up studies, this hypothesis cannot be confirmed, and the possibility of compensatory biochemical pathways cannot be ruled out. Overall, ALOX15 produces lipid ligands responsible for both activation and inhibition of transcription factors such as PPAR β and PPAR γ , and was shown to both protect from and exacerbate multiple sclerosis, although the exact mechanisms remain unelucidated.

With regard to other neurodegenerative diseases, ALOX15 has been implicated in periventricular leukomalacia; indeed, rat premyelinating oligodendrocytes, the oxidative damage of which has been implicated in the pathogenesis of periventricular leukomalacia, were susceptible to AA-stimulated cell death, and this effect was dependent on ALOX15 (REF.³¹⁶). In cerebrospinal fluid of patients with Alzheimer disease ($n = 30$), significantly increased levels of 12(S)-HETE and 15(S)-HETE were reported compared with healthy age-matched and sex-matched individuals ($n = 20$, $P < 0.001$)³¹⁷. ALOX15 activation and 12(S)-HETE were also implicated in the development and progression of Parkinson disease, as pharmacological inhibition (baicalein) or *Alox15* knockout in mice reduced infarct size (measured as localized dead tissue) following ischaemia induction. These results are in contrast with the anti-inflammatory roles of ALOX15 in ischaemia^{278,279}; however, overall, ALOX15 can be considered a feature of autoimmune pathological conditions that is increased at disease sites, exerts both pro-inflammatory and anti-inflammatory effects and propagates protein damage through RCS adduction^{248,252}.

Tumour progression. The molecular basis of tumour metastasis is well established, and inflammation is known to have a role in tumour progression^{318–321}. ALOX15 has been reported to have both tumour-promoting and tumour-suppressing activities³²². Inactivation of the anti-apoptotic enzyme and endogenous ALOX15 inhibitor GPX4 (REF.²⁹²) in 4-hydroxy-tamoxifen (TAM)-inducible *Gpx4* knockout murine embryonic fibroblasts (MEFs) results in rapid cell death^{323,324}. Tumour cells transformed from *Gpx4*-deficient MEFs spontaneously formed viable tumour spheroids in Matrigel, and subcutaneous implantation of transformed *Gpx4* knockout MEFs into C57BL/6 mice

resulted in tumours with the same growth trajectory, weight and volume as tumours from wild-type cells but with increased microvessel density, denoted by increased CD31 expression, and, simultaneously, significantly increased proliferation and apoptosis ($P < 0.05$)³²³. Increased microvessel density and decreased vascular diameter observed in these mice were reversed by the pharmacological inhibition of ALOX15 with baicalein, suggesting that ALOX15 is an important regulator of tumour angiogenesis through the production of 13(S)-HODE, a potent promoter of the pro-angiogenic growth factor basic fibroblast growth factor (bFGF)³²³. Transfection of HCT-116 colon carcinoma cells with *ALOX15* induced pro-neoplastic effects such as extracellular signal-related kinase (ERK) 1 and ERK2 phosphorylation, decreased p21 expression and increased cell proliferation³²⁵. Subsequent ALOX15 inhibition (using nordihydroguaiaretic acid) in *ALOX15*-overexpressing HCT-116 cells impaired ALOX15-driven ERK1 and ERK2 phosphorylation and decreased cell proliferation, indicating that the pro-neoplastic effects observed in colorectal cancer cells are mediated by ALOX15. Following stable and transient *ALOX15* overexpression in other colorectal cancer cell models (HCT-116 and HT-29 cells, respectively), 13(S)-HODE generated from ALOX15 activity inhibited the anti-apoptotic effect of the inflammatory transcription factor NF- κ B through MAPK-mediated activation of PPAR γ , resulting in anti-carcinogenic effects^{326,327}. ALOX15 oxygenates 12C, 13C and 15C in long-chain PUFAs, with species-specific preferences in substrate and target carbon, resulting in situation-specific production of bioactive metabolites³²⁸; 12(S)-HETE, 13(S)-HODE and 15(S)-HETE show pro-neoplastic properties, including angiogenesis promotion³²⁹, whereas 13(S)-HODE exhibits anti-carcinogenic activity and promotes apoptosis^{326,327}. This substrate-product promiscuity could potentially affect the balance between the pro-inflammatory and anti-inflammatory activities of ALOX15 in each species, and might explain the existence of a pro-tumoural and anti-tumoural role of ALOX15 in colorectal cancer models. In the context of the varied role of ALOX15 in tumorigenesis, the typically pro-resolving, anti-inflammatory lipid mediator products of ALOX15 (eicosanoids, lipoxins, resolvins and maresins) have shown anti-carcinogenic activity in inflammation-associated cancers (such as colon and pancreatic cancer)^{330–332}. In a study in which liver

cancer was induced by subcutaneous injection of H22 hepatocarcinoma cells into BALB/c mice, lipoxin A₄ produced by ALOX15 and ALOX5 in regulatory T cells was shown to have pro-carcinogenic activity in early-stage liver cancer progression but anti-carcinogenic activity in late-stage liver cancer progression²⁵⁴. This varied role of ALOX15 metabolites is associated with inflammatory activity and was proposed to be linked to a fine-tuning of inflammation in the tumour microenvironment²⁵⁴.

Overexpression of *ALOX15* has also been reported in human prostate cancer, high-grade prostatic intraepithelial neoplasia, and murine prostate cancer models^{333–335}. In a humanized mouse model in which human *ALOX15* was selectively overexpressed in the prostate of mice, focal and progressive prostatic hyperplasia with nuclear atypia, which is typical of murine prostatic intraepithelial neoplasia, was observed, indicating a role of human ALOX15 in prostate tumour initiation³³⁵. Conversely, results from a study in which BALB/c mice were injected with mammary gland and Lewis lung carcinoma cells showed that overexpression of *ALOX15* under an endothelial cell-specific promoter conferred protection from metastasis through decreased tumour growth, although increased microvessel density and vascularization of tumours was promoted in endothelial cells of *ALOX15*-overexpressing transgenic mice³³⁶. This discrepancy between models might be ascribed to the limitation of endothelial-specific *ALOX15* overexpression in murine models, highlighting the need to investigate the role of ALOX15 in models with global overexpression of ALOX15.

Consensus on the role of ALOX15 in tumour progression still needs to be reached; however, ALOX15 has a pro-angiogenic role across tumour models (colon cancer, mammary gland and Lewis lung carcinoma) and shows pro-tumorigenic activity, promoting both tumour initiation (prostate cancer) and early tumour progression (liver cancer). Conversely, in some models, anticancer effects of ALOX15 activity were reported and were mainly ascribed to the anti-inflammatory activity of the lipid metabolites of ALOX15. Overall, the role of ALOX15 in tumorigenesis is varied, and can be attributed to the tumour microenvironment and the availability of PUFA substrates, which ultimately dictates the products that ALOX15 generates, and modulates the tumorigenic activity of ALOX15.

Linking infertility and disease — directions for future investigations

Multiple lines of evidence suggest that the health status of infertile men is undeniably poor; male infertility diagnosis could be a novel indicator of men's overall somatic health. A complex interplay exists between fertility and overall health, but the underlying linking mechanisms remain elusive. Lipid damage mediated by ALOX15 might provide the first of these links. Lipid damage, RCS production and ROS have a role as both hallmarks and antagonists in the medical comorbidities of male infertility, and in male infertility itself. Similarly, chronic inflammation, which is a common factor among the comorbidities of male infertility, starts with redox imbalances, and can then lead to increased ROS production³³⁷. Ageing and age-related diseases are underpinned by ROS-driven chronic inflammation and redox imbalances, which are exacerbated by an age-related decline in antioxidant capacity, generating further ROS in a self-fulfilling vicious cycle³³⁸. ROS are ubiquitously reported in the pathogenesis of human disease. Historically, antioxidant intervention to restore the redox balance has yielded little therapeutic utility in infertility, indicating the need for new therapeutic targets. Lipid damage as well as RCS and RCS mediators (LOXs, COXs and CYPs) are emerging as biomarkers and core contributors to ROS propagation, and the introduction of improved lipid analysis technologies (such as mass spectrometry) will enable researchers to understand the therapeutic potential of these markers in disease intervention. Lipid damage mediated by ALOX15 might be an unexploited link between loss of fertility and subsequent chronic illness. Similar paradigms have already been identified between other diseases and related comorbidities; for example, increased fatty acid oxidation induced by insulin resistance and consequent increased mitochondrial ROS in arterial endothelium and myocardium are considered a molecular link between diabetes mellitus and comorbid cardiovascular disease³³⁹. ALOX15 is the most well characterized enzymatic contributor to lipid peroxidation in the male germline and, therefore, is an attractive candidate to help understanding why the health status of men with an infertility diagnosis is poorer than that of the general population. Future research on ALOX15 and other lipid modulating enzymes such as COXs, CYPs and acyltransferases will provide empirical evidence of the

contribution of these factors to male infertility, incident comorbidities and the mechanistic link between these diseases. Following the clinical journey of men with a primary diagnosis of male infertility could help elucidate how an infertility diagnosis precipitates into subsequent comorbidities. Additionally, late-life neurodegenerative diseases such as Alzheimer disease and cerebellar ataxia share common aetiologies with male infertility, including RCS propagation and lipid damage, loss of protein homeostasis and aberrant autophagy

processes and, therefore, should be assessed as potential late-life comorbidities in future retrospective and cohort studies.

Conclusions

Male infertility accounts for a substantial proportion of infertility instances worldwide. New evidence indicates a global decline in sperm counts and an increasing incidence of male reproductive system abnormalities; thus, a molecular understanding of sperm development and function, and how these processes are linked to

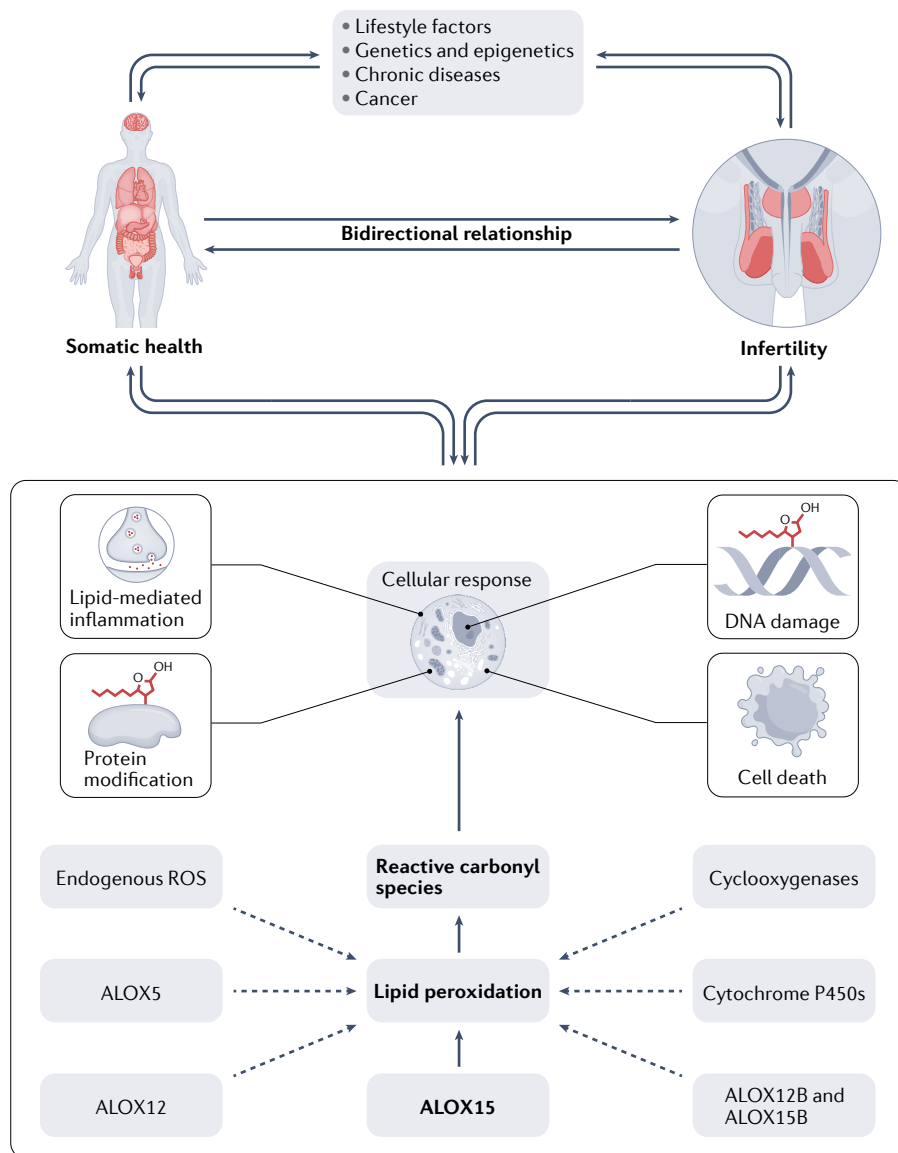


Fig. 4 | Lipid damage as a molecular link between male infertility and somatic health. Somatic health and infertility are underpinned by shared, and often compounding, factors, such as lifestyle, genetics and epigenetics, chronic disease and cancer. Lipid peroxidation might be a potential molecular conduit linking male infertility and somatic health. Lipid peroxidation might have non-enzymatic or enzymatic origins and lipoxygenase enzyme arachidonate 15-lipoxygenase (ALOX15) is the best characterized enzymatic contributor to lipid peroxidation in the male germline. ALOX15 and ALOX15 metabolites have been observed to have roles in numerous comorbidities of male infertility. Specifically, lipid metabolites of ALOX15 have putative roles in DNA damage, protein modification and misfolding, lipid-mediated inflammation and cell death. ROS, reactive oxygen species.

somatic health across life is urgently needed. Considering the unique sensitivity of spermatozoa to endogenous and exogenous oxidative stress, male fertility might be a potential indicator to alert clinicians about patients' risk of future chronic illness.

A connection is being established between the adverse effects of oxidative stress and RCS propagation on germ cells and spermatozoa (through lipid peroxidation), and the role of lipid peroxidation in the pathophysiology of numerous chronic somatic diseases. Lipid oxidation processes might have a role in the undeniably complex, bidirectional relationship between infertility and incident comorbidities; moreover, ALOX15 might warrant further exploration as a biomarker of different comorbidities, and/or a modulating enzyme linking infertility and incident comorbidities (FIG. 4). Additional research into the functions of other lipid-modulating enzymes in disease states and infertility, and as markers of potential future disease in infertile men is required; the characterization of LOXs activity in human spermatozoa provides a starting point for this area of research. In a scenario in which seminal and systemic lipid peroxidation, enzyme activity and the subsequent metabolic footprint could provide a defined measure of comorbidity risk for infertile men, health-care practitioners could provide patients with informed strategies for improving long-term health outcomes. Moreover, focused research in this area is needed to understand why reproductive health tends to deteriorate decades ahead of somatic health, and how this deterioration can be prevented in future generations.

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<https://doi.org/10.1038/s41585-022-00640-y>

Published online 13 September 2022

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Acknowledgements

The authors gratefully acknowledge the research contributions of all laboratories working on the 'canary in the coal mine — male reproductive health' paradigm and M. O'Bryan for bringing this issue to the attention of the Society for Reproductive Biology (SRB), Australia and New Zealand. The authors also acknowledge the work of the Male Reproductive Health Initiative of the European Society of Human Reproduction and Embryology. The authors acknowledge funding from the National Health and Medical Research Council (NHMRC), Australia, awarded to B.N., E.G.B. and R.J.A. (APP1163319).

Author contributions

E.G.B., N.D.B. and R.J.A. researched data for the article. E.G.B., N.D.B., B.N., S.D.R., J.E.S. and J.L.H.W. contributed substantially to discussion of the content. E.G.B. and N.D.B. wrote the article. All authors reviewed and edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Urology thanks M. Kurpisz and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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