Human Reproduction Update, Vol.24, No.1 pp. 86–105, 2018
Advanced Access publication on November 10, 2017 doi:10.1093/humupd/dmx033

human reproduction update

Diabetes-induced hyperglycemia impairs male reproductive function: a systematic review

Constanze C. Maresch [1,2,*], Dina C. Stute[1], Marco G. Alves[3], Pedro F. Oliveira[3,4,5], David M. de Kretser[2], and Thomas Linn[1]

[1]Clinical Research Unit, Centre of Internal Medicine, Justus-Liebig-University, Giessen, Germany [2]Hudson Institute of Medical Research and Department of Anatomy & Developmental Biology, Monash University, Melbourne, Australia [3]Department of Microscopy, Laboratory of Cell Biology, and Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal [4]Department of Genetics, Faculty of Medicine, University of Porto, Porto, Portugal [5]I3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

*Correspondence address: Clinical Research Unit, Centre of Internal Medicine, Justus-Liebig-University, Klinikstrasse 33, Giessen, Germany. Tel: +49-641-99-39751; E-mail: Constanze.C.Maresch@chiru.med.uni-giessen.de orcid.org/0000-0002-4685-535X

Submitted on March 30, 2017; resubmitted on October 10, 2017; editorial decision on October 12, 2017; accepted on October 16, 2017

TABLE OF CONTENTS

BACKGROUND: Hyperglycemia can result from a loss of pancreatic beta-cells or a decline in their function leading to decreased insulin secretion or may arise from insulin resistance and variable degrees of inadequate insulin secretion resulting in diabetes and related comorbidities. To date several reviews have addressed the issue of diabetes-related male infertility but most have focused on how metabolic syndrome causes the decline in male fertility. However, a comprehensive overview as to how diabetes-induced hyperglycemia impairs

male fertility is missing. Impaired regulation of glucose and the resultant hyperglycemia are major threats to the health of individuals in modern societies especially given the rapidly rising prevalence affecting an increasing number of men in their reproductive years. Consequently, diabetes-induced hyperglycemia is likely to contribute to a decline in global birth rates especially in those societies with a high diabetic prevalence.

OBJECTIVE AND RATIONALE: This systematic review addresses and summarizes the impact of hyperglycemia on male reproductive health with a particular emphasis on the molecular mechanisms that influence the testis and other parts of the male reproductive tract.

SEARCH METHODS: A systematic search of the literature published in the MEDLINE-Pubmed database (http://www.ncbi.nlm.nih.gov/pubmed) and Cochrane Library (http://www.cochranelibrary.com) was performed, as well as hand searching reference lists, from the earliest available online indexing year until May 2017, using diabetes- and male fertility-related keywords in combination with other search phrases relevant to the topic of hyperglycemia.

Inclusion criteria were: clinical studies on type 1 diabetic (T1D) men and studies on T1D animal models with a focus on reproductive parameters. Case reports/series, observational studies and clinical trials were included. Studies on patients with type 2 diabetes (T2D) or animal models of T2D were excluded to distinguish hyperglycemia from other metabolic effects.

OUTCOMES: A total of 890 articles were identified of which 197 (32 clinical, 165 animal studies) were selected for qualitative analysis. While the clinical data from men with hyperglycemia-induced reproductive dysfunction were reported in most studies on T1D, the study designs were variable and lacked complete information on patients. Moreover, only a few studies (and mostly animal studies) addressed the underlying mechanisms of how hyperglycemia induces infertility. Potential causes included impaired function of the hypothalamic-pituitary-gonadal axis, increased DNA damage, perturbations in the system of advanced glycation endproducts and their receptor, oxidative stress, increased endoplasmatic reticulum stress, modulation of cellular pathways, impaired mitochondrial function and disrupted sympathetic innervation. However, intervention studies to identify and confirm the pathological mechanisms were missing: data that are essential in understanding these interactions.

WIDER IMPLICATIONS: While the effects of regulating the hyperglycemia by the use of insulin and other modulators of glucose metabolism have been reported, more clinical trials providing high quality evidence and specifically addressing the beneficial effects on male reproduction are required. We conclude that interventions using insulin to restore normoglycemia should be a feasible approach to assess the proposed underlying mechanisms of infertility.

Key words: hyperglycemia / diabetes mellitus type 1 / blood glucose / insulin / male infertility / testis / prostate / epididymis / activins / poly(ADP-ribose) polymerases

Introduction

In 2010, an estimated 48.5 million couples worldwide were infertile with male factor infertility contributing to ~40–50% of these cases, and with as many as 2% of all men exhibiting suboptimal sperm parameters (Mascarenhas *et al.*, 2012; Kumar and Singh, 2015).

Hyperglycemia is characterized by blood glucose levels exceeding normal levels and is diagnosed either by impaired fasting blood glucose levels (IFG) or by impaired glucose tolerance (IGT): IFG is a state with blood glucose levels repeatedly exceeding normal blood glucose concentrations above 7 mmol/l, while IGT is a state with blood glucose levels greater than 11 mmol/l 2 h after a 75 g oral glucose load. IGF and IGT are independent parameters for the diagnosis of diabetes mellitus (DM) and have been used as a diagnostic criterion in recent studies of diabetes as a cause of male factor infertility (World Health Organisation, 2006; Alves *et al.*, 2013a, 2013b). These studies suggested a negative impact of DM on erectile and ejaculation function, as well as a reduction in semen volume, sperm counts, sperm motility and abnormal sperm morphology (Alves *et al.*, 2013b). Given that the global burden of diabetes is constantly increasing with an estimated prevalence of 422 million people in the year 2014, there will be an increasing number of men of reproductive age, as there is a rise in the number of childhood and adolescent males with DM (Silink, 2002; Wild *et al.*, 2004; Agbaje *et al.*, 2007; Guariguata *et al.*, 2014; World Health Organization, 2016). Furthermore, male infertility is now recognized as a sign of male health impairment (Eisenberg *et al.*, 2015; Ventimiglia *et al.*, 2015), particularly when sexual dysfunction is present (Lotti *et al.*, 2016).

Glucose metabolism in health and disease

Under physiological conditions, carbohydrates, mainly consisting of starch or disaccharides such as sucrose and lactose, account for the main source of energy income in the body (Lunt and Vander Heiden, 2011). Through intestinal digestion these carbohydrates are reduced to monosaccharides, such as glucose, as well as low amounts of fructose and lactose, and are resorbed in enterocytes and hepatocytes, where they are metabolized further via various pathways, including glycolysis, the pentose-phosphate-pathway, hexosamine pathway and glycogenesis (Fig. 1) (Zierler, 1999; Hay, 2016). Further pathways for glucose metabolism (Fig. 2), which have also repeatedly been associated with hyperglycemia-induced damage, include the polyol-pathway and the formation of advanced glycation endproducts (AGEs), as well as the activation of protein kinase C (PKC) (Brownlee, 2001). Extensive reviews on glucose metabolic pathways can be found in Hay (2016) or Brownlee (2001).

In order to maintain glucose homeostasis in all cells, glucose uptake is mainly regulated by glucose transporters and glucose regulatory hormones. Glucose transporters comprise sodium-dependent (sodium glucose co-transporter, SGLT) and facilitative glucose transporters

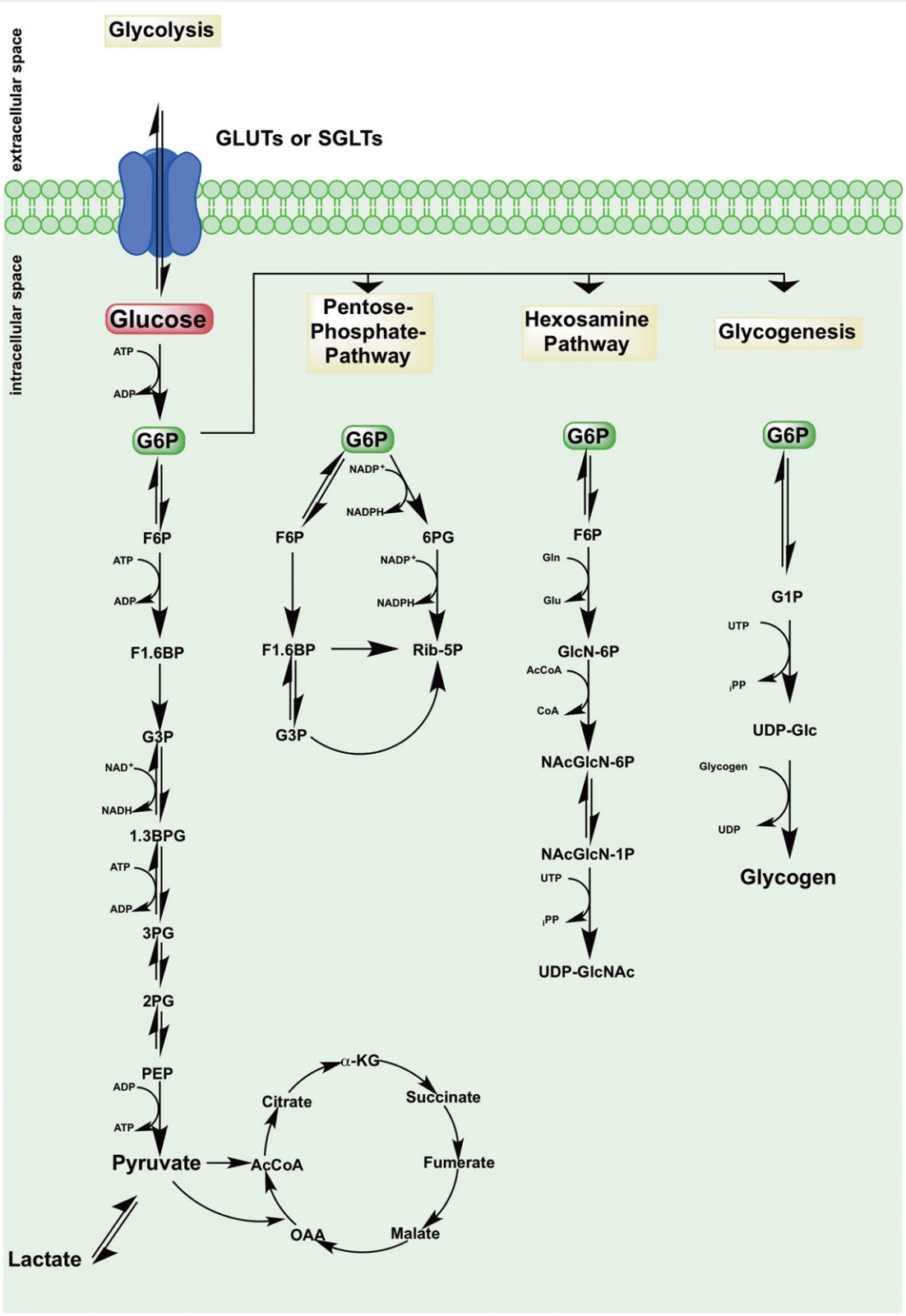

Figure 1 Glucose metabolism pathway under physiological conditions. Glucose enters the intracellular space through receptors GLUT 1–4, to enter into the glycolysis pathway. The first metabolite glucose-6-phosphate (G6P) is metabolized further via the glycolysis pathway or via the branching pentose phosphate, hexosamine pathway or gylcolysis. 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; 6 PG, 6-phosphogluconate; 6PGDH, 6-phosphogluconate dehydrogenase; 6PGL, 6-phosphogluconolactonase; α-KG, α-ketoglutarate; AcCoA, acetyl-CoA; F1,6BP, fructose-1,6-bisphosphate; F2,6BP, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate; G1P, glucose-1-phosphate; G3P, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; GlcN-6 P, glucosamine-6-phosphate; GLUT, glucose transporter; NAcGlcN-1 P, *N*-acetyl d-glucosamine-1-phosphate; NAcGlcN-6 P, *N*-acetyl d-glucosamine-6-phosphate; OAA, oxaloacetate; PPi, inorganic phosphate; Rib-5 P, ribose-5-phosphate; UDP-Glc, UDP-glucose; UDP-GlcNAc, UDP-*N*-acetylglucosamine.

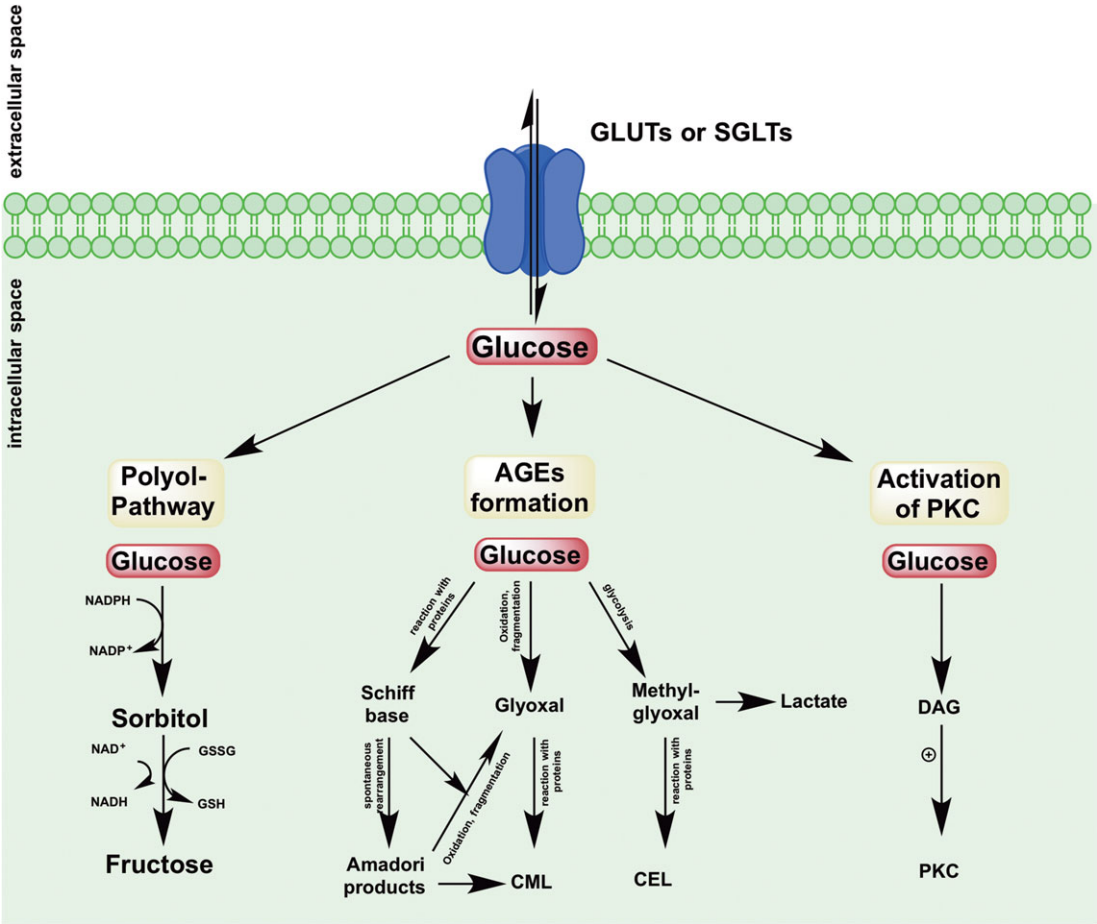

Figure 2 Glucose metabolism pathways that are often associated with hyperglycemia-induced damage. Glucose enters the intracellular space through GLUTs or SGLTs into the polyol-pathway, the AGE-formation pathway or into the activation of PKC. AGEs, advanced glycation endproducts; CEL, N(ε)-(carboxyethyl)lysine; CML, N(ε)-(carboxymethyl)lysine; DAG, diacylglycerol; GLUT, glucose transporter; GSH, reduced glutathione; GSSG, glutathione disulfide; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PKC, protein kinase C; SGLT, sodium-glucose linked transporter.

(GLUTs), which are sodium-independent (reviewed in (Baud *et al.*, 2016; Mueckler and Thorens, 2013)).

Besides the glucose transporters, glucose homeostasis is also maintained by the close interaction of glucose-regulatory hormones, which include insulin, glucagon, amylin, glucagone-like-peptide-1 (GLP-1), glucose-dependent insulinotropic peptide, epinephrine, cortisol and growth hormone (GH) (Aronoff *et al.*, 2004).

Causes of abnormal glucose regulation and associated mechanisms

Glucose dysregulation resulting in hyperglycemia can have several causes, some of which are acquired and others congenital diseases. Acquired disorders of glucose metabolism are hyperglycemia, caused by absolute or relative insulin deficiency; hypoglycemia, due to hyperinsulinism as a consequence of a pancreatic islet cell tumor or the lack of insulin antagonists, such as glucocorticoids, in adrenal insufficiency; carbohydrate malabsorption; and neonatal jaundice caused by a deficiency in glucuronyltransferase activity.

Other than acquired defects in glucose metabolism, congenital glucose disorders mainly affect enzyme functions leading to severe and life-threatening diseases in homozygous carriers. These diseases include galactosemia, fructose intolerance, glycogenosis and inherited hemolytic anemia.

Among these diseases, DM is the disorder that has been studied most extensively in terms of glucose dysregulation thus providing insight into glucose metabolism. Diabetes comprises a group of chronic, metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action (ADA, 2012). Left untreated, diabetes leads to chronic hyperglycemia, which is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels (Chavakis *et al.*, 2002; Schmidt *et al.*, 2009; ADA, 2012; Alves *et al.*, 2013a; Hombrebueno *et al.*, 2014; Lo *et al.*, 2015; Venegas-Pino *et al.*, 2016). Besides these complications, DM has been

discussed in recent years in the context of male reproductive dysfunction since it can lead to erectile and ejaculatory disorders, as well as a reduction in semen volume, sperm counts, sperm motility and abnormal sperm morphology (Alves et al., 2013a). Being one of the most prominent public health threats in modern societies with a rising prevalence, DM leads to an increase in hyperglycemia-related disorders (Li et al., 2015).

Several hypotheses about how hyperglycemia causes diabetic complications at a biochemical level have been described, including increased polyol and hexosamine pathway flux, increased AGE formation and activation of PKC isoforms (Brownlee, 2001).

Testicular glucose metabolism

The maintenance of spermatogenesis requires the production of lactate from glucose by the Sertoli cell. Therefore, blood-to-germ cell transport of glucose and other metabolic intermediates from the basal into the adluminal compartment is highly controlled, particularly owing to the presence of the blood-testis-barrier (BTB) (Riera et al., 2009).

To date expression of Glut1, Glut2, Glut3, Glut8 and recently also Glut4 has been demonstrated in the testis (Ulisse et al., 1992; Burant and Davidson, 1994; Kokk et al., 2004; Carosa et al., 2005; Verma and Haldar, 2016), with Glut1 and Glut3 exhibiting synergistic roles in the maintenance of glucose uptake (Carosa et al., 2005; Galardo et al., 2008). To the best of our knowledge, the presence of sodium dependent glucose transporters within the testis has not been described. Besides the facilitated glucose transport, it has also been reported that glucose uptake and lactate production by Sertoli cells are regulated by various hormones such as FSH and testosterone (Galardo et al., 2008; Oliveira et al., 2012; Rato et al., 2015b). Moreover, it was shown that the insulin family of growth factors, comprising insulin, insulin-like growth factors I (IGF1) and II (IGF2) and insulin receptors (INSR) as well as type-I insulin-like growth factor receptor (INS1R), are crucial for the development and function of the testis (Nakayama et al., 1999; Pitetti et al., 2013). Most interestingly, IGF1 as well as INS1R were shown to be expressed by Sertoli cells, Leydig cells, spermatogonia and spermatocytes. Thus, they are involved in regulation of the energy metabolism of Sertoli cells by regulating the uptake of nucleotides (ATP, GTP, UTP), and the secretion of transferrin, pyruvate, and lactate, suggesting that insulin is actively involved in initiation and maintenance of spermatogenesis (Griswold and Merryweather, 1982; Skinner and Griswold, 1982; Jutte et al., 1983; Borland et al., 1984; MacLean et al., 2013; Griffeth et al., 2014).

Testicular cells have several metabolic features that tightly control the blood-to-germ cell transport of glucose and other metabolic intermediates. Glucose transport is further regulated via hormonal action of the hypothalamic-pituitary-gonadal (HPG) axis and the presence of the BTB formed by specialized cell junctions between Sertoli cells which have the ability to produce lactate at a very high rate (Alves et al., 2013b). Since these unique characteristics of glucose metabolism within testicular cells make them prone to changes under diabetic conditions, several clinical and animal studies have focussed on evaluating the molecular mechanisms responsible for the alterations induced in male reproductive potential. While much remains to be determined, it is likely that both the glucose sensing machinery as well as the hormonal control of these cells work in concert to counteract the effects of hyperglycemia and thus play a crucial role in the prevention of subfertility and/or infertility associated with DM (Sjoberg et al., 2013; Alves et al., 2013b; La Vignera et al., 2015).

Besides the insights into possible mechanisms linking metabolic diseases with male factor subfertility and/or infertility, these data have not distinguished the influence of hyperglycemia itself from other metabolic changes such as obesity or insulin resistance. Hence, the impact of metabolic changes on male reproductive health are often associated with dyslipidemia and subsequent lipid peroxidation, as well as inflammation, conditions that are not necessarily related to hyperglycemia *per se* (Kasturi et al., 2008). Moreover, recent studies have uncovered new mechanisms involved in hyperglycemia-induced male subfertility/infertility.

Therefore, this systematic review validates studies using T1D as the appropriate disorder to examine the impact of hyperglycemia *per se* on male reproductive health. The contribution of selected studies to relevant issues of medical practice is examined. Molecular pathways altered by hyperglycemia resulting from the expected increase in glucose transport into testicular cells are discussed. Potentially harmful mechanisms, including enhancement of the activin family of proteins and reduced activity of poly-ADP-ribose polymerase, are discussed. Finally, future directions for the study of molecular mechanisms responsible for these complications as well as options for the treatment of hyperglycemia-induced male infertility are presented.

Methods

Protocol and registration

The protocol of the present study has been registered (PROSPERO ID number: CRD42017070203) in the PROSPERO registry (http://www.crd.york.ac.uk/PROSPERO), an international database for the prospective registration of systematic reviews in health and social care.

Information sources

We conducted a systematic search of the literature published in the MEDLINE-Pubmed database (http://www.ncbi.nlm.nih.gov/pubmed) and Cochrane Library (http://www.cochranelibrary.com), as well as hand searching reference lists, from the earliest available online indexing year until May 2017, in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Liberati et al., 2009).

We applied the following inclusion filters: Classical Article, Clinical Study, Comparative Study, Congresses Dataset, English Abstract, Evaluation Studies, Introductory Journal Article, Journal Article, Letter, Meta-Analysis, Multicenter Study, Observational Study, Abstract and English. The search strategy used diabetes-related keywords and words related to male fertility: 'Hyperglycemia' OR 'DM, Type 1' OR 'Blood Glucose' OR 'Insulin' OR 'Male Infertility' OR 'Testis' OR 'Prostate' OR 'Epididymis' OR 'Sperm' OR 'Semen' OR 'Activins' OR 'Poly(ADP-ribose) Polymerases' in combination with other search phrases relevant to the topic of hyperglycemia.

Eligibility criteria, search and study selection

The titles and abstracts of all the articles were screened for eligibility by C.C.M. and T.L., who are specialists in male (in)fertility and diabetes. We included original interventional, case-control, cross-sectional and observational prospective and retrospective studies in which T1D was

defined by hyperglycemia (blood glucose > 11.1 mmol/l) in the absence of obesity. The primary outcomes of these studies were semen quality (volume, motility, morphology, sperm count or concentration, sperm DNA damage or chromatin integrity, sperm aneuploidies and hormonal level) or testicular funtion (morphology or spermatogenesis). We excluded studies on T2D patients or animal models, studies that investigated both T1D and T2D but did not distinguish the outcome clearly, and review articles. Once compliance with all the inclusion/exclusion criteria had been verified, the full text of the selected articles was evaluated for the level of evidence of the results.

Data extraction

We extracted the following information from each study: author/s, year of publication, journal, title of the article, location of the study, age, infertility problem, number of patients or animals (sample size), study design, exposure (intervention), primary outcomes and major findings or principal conclusion. Each report was double-checked by C.C.M. and T.L. in terms of feasibility and fulfillment of inclusion criteria.

Quality assessment in clinical trials

We evaluated and scored the quality of the clinical trials using both customized and published grading criteria (Burns *et al.*, 2011). The quality scores were assessed in parallel by C.C.M. and T.L., and discrepancies were re-evaluated together. With this system, we assessed the quality of individual studies using the following grading criteria: (A) Trial in full accordance with the aim of the review, T1D characterized by HbA1c or repeated glucose measurements, duration of diabetes and daily insulin dosage; (B) Trial comparing normal with elevated HbA1c or glucose measurements irrespective of diabetes type, incomplete characterization of T1D; (C) Trial comparing diabetics with non-diabetics without giving HbA1c or glucose, or not giving diabetes type in comprehensible way; (D) Trial not related to aim of the review or only abstract available or with incomplete information on diabetic patients or referenced information which is not available in given source. Evidence levels were attributed as follows: (1) RCTs, prospective, homogeneity of results, interventional; (2) Cohort studies with acceptable quality, number of probands and controls adequate to hypothesis, and reasonable homogeneity of results; (3) Case-control or low-quality cohort study; (4) Case report, cross-sectional study; (5) Expert opinion. For example, studies with scores C5 and D1 were considered of low quality, while studies with A4 to B1 were considered of acceptable quality. Furthermore, risk of bias was evaluated according to the Cochrane Collaboration (http://www.cochrane.org). Three trials were eligible for complete bias analysis, two of which were classified with low and one with high risk of bias.

Results

Study selection

We identified 808 articles after a primary search of MEDLINE-Pubmed and 358 from other sources (Cochrane Library and reviews references) (Fig. 3). By analyzing the titles and abstracts (n = 890), we screened and excluded 598 for reasons of the scope of the study. A total of 292 articles were collected as full texts so that the inclusion/exclusion criteria and quality could be assessed: 81 articles were excluded because they did not meet the inclusion/exclusion criteria, and 14 articles for which no full text was available. After applying all the eligibility parameters, 197 articles were included for qualitative analysis.

Summary of selected studies and design

The articles included 32 clinical and 165 animal studies conducted in 15 and 30 countries, respectively: Argentina, Australia, Brazil, Canada, China, Egypt, Finland, Germany, India, Iran, Israel, Italy, Japan, Cuba, Kuwait, Malaysia, Mexico, The Netherlands, Nigeria, Poland, Portugal, Romania, Russia, Saudi-Arabia, South Africa, South Korea, Spain, Taiwan, Tunisia, Turkey, UK, Ukraine, United Arab Emirates, USA.

General, summarized information on the studies is given in Tables I–III.

Clinical studies were conducted on a total of 5598 T1D individuals with a mean age of 33 ± 11 years and a mean diabetes duration of 12 ± 6 years. There was 1 interventional, 3 cohort and 22 case–control as well as four case report/cross-sectional studies.

Animal studies were conducted on a mean number of 38 ± 21.0 animals per study. The mean diabetes duration was 8 ± 9 weeks. There were 56 observational and 109 intervention studies.

Detailed information on the studies is given in Supplementary Tables 1 and 2.

Hyperglycemia-related male infertility

The notion that hyperglycemia can compromise male fertility has long been described. Physicians in the 11th century already noticed that men with dysregulated glucose metabolism presented problems related to their sexual functions and difficulties in fathering offspring, describing this condition as a “collapse of the sexual functions” of the male. Accordingly, male infertility is now recognized as a sign of male health impairment (Ventimiglia *et al.*, 2015), particularly when sexual dysfunction is present (Lotti *et al.*, 2016).

Clinical studies

Notably, *in vivo* maintenance of spermatogenesis relies on the uptake and use of glucose (Zysk *et al.*, 1975), although its concentration in the tubular fluid is very low (Robinson and Fritz, 1981). For many years, it was suggested that hyperglycemia was a dysfunction associated mostly with ageing, but this concept has changed. It is now assumed that hyperglycemia affects an increasing number of children, adolescents, young adults and men of reproductive age (reviewed in (Alves *et al.*, 2013a, 2013c)). Of these, ~50% of male diabetics presented some degree of subfertility or infertility (La Vignera *et al.*, 2009). The clinical manifestation of a large spectrum of conditions depended on several factors including age, duration of the disease, the frequency and severity of hyperglycemic/hypoglycemic events, and the degree of glucose control. Unfortunately, this has resulted in heterogeneous patient cohorts that make clinical studies difficult to compare. Here, we have identified 32 clinical studies that focused on the effects of hyperglycemia in resemblant interventional, cohort, case–control or cross-sectional setups. Results from these investigations showed that hyperglycemia was associated with a reduced number of offspring (Holstein *et al.*, 2012; Sjoberg *et al.*, 2013; Wiebe *et al.*, 2014), going along with multiple alterations in the function of the reproductive tract of male diabetics. These alterations included non-testicular functions, such as retrograde ejaculation (RE) (Kurbatov *et al.*, 2015; Kam *et al.*, 2017) and erectile dysfunction (Schiavi *et al.*, 1985; Wessells *et al.*, 2011), as well as alterations in sperm parameters and hormone levels.

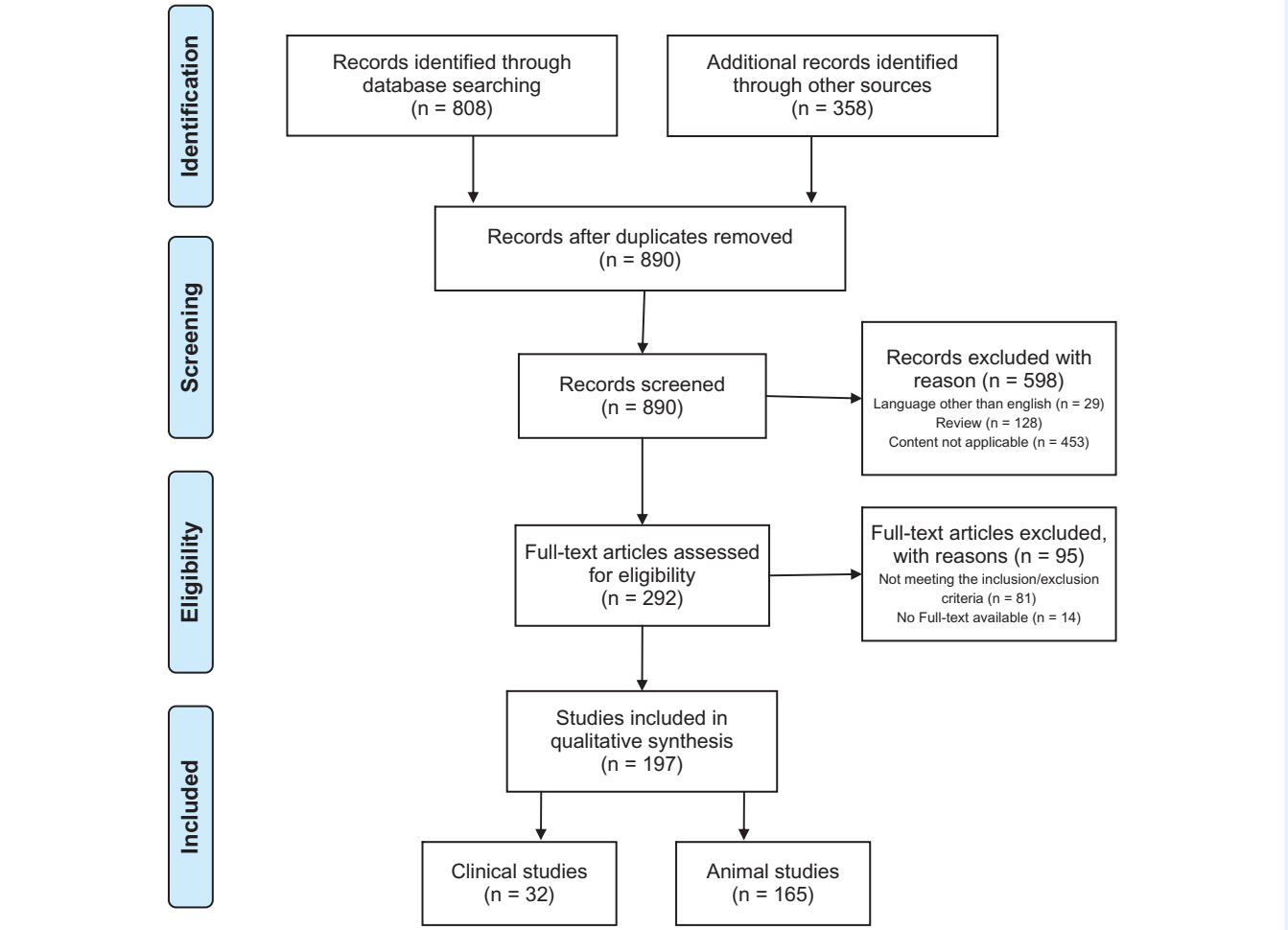

Figure 3 Flow chart of the literature search and selection process.

While health detriments were clearly observed in diabetic men, the effect of hyperglycemia on sperm quality was controversial. Albeit the majority of trials (n = 9) with a total of 132 patients reported a normal sperm count and semen volume (Padron *et al.*, 1984; Murray *et al.*, 1988; Shrivastav *et al.*, 1989; Niven *et al.*, 1995; Agbaje *et al.*, 2008; Mallidis *et al.*, 2009a, 2009b; Paasch *et al.*, 2011; Roessner *et al.*, 2012), only two studies including 34 patients found reductions that occurred in the absence of diabetic complications (Garcia-Diez *et al.*, 1991; Agbaje *et al.*, 2007). Poor glucose control was significantly associated with impairment of sperm motility in 5 studies with 193 patients (Ali *et al.*, 1993a; Niven *et al.*, 1995; Baccetti *et al.*, 2002; Lopez-Alvarenga *et al.*, 2002; La Vignera *et al.*, 2015). In fact, a more detailed study concerning sperm of T1D men showed that only some parameters associated with sperm motility were altered (i.e. linearity and linear index of straightness of swimming), while others remained unchanged (i.e. track speed, path velocity, progressive velocity, among others) (Niven *et al.*, 1995). Further, a series of 3 publications with 100 patients indicated that a history of diabetic neuropathy increased the total number of pathologic outcomes in sperm analysis (Ali *et al.*, 1993a, 1993b, 1993c).

More recently, a proteome analysis of sperm from T1D men found a distinct profile when compared to that of individuals with normal glycemia (Paasch *et al.*, 2011). Another sperm analysis from T1D men showed normal standard analysis parameters, such as sperm count, but a higher level of chromatin damage (Agbaje *et al.*, 2007), which may compromise the fertility potential of those individuals to a larger extent.

The effect of hyperglycemia on hormonal dysregulation was confirmed in some studies (Baccetti *et al.*, 2002; Lopez-Alvarenga *et al.*, 2002; Grossmann *et al.*, 2008; Maric *et al.*, 2010), but not in others (Cohen *et al.*, 1984; Padron *et al.*, 1984; van Dam *et al.*, 2003; Leal *et al.*, 2012; Rocha *et al.*, 2014). A single study with individuals exposed to hyperglycemia displayed an abnormal feedback in the HPG-axis linked to a reduced sensitivity in the pituitary to GnRH stimulation, however, it did not become clear to what extent this contributed to a significant impairment of fertility (Baccetti *et al.*, 2002). Blood levels of testosterone, LH, and FSH were rarely changed in the absence of concomitant diseases. By contrast, poorly controlled T1D in association with characteristic diabetic complications, such as neuropathy or renal glomerulopathy, resulted in consistent

Table I Summary characteristics from human studies on diabetes-induced hyperglycemia and male reproductive function.

Total number of T1D patients (*n*)	5501[a]
Median number of T1D patients (min–max)	23 (1–2819)
Age range (mean ± SD) years	33 ± 12
Type of study (Evidence Level) (n)	
Interventional (Level 1)	2
Cohort (Level 2)	2
Case-control (Level 3)	23
Case report, cross sectional (Level 4)	5
Mean diabetes duration (mean ± SD) years	11 ± 6
Reproduction outcome (no. of studies[b])	
Non-testicular functions	
Retrograde ejaculation	1
Erectile dysfunction	1
PSA reduced with poor control	1
Sperm parameters	
Reduced count/volume	2
Reduced count/volume with neuropathy	1
count/volume	9
Reduced motility with poor glucose control	3
Hormones	
T reduced with neuropathy/ renal disease	3
Rarely or no changes of T, FSH, LH	5
LH reduced with poor glucose control	1
Male fertility	
No. of offspring reduced	3

T1D, type one diabetes, T, testosterone, PSA, prostate-specific antigen.
[a]Each study counts single.
[b]Each study counts multiply, when multiple outcomes were observed.

Table II Evidence-based statements derived from 32 clinical trials on males with T1D compared with non-diabetic control subjects.

Statement	Report with highest evidence level
Erectile dysfunction and T1D	1
Reduced testosterone blood levels and diagnosis of diabetic nephropathy or neuropathy	2
Subfertility and T1D	2
Reduced number of offspring in T1D	2
Reduced sperm count and diagnosis of diabetic neuropathy	3
Reduced sperm motility associated with poor glucose control	3

Table III Summary characteristics from animal studies.

Mean number of animals (n)	38 ± 21.0
Mean diabetes duration (weeks)	8 ± 9.10
Type of study (no. of studies)	
Observational	56
Intervention	109
Insulin	25
Hormone replacement (testosterone, estrogen, oxytocin, etc.)	10
Anti-oxidants (plant extracts, vitamins, etc.)	66
Exercise	1
High energy diet	1
Others	7
Reproduction outcome (no. of studies)	
Reproductive function (general)	28
Testicular disruption	54
Altered sperm parameters	21
Disrupted spermatogenesis	17
Epididymal dysfunction	5
Prostatic dysfunction	19
Hormonal dysregulation	34
Ejaculation dysfunction	3
Erectile dysfunction	2
Apoptosis	14
Seminal vesicle, coagulating gland or penile dysfunction	6

reduction of androgen blood levels (Baccetti *et al.*, 2002; Maric *et al.*, 2010).

Unfortunately, most studies did not take into account the modern medical care of T1D patients, such as novel glucose measuring technologies, self-managed glucose control or flexible concepts of insulin therapy.

Taken together, clinical studies revealed a lack of solid consensus and even some contradictions concerning the effect of T1D and the hyperglycemic state on male fertility. This is caused by a variability of factors, such as the duration of exposure to glucose dysregulation, the age of the individuals, the techniques used to determine semen quality and testicular morphology, dietary habits, lifestyle factors and many others. In addition, the molecular mechanisms by which hyperglycemia may alter testicular functioning are not easy to unveil in humans, partly owing to ethical concerns against testis biopsy. Therefore, the vast majority of the clinical studies lack a molecular approach to investigating the effects of hyperglycemia, glucose transport and metabolism in sperm and testicular cells and, thus, those mechanisms remain largely unknown. Therefore, the use of animal models has been crucial to provide a more detailed molecular insight on the effects of hyperglycemia on the male reproductive system and fertility.

Animal studies

The first studies using diabetic rat models reported a decrease in male fertility after mating with wild-type females (Frenkel *et al.*, 1978). These studies also revealed that male diabetic rodents have

diminished body and reproductive organ weight, lower testicular and epididymal sperm content, and lower sperm motility (Hassan et al., 1993; Soudamani et al., 2005; Scarano et al., 2006). Moreover, the BB Wistar rats, a model that spontaneously develops T1D and thus a good model to study the effects of hyperglycemia through time, showed severe gonadal dysfunction and compromised male fertility (Murray et al., 1981, 1983) with histological studies revealing a reduction in the size of the tubules and an increased epididymal lumen (Soudamani et al., 2005) as hallmarks for hyperglycemia-induced reproductive dysfunction. Sperm changes, particularly decreased concentration and motility, were also described in Goto-Kakizaki (GK) diabetic rats (Amaral et al., 2006). Rats with diabetes induced using the toxin Streptozocin (STZ) presented altered sex behavior, decreased gonadal weight, decreased sperm quantity and sperm motility (Hassan et al., 1993), also highlighting that diabetic individuals present several problems in epididymal function (Soudamani et al., 2005). In fact, it was shown that DM causes a regression of the epididymis, decreasing the absolute weight of caput, corpus and caudal regions, accompanied by a reduction in tubular diameter and a lumen devoid of spermatozoa (Soudamani et al., 2005). Still, STZ-induced hyperglycemic rats sustain overall a fertilizing ability in spite of pathologies of a variable number of sperm parameters (Scarano et al., 2006). Moreover, tight glycemic control was able to restore sperm counts and motility in the STZ-induced diabetic model (Seethalakshmi et al., 1987), as well as the morphology of the epididymis (Soudamani et al., 2005). Although the molecular mechanisms involved were not investigated, these studies illustrate that hyperglycemia, or poor glycemic control, are (at least in part) responsible for the deleterious effects of DM in male reproductive health. Indeed, uncontrolled glycemia was shown to be a major contributor to testicular dysfunction in diabetic male rats (Cameron et al., 1990).

Hyperglycemic rats were also reported to have altered steroidogenesis (Kim and Moley, 2008), exhibiting a decrease in serum testosterone levels and negative outcomes after mating (Tsounapi et al., 2017a). Reduction of testosterone was associated with an enhancement of testicular glycogen synthesis, suggesting that hyperglycemia induces a metabolic and hormonal reprogramming in the testis, partly through decreased enzyme activity of phosphofructokinase 1 and lactate dehydrogenase (Rato et al., 2015a). DM is reported to increase cellular glucose uptake and to reduce the production of lactate by testicular cells, while also increasing the levels of cholesterol, non-esterified fatty acids, triglycerides and phospholipids in rat testicular tissues (Sharaf et al., 1978). The biochemical responses of Sertoli and peritubular cells exposed to diabetic glucose levels showed that their metabolic machinery was indeed modulated by glucose (Hutson, 1984). In addition, hyperglycemia also induces severe alterations in lipid metabolism, particularly the hydrolysis of triglycerides, fatty acyl esters of cholesterol, and steroid hormones, and studies have proved that a normal lipid metabolism is essential for normal spermatogenesis (Chung et al., 2001). High-energy diets inducing mild hyperglycemia were reported to compromise male fertility (reviewed in (Rato et al., 2014a)). It was recently shown that mild hyperglycemia in prediabetic rats results in increased abnormal sperm morphology (Rato et al., 2013).

The oxidative balance of the male reproductive tract has also been studied and it was shown that hyperglycemia induces oxidative stress (OS) and initiates apoptosis, among other deleterious effects (Amaral et al., 2008). Indeed, it is known that hyperglycemia induces cellular OS due to excessive reactive oxygen species (ROS) production and decreased antioxidant defences (Tabak et al., 2011). The production of ROS has severe deleterious effects on sperm quality and function (for review see (Aitken et al., 2016)). In addition, high levels of ROS are detrimental to fertility potential in natural and assisted conception (Agarwal et al., 2003). Hyperglycemia-induced superoxide overproduction inhibits, among others, glyceraldehyde-3-phosphate dehydrogenase, PKC and aldose reductase in endothelial cells (Nishikawa et al., 2000), which results in lower antioxidant defences, overproduction of ROS and mitochondrial dysfunction. Mitochondrial biogenesis is also sensitive to ROS production (Yu et al., 2006) and hyperglycemia inhibits the testicular antioxidant capacity in mitochondria (Palmeira et al., 2001). Mild hyperglycemia decreases mitochondrial DNA content, testicular mitochondrial complex III and adenylate energy charge, which were reported to be followed by a repression of respiratory capacity and increased OS (Rato et al., 2014b). This effect was suggested to be modulated by peroxisome proliferator-activated receptor γ coactivator 1 α and Sirtuin 3 (Rato et al., 2014b), which may be essential to control the hyperglycemia-mediated effects on testis.

In STZ-induced hyperglycemic rats, an upregulation of the pro-apoptotic factors Bax, Bad and c-Jun N-terminal kinases was also reported, which was correlated with an increase in germ cell death (Koh, 2007a, 2007b). OS markers were also increased in the epididymis of prediabetic rats, illustrating that sperm are exposed to ROS during storage, even in mild hyperglycemic conditions (Dias et al., 2016). In addition, sperm cells are highly susceptible to ROS since their cell membranes are rich in polyunsaturated fatty acids (Aitken et al., 2012) and apoptosis is known to be mediated by OS and thus exerts an effect in sperm (Grunewald et al., 2008; Aitken and Koppers, 2011). Indeed, hyperglycemia-mediated ROS are suggested to be a main mechanism by which DM affects male reproductive health (Roessner et al., 2012) and high levels of ROS were detected in the ejaculates of diabetic men (Roessner et al., 2012). STZ-induced hyperglycemia promotes impaired sperm parameters, with an increase of total oxidant levels accompanied by a decrease in antioxidant levels (Kilarkaje et al., 2014), illustrating that oxidative unbalance is a key hallmark for hyperglycemia in testis. This is supported by multiple studies showing that intervention using antioxidative agents was able to rescue hyperglycemia-induced oxidative damage in the male reproductive tract (Khaneshi et al., 2013; Sebai et al., 2015; Tsounapi et al., 2017a,b; Shi et al., 2017; Yigitturk et al., 2017). Summarized findings from these investigations are presented in Table III, while Table IV provides an overview on the observed reproductive dysfunction and postulated mechanisms.

Glucose receptor expression

Even though sodium dependent and facilitative glucose transporters are the main transport mechanisms for glucose to pass into the intracellular space, surprisingly little research has been done on the regulation of these transporters in the context of hyperglycemia-induced male infertility. Kim and Moley (2008) have described the presence of Scl2a8, the gene encoding Glut8, in the intra-cellular compartment within the testis, as well as in the midpiece, principal piece, and the acrosomal region of sperm of diabetic mice, with testicular expression being reduced compared to wild-type controls. This goes

Table IV Animal studies on the impact of hyperglycemia on reproductive function and postulated mechanisms.

Observed reproductive dysfunction and postulated mechanisms	Detailed evidence	References
Sexual disturbance (in terms of libido, potency, fertility, sexual motivation, number of offspring)	• Reduced fertility • Lower prolificacy • Reduced libido • Low sexual motivation and mating activity • Decrease intracavernous pressure • Lower fertilization rates • Reduced embryo development rates • Ejaculatory dysfunction • Reduced reproductive organ weight	Ballester *et al.* (2004), De Young *et al.* (2004), Scarano *et al.* (2006), Kim and Moley (2008), Yonezawa *et al.* (2009), Pontes *et al.* (2011), Bondarenko *et al.* (2012), Schoeller *et al.* (2012)
Disrupted seminiferous tubular morphology (i.e. disruption in spermatogenesis, spermatogenic arrest, reduced tubular diameter, reduced tubular lumen, germinal arrest)	• Changes in spermatogenetic epithelium • Changes in type I collagen amino acid content • Spermatogonial germ cells are unable to mature into spermatocytes and spermatids • Decreased mean seminiferous tubule diameter and mean testicular biopsy score • Decreased germinal epithelium height • Increase apoptosis rate • Reduced proliferating cell nuclear antigen index • Germ cell depletion • Disrupted occludin distribution pattern	Soudamani *et al.* (2005), Amaral *et al.* (2009), Gomez *et al.* (2009), Ricci *et al.* (2009), Navarro-Casado *et al.* (2010), Aktas *et al.* (2011), Mohasseb *et al.* (2011), Arikave *et al.* (2012), Bondarenko *et al.* (2012), Kianifard *et al.* (2012), Schoeller *et al.* (2012, 2014), Trindade *et al.* (2013), Donmez *et al.* (2014), Shayakhmetova *et al.* (2014)
Disruption of the testicular interstitial area (i.e. loss of interstitial tissue, vacuolization)	• Decrease in number and function of Leydig cells • Edema in interstitial tissue • Hypertrophic interstitial compartment	Ballester *et al.* (2004), Chandrashekar and Muralidhara (2009), Ricci *et al.* (2009), Mohasseb *et al.* (2011), Kianifard *et al.* (2012), Trindade *et al.* (2013)
Impaired function of the HPG-axis (i.e. changes in testosterone, LH, FSH, prolactin, GnRH and leptin levels)	• Reduced serum levels of FSH, LH and testosterone • Reduced pituitary LH levels • Reduced prolactin and growth hormone serum levels	Hutson *et al.* (1983), Benitez and Perez Diaz (1985), Seethalakshmi *et al.* (1987), Ballester *et al.* (2004), Aybek *et al.* (2008), Ribeiro *et al.* (2008), Favaro *et al.* (2009), Burul-Bozkurt *et al.* (2010), Mohasseb *et al.* (2011), Pontes *et al.* (2011), Porto *et al.* (2011), Bose *et al.* (2012), Kianifard *et al.* (2012), Schoeller *et al.* (2012, 2014), Trindade *et al.* (2013)
Pertubations in the AGE/RAGE axis	• Increase in RAGE protein in sperm and seminal plasma • Increased levels of CML in sperm	Mallidis *et al.* (2007a), O'Neill *et al.* (2010)
Oxidative stress	• Increase in neuronal NO synthase and urine nitrite and nitrate concentration • Elevated hydroperoxide, malondialdehyde and caspase-3 activity • Reduced superoxide dismutase and glutathione peroxidase enzymatic activities • Reduced manganese superoxide dismutase • Reduced activity of aldehyde dehydrogenase, tricarboxylic acid cycle enzymes • Enhanced activities of oxidative phosphorylation enzymes • Perturbations in calcium homeostasis and membrane potential • Cyp2e1 mRNA over-expression in testes	De Young *et al.* (2004), Shrilatha and Muralidhara (2007), Aybek *et al.* (2008), Chandrashekar and Muralidhara (2009), Gumieniczek and Wilk (2009), Ricci *et al.* (2009), Burul-Bozkurt *et al.* (2010), Mohasseb *et al.* (2011), Zhao *et al.* (2011), Shayakhmetova *et al.* (2014)
Significantly affected sperm parameters (i.e. volume, motility, morphology)	• Reduced sperm production • Lower sperm concentration • Reduced motility • Increase in sperm head detachments • poorer CASA parameters • Reduced seminal vesicle fluid • Increase in teratozoospermia	Scarano *et al.* (2006), Shrilatha and Muralidhara (2007), Kim and Moley (2008), Singh *et al.* (2009), Navarro-Casado *et al.* (2010), Schoeller *et al.* (2012), Mangoli *et al.* (2013)

Continued

Table IV *Continued*

Observed reproductive dysfunction and postulated mechanisms	Detailed evidence	References
Increased ER-Stress	• Increase in Chop expression and caspase-12 activation	Zhao *et al.* (2011)
Increased DNA damage (sperm nDNA, mtDNA fragmentation)	• Affected sperm DNA integrity and DNA ploidy • Increased nDNA damage • Reduced DNA integrity	Shrilatha and Muralidhara (2007), O'Neill *et al.* (2010), Bose *et al.* (2012), Mangoli *et al.* (2013)
Modulation of cellular pathways (i.e. cyclic AMP, ERK/JNK, GLUT, MAPK)	• Changed expression of Glut-3 hexose, transporter, c-kit, Igf-I, androgen receptors, tyrosine phosphorylation in Leydig cells • Changed expression of c-kit, Igf-I, insulin, and Fsh receptors in the seminiferous tubules • Reduced testicular expression of Slc2a8 and Slc2a9b • Reduced 3β-hydroxysteroid dehydrogenase expression in Leydig cells • Decreased activity of total (t)-ERK and phosphor (p)-ERK immunoreactivities • Increased p-JNK immunoreactivity • Decrease in testicular Zn level • Increased Bax/Bcl-2 ratio • Increase in activation of p38 MAPK and p53 protein	Benitez and Perez Diaz (1985), Ballester *et al.* (2004), Mallidis *et al.* (2007b), Kim and Moley (2008), Gomez *et al.* (2009), Zhao *et al.* (2011); Donmez *et al.* (2014)
Impaired mitochondrial function	• Decreased calcium load • Hyperplasia	Ribeiro *et al.* (2008); Chandrashekar and Muralidhara (2009); Zhao *et al.* (2011); Kianifard *et al.* (2012); Schoeller *et al.* (2012)
Disturbances in the lower reproductive tract (epididymis, prostate, seminal vesicle)	• Reduced seminal vesicle weight • Decrease in the absolute weight of caput, corpus, and cauda epididymis • Reduction in the size of the tubule and lumen of the epididymal segments with an increase in interstitial stroma • principal cells packed tightly with clumping of nuclei • Epididymal lumen devoid of spermatozoa • Decreased prostatic weight • Reduced epithelial cell proliferation in all lobes • Diminished prostatic acid phosphatase • Reduction in androgen-binding protein, sialic acid, glycerylphosphoryl choline, and carnitine • Defective sperm maturation	Seethalakshmi *et al.* (1987), Soudamani *et al.* (2005), Morrison *et al.* (2006), Scarano *et al.* (2006), Ribeiro *et al.* (2008), Favaro *et al.* (2009), Singh *et al.* (2009), Yonezawa *et al.* (2009), Navarro-Casado *et al.* (2010), O'Neill *et al.* (2010), Pontes *et al.* (2011), Porto *et al.* (2011)
Disrupted sympathetic innervation	• Increased uptake of tritiated noradrenaline which decreased with age • Increased release of tritiated noradrenaline in response to various stimuli	Morrison *et al.* (2006)

AGE, advanced glycation endproduct; Bax, bcl-2 associated X protein; Bcl-2, B-cell lymphoma 2; CASA, computer-assisted semen analysis; Chop, C/EBP homologous protein; c-kit, tyrosine-protein kinase Kit; CML, N(ε)-(carboxymethyl)lysine; Cyp2e1, Cytochrome P450 2E1; ERK, extracellular signal–regulated kinases; Glut, glucose transporter; GnRH, gonadotropin-releasing hormone; HPG, hypothalamic-pituitary-gonadal; Igf-I, insulin-like growth factor 1; JNK, c-Jun N-terminal kinases; MAPK, mitogen-activated protein kinase; NO, nitric oxide; RAGE, receptor for AGEs; Scl2a, solute carrier family 2a.

along with a significant reduction in Scl2a8 mRNA. In contrast, a change of Glut8 due to hyperglycemia was not confirmed in rat testis (Gomez *et al.*, 2009). Further, Slc2a9, the gene encoding Glut9, was not present within the testis, while it was localized in the midpiece and acrosomal region of sperm, with a significant reduction in type I diabetic mice (Kim and Moley, 2008).

Activin signaling

Activins and the antagonistic inhibins are both members of the transforming-growth-factor β (TGF-β) superfamily and were first described based on their ability to regulate FSH synthesis and secretion by the anterior pituitary (Dias *et al.*, 2009; Barakat *et al.*, 2012). Both are highly conserved across different species, and critical for germ and Sertoli cell development as well as for adult testis function.

Members of the TGF-β superfamily are known to signal through the formation of a multimeric receptor complex formed of two distinct receptor subunit classes (Barakat *et al.*, 2012; Loveland and Hedger, 2015; Young *et al.*, 2015), further enabling the phosphorylation of downstream signaling molecules such as regulatory Smads (R-Smads) 2 and 3. Being phosphorylated, these Smads are able to bind to the common Smad4 in order to build a complex, which can

translocate into the nucleus to affect gene transcription (Barakat *et al.*, 2012; Loveland and Hedger, 2015; Young *et al.*, 2015).

Both activins and inhibins have been well described for their role in the regulation of reproduction, and have also been shown to function in both a paracrine and autocrine manner in each organ of the hypothalamic-pituitary-testis axis. Activin is known to stimulate GnRH release from the hypothalamus and FSH release from the pituitary. It can further increase the number of GnRH receptors on the surface of gonadotrophes and augment the GnRH-mediated transcriptional activation of GnRH receptors in order to enhance the pituitary response to GnRH (Barakat *et al.*, 2012). In the testis itself, the local production of activin and the control of its signaling pathway are critical for germ and Sertoli cell development as well as for adult testis function (Barakat *et al.*, 2012). The role of the activins in reproductive biology has been extensively reviewed recently (Wijayarathna and de Kretser, 2016).

Little is known of the actions of the activin family of proteins with regard to hyperglycemia. Several studies have proposed an important role for certain members of the TGF-β superfamily in the pathogenesis of diabetes by maintaining β-cell function under physiological conditions. Specifically, activins A and B, as well as their binding protein follistatin, have been shown to be involved in the regulation of glucose metabolism and inflammation (Szabat *et al.*, 2010; Andersen *et al.*, 2011; Hashimoto and Funaba, 2011; Chen *et al.*, 2013; Ofstad *et al.*, 2013; Wu *et al.*, 2013). Moreover, since gene expression of the activin βB subunit, as well as that of the canonical activin receptors ALK4, ActRIIA and ActRIIB, has been demonstrated in the pancreatic bud in mice (Dichmann *et al.*, 2003), and the activin βA subunit was localized in α, β and δ cells of the pancreatic islet (Ogawa *et al.*, 1993; Yasuda *et al.*, 1993), the involvement of activins in the development and onset of diabetes seems likely. Hashimoto and Funaba reported that activins A and B are regulators of the differentiation and activation of pancreatic β-cells and that they are, moreover, involved in controlling the response of insulin target cells (Hashimoto and Funaba, 2011). Additionally, they have been proposed to modulate events involved in insulin sensitivity in a tissue-dependent manner and, therefore, regulate whole-body insulin responsiveness (Hashimoto and Funaba, 2011). Hence, activin A is capable of reducing target tissue sensitivity to insulin, while activin B was shown to enhance insulin gene transcription (Hashimoto and Funaba, 2011; Wu *et al.*, 2012). This is further supported by the finding that activin enhances glucose stimulated insulin secretion, and augments the expression of genes characteristic of mature β-cells, such as insulin, solute carrier family 2 (Scl2a2 or Glut2) and Pc2, which suggests that activin is actively involved in β-cell differentiation.

In this context, recent work from our group (Maresch *et al.*, 2017) suggests that hyperglycemia leads to testicular disruption accompanied by an upregulation of testicular activin A levels and, in turn, to a dysregulation of the downstream signaling. We have found that under severe hyperglycemic conditions, the activin type II receptors are significantly downregulated followed by a specific upregulation of pSmad3 and Smad4. These findings further support a significant role for the activins in hyperglycemia-induced changes in male reproductive biology.

Hyperglycemia and epididymal function

While the testis is regarded as an immune privileged organ owing to the presence of the BTB, cells within the epididymis are more susceptible to metabolic induced damage. The epididymis is composed of the caput, mid-piece and cauda and is important for the maturation and motility of spermatozoa, the latter increasing during epididymal transit. As an androgen-dependent organ, it is influenced by testicular testosterone secretion and obviously is dependent on the testis for the production of sperm and thus remains under the direct and indirect influence of testicular pathologies. To date, only a few studies have investigated epididymal pathologies in the context of hyperglycemia-induced changes, showing that high levels of circulating blood glucose affect the histologic architecture of the epididymal duct, specifically resulting in a reduced germ cell population, loss of stereocilia, clumping of epithelial cells and lipid vacuolization along with inflammatory infiltrations, exfoliated cells and round spermatids with cribriform changes (La Vignera *et al.*, 2015; Korejo *et al.*, 2016). These changes may, in part, be related to OS (Mallidis *et al.*, 2007a; Maremanda *et al.*, 2016) and subsequent hypozincemia (Maremanda *et al.*, 2016). Hypozincemia is a common comorbidity in diabetic patients and was recently investigated in the context of diabetes-induced male reproductive dysfunction. Results from this study show that diabetic rats had reduced zinc contents in serum, testis, and sperm with a subsequent increase in OS. Oral zinc supplementation was able to attenuate these changes (Maremanda *et al.*, 2016).

Hyperglycemia in further parts of the reproductive tract

In the context of male reproductive dysfunction, the majority of studies have investigated changes in testicular morphology and spermatogenesis. Studies investigating hyperglycemic changes in the distal parts of the reproductive tract, such as the efferent ducts, seminal vesicles or the prostate are rare. Hyperglycemic changes within the prostate were previously related to changes in metabolic status in the onset of benign prostatic hyperplasia, possibly leading to subsequent sperm abnormalities (Vignozzi *et al.*, 2014). Studies examining hyperglycemia-induced prostatic changes have shown that this condition interferes with prostate growth and leads to atrophic changes in the prostate gland (Ribeiro *et al.*, 2008; Favaro *et al.*, 2009; Porto *et al.*, 2011). These effects were suggested to be zone dependent because of differences in lobe response in diabetic rodents (Favaro *et al.*, 2009; Porto *et al.*, 2011). Histological examination revealed that hyperglycemia was related to atrophy in prostatic secretory epithelial cells, stromal hypertrophy, an increase in inflammatory cells, and prostatic intraepithelial neoplasia, as well as dilated organelles in the secretory process (Cagnon *et al.*, 2000; Carvalho *et al.*, 2003; Ribeiro *et al.*, 2008). Recent studies showed further that hyperglycemia in diabetic men was inversely related to serum levels of prostate-specific antigen (PSA). This was found to be independent of age, BMI, androgen levels, and medication use as well as the level of hyperglycemia, suggesting a direct effect of hyperglycemia on PSA levels (Ozbek *et al.*, 2014; Sarma *et al.*, 2015).

Besides effects on the prostate, hyperglycemia resulted in a reduction in seminal vesicle weight in diabetic rodents (Hutson *et al.*, 1983; Schoeller *et al.*, 2012) and a higher fundus-to-body antero-posterior diameter ratio with a lower pre- and post-ejaculatory difference in diabetic men (La Vignera *et al.*, 2013). This might be related to changes in the sympathetic innervation of the seminal vesicle (Morrison *et al.*, 2006) or an increase in OS (Tsounapi *et al.*, 2017a).

It has been known since 1963 that RE occured commonly in men with diabetes, probably contributing to infertility (Greene et al., 1963; Bourne et al., 1971; Fedder et al., 2013; Kam et al., 2017). Thus, diabetics were found to present more frequently with RE compared to their age-matched controls. While this observation could not be associated with BMI, waist circumference, blood pressure, HbA1c, high-density lipoprotein cholesterol, triglycerides, fasting glucose or s-testosterone, it seemed to progress with the duration of the disease (Fedder et al., 2013). Changes in the sympathetic innervation of the seminal vesicle, possibly in the context of diabetic autonomic neuropathy, are thought to be causative for this pathology (Morrison et al., 2006; Fedder et al., 2013).

Discussion

T1D, associated with hyperglycemia, is one of the most prominent public health threats in modern societies with a rapidly rising prevalence. This is accompanied by a rise in the number of childhood and adolescent males with T1D, affecting an increasing number of men of reproductive age (Guariguata et al., 2014).

Up to the present time, a large number of studies have described a negative impact of DM on male reproductive function leading to degenerative changes within the testis and epididymis, erectile and ejaculatory dysfunction, as well as a reduction in semen volume, sperm counts, sperm motility and altered sperm morphology. However, these data have not discriminated the influence of hyperglycemia itself from other metabolic traits such as obesity or insulin resistance. Hence, the impact of metabolic changes on male reproductive health are often associated with dyslipidemia and inflammation, conditions that are not always related to hyperglycemia *per se* (Kasturi et al., 2008).

Therefore, the present review of clinical and animal studies provides the most comprehensive analysis to date of the associations between hyperglycemia and male factor infertility.

A systematic search yielded a series of case–control studies suggesting that uncomplicated T1D was not associated with the hallmarks of male infertility or hypogonadism. However, poorly controlled blood glucose in conjunction with late stage diabetic complications reduced both sperm counts and testosterone blood levels. Intervention studies with T1D patients, informing on testicular mechanisms and applicable for healthcare practice guidelines, were hardly identified.

Observed changes in animal models included testicular disruption, spermatogenic dysfunction, increased germ-cell apoptosis, hormonal dysregulation and altered sperm parameters, as well as a dysfunction in distal reproductive organs. While the analyzed clinical studies rarely investigated the underlying mechanisms of the observed pathologies, data from animal studies suggested that impaired function of the HPG-axis, increased DNA damage, perturbations in the AGE/RAGE system, OS, increased ER-stress, modulation of cellular pathways, impaired mitochondrial function and disrupted sympathetic innervation were reported causes.

Future treatment aspects in the protection from diabetes-induced sperm failure

Insulin

Testicular effects of local insulin expression. Small amounts of insulin are secreted from Sertoli cells in an autocrine-paracrine manner (Perrard-Sapori et al., 1987; Griffeth et al., 2014), with a recent study suggesting that the insulin might also be released into the blood stream under the regulation of the HPG-axis (Schoeller et al., 2012). Moreover, there is evidence that insulin can regulate human sperm fertilization capacity through regulation of glucose metabolic pathways during sperm acrosome formation and capacitation (Aquila et al., 2005).

The regulation of insulin levels directly impacts on the expression of INSR, as well as IGF1R, leading to the activation of downstream signaling pathways involved in proliferation, differentiation, metabolism and cell survival (Avruch, 1998; Taniguchi et al., 2006). Thus, upregulation of insulin results in the downregulation of Insr and Igf1r, and vice versa (Friedman et al., 1997; Franko et al., 2012; Vigneri et al., 2016). A recent report suggests that the lack of Insr and Igr1r in Sertoli cells results in a 75% reduction in testis size and daily sperm production (Pitetti et al., 2013). However, there is no clear evidence if blood insulin levels, either high or low, will affect fertility in the same way as testicular insulin synthesized by Sertoli cells.

Treatment for hyperglycemia. A progression of diabetic vascular complications despite reversal of hyperglycemia was observed in the EDIC (Epidemiology of Diabetes and Complications) study (Diabetes et al., 2015). This durable effect of prior hyperglycemia on the progression of body-wide vasculopathy was defined as metabolic memory. Enhanced glycation reactions, OS, and induction of the innate immune system were shown to facilitate the phenomenon. On the other hand, metabolic memory is also operative in persistent salutary effects of a period of intensive insulin therapy after differences in glycemia between the original intensive and conventional treatment cohorts have disappeared (Writing Team for the Diabetes, Complications Trial/Epidemiology of Diabetes, and Complications Research, 2002). In a series of identically designed large controlled trials, intensive insulin treatment aimed at near-normal blood glucose levels, while conventional treatment was performed to deflect severe metabolic disturbances, such as ketoacidosis and weight loss. Apart from empowering the diabetic patient to organize treatment, another essential precondition of an intensive insulin regimen is its implementation early in the course of diabetes. If such intervention is delayed or near-normal glycemia is not attained, the momentum of complications is harder to slow down. Metabolic memory is now accepted as part of the pathogenesis of retinopathy and other micro- and macrovascular complications of diabetes. However, until now its role in testicular pathogenesis has been poorly investigated, with studies in ruminants suggesting that, depending on its availability, glucose impacts on the reproductive axis via insulin and leptin secretion, which in turn regulate GnRH output (Blache et al., 2006).

As there are no established guidelines of medical care in diabetes-induced infertility, other hormonal approaches with GH and hCG were used in medical practice (Moss et al., 2013). GH was shown to stimulate IGF1 formation, improving sperm maturation in a paracrine-autocrine manner, while hCG triggers Leydig cell production of testosterone. Clomiphene and aromatase inhibitors appeared not to negatively impact sperm and were even shown to be beneficial in the treatment of male infertility, but were not tested for the treatment of hyperglycemia (Chua et al., 2013). Further, exogenous testosterone and anabolic steroids obviously had detrimental effects as they suppressed the HPG-axis. Hence, none of these options can be recommended in conditions of hyperglycemia at present owing to the lack

of quality research data. By contrast, insulin efficacy in handling hyperglycemia is profoundly supported by human studies and safe in routine clinical use. As such, a well-conducted trial of up-to-date insulin treatment, taking metabolic memory effects into account and examining male fertility as primary objective, will be most warranted.

Oral hypoglycemic agents

Besides insulin as the major glucose regulatory agent, oral hypoglycemic agents have been discussed in the control of hyperglycemia and subsequent male reproductive dysfunction. As such, metformin, known for its ability to suppress gluconeogenesis in the liver, was previously shown to improve semen characteristic in patients with metabolic syndrome owing to a reduction in insulin resistance, sex hormone-binding globulin levels, as well as an increase in serum androgen levels (Morgante *et al.*, 2011). This is further supported by evidence from an animal study in rats showing that metformin is able to abrogate not only the decrease in testis weight, but also the decrease in number of spermatogonia, Sertoli cells and Leydig cells (Yan *et al.*, 2015). *In vitro* experiments from our group on metformin-treated Sertoli cells further supported the benificial effects of metformin as it provides nutritional support by increasing lactate production and has an anti-apoptotic effect on developing germ cells (Alves *et al.*, 2014).

Research on further oral hypoglycemic agents, such as glitazones, alpha-glucosidase or SGLT2 inhibitors, GLP-1 analogs, or sulphonylureas, in the context of male reproductive health remains scarce. Pioglitazone was shown to improve erectile function in rats, however, to the best of our knowledge there are no studies on diabetic animals or patients to date (Aliperti *et al.*, 2014). Moreover, as seminal alpha-glucosidase has been used as a clinical marker of epididymal function (Cooper *et al.*, 1990), the use of alpha-glucosidase inhibitors in the treatment of diabetes-induced reproductive dysfunction is indicated, but has not been adapted in controlled studies so far.

Even though some oral hypoglycemic agents were shown to improve male reproductive potential, results have to be taken with caution in terms of hyperglycemia-induced male reproductive dysfunction as these drugs were approved as anti-diabetic agents in the treatment of T2D, which often occurs in the context of the metabolic syndrome and, thus, other comorbidities. Therefore, these drugs would need a thorough re-examination in the context of male reproductive dysfunction.

Follistatin

Recent evidence on the underlying mechanisms of hyperglycemia-induced male reproductive dysfunction identified a dysregulation within the activin family of proteins as possible target for future treatments (Maresch *et al.*, 2017). In light of this finding, follistatin, a key regulator of the biological actions of the activins, should be evaluated as a therapeutic agent. Originally described as FSH suppressing Protein (Robertson *et al.*, 1987; Ueno *et al.*, 1987), follistatin has the capacity to bind to activins A and B with high affinity, negatively regulating their bioactivity (Nakamura *et al.*, 1991). This ability of follistatin was previously used to counteract the actions of the activins in the treatment of diseases and pathologies that are associated with activin upregulation. Hence, subcutaneous follistatin treatment attenuated the radiation-induced fibrotic response in irradiated mice (Forrester *et al.*, 2017), while follistatin gene therapy proved to be useful in the treatment of inclusion body myositis in men (Mendell *et al.*, 2017). In mouse models of cancer cachexia, overexpression of follistatin via subcutaneous vector injection ameliorated the cachectic effects of activin A (Chen *et al.*, 2014).

To date, there are no therapeutic attempts to target the regulation of activin A in reproductive disorders. Therefore, advancements in this area would definitely be beneficial to the field of reproductive pathology. In this respect, the finding that testicular activin A signaling is disrupted in type 1 diabetic males makes follistatin an interesting target for the investigation within therapeutic purposes.

Poly-ADP-ribose-polymerase inhibitors

While erectile dysfunction is an example of a common disorder clearly linked to macrovascular pathology, impairment of spermatogenesis may be extrapolated from direct biochemical consequences of excessive glucose, as delineated before.

The process of spermatogenesis is characterized by the transient occurrence of physiological DNA strand breaks caused by the transition from histone- to protamine-based chromatin. Notably, these processes involve the formation of poly(ADP-ribose) (PAR) and thereby induce DNA damage signaling (Meyer-Ficca *et al.*, 2011). Generated through the binding of PAR polymerases 1 and 2 (PARP1 and PARP2, respectively) to single strand DNA breaks, poly(ADP-ribosyl)ation is known to be involved in multiple cellular processes including DNA repair, genomic stability, and programmed cell death under physiological conditions (Kiss and Szabo, 2005). Under pathophysiological conditions, however, activation of PARP leads to cellular dysfunction and necrotic cell death, due to a reduction in the rate of glycolysis and mitochondrial respiration (Kiss and Szabo, 2005). In this context, several studies in diabetic subjects have identified an increase in PARP related to oxidative and nitrosative stress, as well as genomic instability (Kiss and Szabo, 2005; Horvath *et al.*, 2009). Investigations in the male reproductive tract further show an upregulation of PARP within the testis of diabetic rats (Abdelali *et al.*, 2016). This occurs in parallel with an increase in diabetes-induced DNA damage in the reproductive tract, as described previously.

Inhibition of PARP was used in numerous studies and effectively ameliorated muscular activity in ischemia reperfusion in diabetic rats (Long *et al.*, 2013), and PARP activity in the blood of T2D patients (Shrikhande *et al.*, 2006). However, to date there is only one study that investigated the effects of PARP inhibitors on the testis, showing no effects in T1D rats (Abdelali *et al.*, 2016). Still, searching for non-glucose lowering agents will be desirable as there will be treatments needed in case of delayed or failing implementation of insulin treatment.

Limitations and strengths

Some limitations of our study should be acknowledged. Although broad search terms were used, and reference lists were hand searched, possibly not all publications have been identified. In addition, our search strategy was limited to Pubmed and Cochrane databases, and not EMBASE or other databases. However, the main scientific journals are indexed in Pubmed database. Higher evidence levels were scarce in clinical trials even following assessment of bias sources and quality scores by two authors independently. The review of basic research studies showed that they opened new pathways of

research on mechanisms involved in the effects of hyperglycemia, but altogether were far from conclusive and left many issues in humans unanswered.

Notwithstanding these limitations, this review is the most up to date and exhaustive review of studies on the impact of hyperglycemia on male reproductive function carried out in accordance with the PRISMA guidelines and a quality-validated, registered protocol. Future studies along the indicated lines are needed to confirm our conclusions.

Conclusions

Hyperglycemia is a major health concern with a growing incidence, affecting an increasing number of men in their reproductive years. While clinical outcomes of hyperglycemia-induced male reproductive dysfunction were covered in studies on T1D, the designs were variable and important patient information was not available. Moreover, only a few studies addressed the underlying mechanisms in the context of hyperglycemia and disrupted fertility, suggesting impaired function of the HPG-axis, increased DNA damage, perturbations in the AGE/RAGE system, OS, increased ER-Stress, modulation of cellular pathways, impaired mitochondrial function and disrupted sympathetic innervation as possible causes (Fig. 4). However, mechanistic studies identifying the pathological details in a conclusive way, were missing and would be essential for the understanding of these interactions.

Given that the fact that insulin is the significant factor in terms of glucose regulation, interventions using insulin to regain normoglycemia should be adapted to prove the suggested underlying mechanisms. Furthermore, new treatment options, such as follistatin or poly-ADP-polymerase inhibition, should be investigated when activin dysregulation or DNA damage were identified as playing a role.

In the future, hyperglycemia should be investigated in more detail on a molecular basis in order to fully understand the impact on male reproductive health.

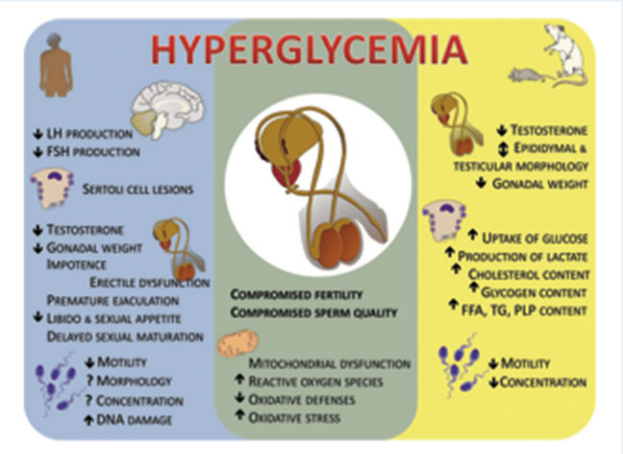

Figure 4 Effects of hyperglycemia on male reproductive tract and reproductive health. The increased glycemia levels impair testicular function by disrupting both steroidogenesis and spermatogenesis. Furthermore, hyperglycemia also impairs male reproductive function, lowering sperm reproductive parameters owing to increased OS. FFA, free fatty acids; PLP, phospholipids; TG, triglycerides; Blue box - clinical studies; Yellow box - animal studies; Green box - intersectional studies; Bidirectional arrows, alteration; Down arrows, decrease; Up arrows, increase;?, non-consensual alteration.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Acknowledgements

The authors would like to thank Dr Peter Stanton for extensively reviewing the manuscript and providing expert advice.

Authors' roles

T.L. is the principal investigator and the corresponding guarantor of research of this document. C.C.M. and T.L. conceptualized the idea of investigating hyperglycemia on male reproduction. C.C.M., D.C.S., M.G.A., P.F.O. and T.L. performed the systematic data base search and drafted the manuscript. D.M.de.K., P.O. and T.L. reviewed the complete manuscript and provided critical comments.

Funding

The State of Hessia (LOEWE-MIBI, T.L.), the Bundesministerium für Bildung und Forschung (BMBF 01DL13015, T.L.), the Deutsche Forschungsgemeinschaft (DFG, 353/17-1, T.L.), the DFG as part of an International Research Training Group project (1871/8), by a National Health and Medical Research Council of Australia (NHMRC) Project Grant (1063843; to D.M.de.K.), by the Victorian Government's Operational Infrastructure Support Program, and by the "Fundação para a Ciência e a Tecnologia"–FCT co-funded by Fundo Europeu de Desenvolvimento Regional—FEDER via Programa Operacional Factores de Competitividade—COMPETE/QREN to Unit for Multidisciplinary Research in Biomedicine (UMIB), Projectos de IC&DT Estratégicos e de Interesse Público (PEst-OE/SAU/UI0215/2014); P.F.O. (SFRH/BPD/108837/2015; PTDC/BBB-BQB/1368/2014) and M.G.A. (SFRH/BPD/80451/2011; PTDC/BIM-MET/4712/2014). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Abdelali A, Al-Bader M, Kilarkaje N. Effects of Trans-Resveratrol on hyperglycemia-induced abnormal spermatogenesis, DNA damage and alterations in poly (ADP-ribose) polymerase signaling in rat testis. *Toxicol Appl Pharmacol* 2016;**311**:61–73.

ADA. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2012;**35**:S64–S71.

Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Ferti Steril* 2003;**79**:829–843.

Agbaje IM, McVicar CM, Schock BC, McClure N, Atkinson AB, Rogers D, Lewis SE. Increased concentrations of the oxidative DNA adduct 7,8-dihydro-8-oxo-2-deoxyguanosine in the germ-line of men with type 1 diabetes. *Reprod Biomed Online* 2008;**16**:401–409.

Agbaje IM, Rogers DA, McVicar CM, McClure N, Atkinson AB, Mallidis C, Lewis SE. Insulin dependant diabetes mellitus: implications for male reproductive function. *Hum Reprod* 2007;**22**:1871–1877.

Aitken RJ, Gibb Z, Baker MA, Drevet J, Gharagozloo P. Causes and consequences of oxidative stress in spermatozoa. *Reprod Fertil Dev* 2016;**28**:1–10.

Aitken RJ, Jones KT, Robertson SA. Reactive oxygen species and sperm function—in Sickness and in Health. *J Androl* 2012;**33**:1096–1106.

Aitken RJ, Koppers AJ. Apoptosis and DNA damage in human spermatozoa. *Asian J Androl* 2011;**13**:36–42.

Aktas CK, Kanter M, Erboga M, Timurkan H. Effects of experimental diabetes on testis proliferations and apoptosis in rats. *J Exp Clin Med* 2011;**28**:94–98.

Ali ST, Shaikh RN, Ashfaqsiddiqi N, Siddiqi PQ. Serum and urinary levels of pituitary–gonadal hormones in insulin-dependent and non-insulin-dependent diabetic males with and without neuropathy. *Arch Androl* 1993a;**30**:117–123.

Ali ST, Shaikh RN, Siddiqi NA, Siddiqi PQ. Comparative studies of the induction of erectile response to film and fantasy in diabetic men with and without neuropathy. *Arch Androl* 1993b;**30**:137–145.

Ali ST, Shaikh RN, Siddiqi NA, Siddiqi PQ. Semen analysis in insulin-dependent/non-insulin-dependent diabetic men with/without neuropathy. *Arch Androl* 1993c;**30**:47–54.

Aliperti LA, Lasker GF, Hagan SS, Hellstrom JA, Gokce A, Trost LW, Kadowitz PJ, Sikka SC, Hellstrom WJ. Efficacy of pioglitazone on erectile function recovery in a rat model of cavernous nerve injury. *Urology* 2014;**84**:1122–1127.

Alves MG, Martins AD, Cavaco JE, Socorro S, Oliveira PF. Diabetes, insulin-mediated glucose metabolism and Sertoli/blood-testis barrier function. *Tissue Barriers* 2013a;**1**:e23992.

Alves MG, Martins AD, Rato L, Moreira PI, Socorro S, Oliveira PF. Molecular mechanisms beyond glucose transport in diabetes-related male infertility. *Biochim Biophys Acta* 2013b;**1832**:626–635.

Alves MG, Martins AD, Vaz CV, Correia S, Moreira PI, Oliveira PF, Socorro S. Metformin and male reproduction: effects on Sertoli cell metabolism. *Br J Pharmacol* 2014;**171**:1033–1042.

Alves MG, Rato L, Carvalho RA, Moreira PI, Socorro S, Oliveira PF. Hormonal control of Sertoli cell metabolism regulates spermatogenesis. *Cell Mol Life Sci* 2013c;**70**:777–793.

Amaral S, Moreno AJ, Santos MS, Seica R, Ramalho-Santos J. Effects of hyperglycemia on sperm and testicular cells of Goto-Kakizaki and streptozotocin-treated rat models for diabetes. *Theriogenology* 2006;**66**:2056–2067.

Amaral S, Mota PC, Lacerda B, Alves M, Pereira Mde L, Oliveira PJ, Ramalho-Santos J. Testicular mitochondrial alterations in untreated streptozotocin-induced diabetic rats. *Mitochondrion* 2009;**9**:41–50.

Amaral S, Oliveira PJ, Ramalho-Santos J. Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive oxygen species. *Curr Diabetes Rev* 2008;**4**:46–54.

Andersen GO, Ueland T, Knudsen EC, Scholz H, Yndestad A, Sahraoui A, Smith C, Lekva T, Otterdal K, Halvorsen B *et al*. Activin A levels are associated with abnormal glucose regulation in patients with myocardial infarction: potential counteracting effects of activin A on inflammation. *Diabetes* 2011;**60**:1544–1551.

Aquila S, Gentile M, Middea E, Catalano S, Ando S. Autocrine regulation of insulin secretion in human ejaculated spermatozoa. *Endocrinology* 2005;**146**:552–557.

Arikave A, Daramola AO, Udenze IC, Akinwolere FM, Olatunki-Bello II, Obika LF. Comparison of streptozotocin-induced diabetic and insulin resistant effects on spermatogenesis with proliferating cell nuclear antigen (PCNA) immunostaining of adult rat testis. *J Exp Clin Med* 2012;**29**:209–214.

Aronoff SL, Berkowitz K, Shreiner B, Want L. Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. *Diabetes Spectrum* 2004;**17**:183–190.

Avruch J. Insulin signal transduction through protein kinase cascades. *Mol Cell Biochem* 1998;**182**:31–48.

Aybek H, Aybek Z, Rota S, Sen N, Akbulut M. The effects of diabetes mellitus, age, and vitamin E on testicular oxidative stress. *Fertil Steril* 2008;**90**:755–760.

Baccetti B, La Marca A, Piomboni P, Capitani S, Bruni E, Petraglia F, De Leo V. Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. *Hum Reprod* 2002;**17**:2673–2677.

Ballester J, Munoz MC, Dominguez J, Rigau T, Guinovart JJ, Rodriguez-Gil JE. Insulin-dependent diabetes affects testicular function by FSH- and LH-linked mechanisms. *J Androl* 2004;**25**:706–719.

Barakat B, Itman C, Mendis SH, Loveland KL. Activins and inhibins in mammalian testis development: new models, new insights. *Mol Cell Endocrinol* 2012;**359**:66–77.

Baud G, Raverdy V, Bonner C, Daoudi M, Caiazzo R, Pattou F. Sodium glucose transport modulation in type 2 diabetes and gastric bypass surgery. *Surg Obes Relat Dis* 2016;**12**:1206–1212.

Benitez A, Perez Diaz J. Effect of streptozotocin-diabetes and insulin treatment on regulation of Leydig cell function in the rat. *Horm Metab Res* 1985;**17**:5–7.

Blache D, Zhang S, Martin GB. Dynamic and integrative aspects of the regulation of reproduction by metabolic status in male sheep. *Reprod Nutr Dev* 2006;**46**:379–390.

Bondarenko LB, Shayakhmetova GM, Matvienko AV, Kovalenko, VM. Diabetes-mediated changes in rat type i collagen and spermatogenesis indices. *Rom J Diabetes Nutr Metab Dis* 2012;**19**:245–254. doi:10.2478/v10255-012-0030-4.

Borland K, Mita M, Oppenheimer CL, Blinderman LA, Massague J, Hall PF, Czech MP. The actions of insulin-like growth factors I and II on cultured Sertoli cells. *Endocrinology* 1984;**114**:240–246.

Bose R, Adiga SK, D'Souza F, Salian SR, Uppangala S, Kalthur G, Jain N, Radhakrishnan RA, Bhat N, Krishnamurthy H *et al*. Germ cell abnormalities in streptozotocin induced diabetic mice do not correlate with blood glucose level. *J Assist Reprod Genet* 2012;**29**:1405–1413.

Bourne RB, Kretzschmar WA, Esser JH. Successful artificial insemination in a diabetic with retrograde ejaculation. *Fertil Steril* 1971;**22**:275–277.

Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;**414**:813–820.

Burant CF, Davidson NO. GLUT3 glucose transporter isoform in rat testis: localization, effect of diabetes mellitus, and comparison to human testis. *Am J Physiol* 1994;**267**:R1488–R1495.

Burns PB, Rohrich RJ, Chung KC. The levels of evidence and their role in evidence-based medicine. *Plastic and Reconstructive Surgery* 2011;**128**:305–310.

Burul-Bozkurt N, Pekiner C, Kelicen P. Diabetes alters aromatase enzyme levels in gonadal tissues of rats. *Naunyn Schmiedebergs Arch Pharmacol* 2010;**382**:33–41.

Cagnon VH, Camargo AM, Rosa RM, Fabiani R, Padovani CR, Martinez FE. Ultrastructural study of the ventral lobe of the prostate of mice with streptozotocin induced diabetes (C57BL/6J). *Tissue Cell* 2000;**32**:275–283.

Cameron DF, Rountree J, Schultz RE, Repetta D, Murray FT. Sustained hyperglycemia results in testicular dysfunction and reduced fertility potential in BBWOR diabetic rats. *Am J Physiol* 1990;**259**:E881–E889.

Carosa E, Radico C, Giansante N, Rossi S, D'Adamo F, Di Stasi SM, Lenzi A, Jannini EA. Ontogenetic profile and thyroid hormone regulation of type-1 and type-8 glucose transporters in rat Sertoli cells. *Int J Androl* 2005;**28**:99–106.

Carvalho CA, Camargo AM, Cagnon VH, Padovani CR. Effects of experimental diabetes on the structure and ultrastructure of the coagulating gland of C57BL/6J and NOD mice. *Anat Rec A Discov Mol Cell Evol Biol* 2003;**270**:129–136.

Chandrashekar KN, Muralidhara. Evidence of oxidative stress and mitochondrial dysfunctions in the testis of prepubertal diabetic rats. *Int J Impot Res* 2009;**21**:198–206.

Chavakis E, Riecke B, Lin J, Linn T, Bretzel RG, Preissner KT, Brownlee M, Hammes HP. Kinetics of integrin expression in the mouse model of proliferative retinopathy and success of secondary intervention with cyclic RGD peptides. *Diabetologia* 2002;**45**:262–267.

Chen JL, Walton KL, Winbanks CE, Murphy KT, Thomson RE, Makanji Y, Qian H, Lynch GS, Harrison CA, Gregorevic P. Elevated expression of activins promotes muscle wasting and cachexia. *FASEB J* 2014;**28**:1711–1723.

Chen WJ, Greulich S, van der Meer RW, Rijzewijk LJ, Lamb HJ, de Roos A, Smit JW, Romijn JA, Ruige JB, Lammertsma AA *et al*. Activin A is associated with impaired myocardial glucose metabolism and left ventricular remodeling in patients with uncomplicated type 2 diabetes. *Cardiovasc Diabetol* 2013;**12**:150.

Chua ME, Escusa KG, Luna S, Tapia LC, Dofitas B, Morales M. Revisiting oestrogen antagonists (clomiphene or tamoxifen) as medical empiric therapy for idiopathic male infertility: a meta-analysis. *Andrology* 2013;**1**:749–757.

Chung S, Wang SP, Pan L, Mitchell G, Trasler J, Hermo L. Infertility and testicular defects in hormone-sensitive lipase-deficient mice. *Endocrinology* 2001;**142**:4272–4281.

Cohen HN, Paterson KR, Wallace AM, Beastall GH, Manderson WG, MacCuish AC. Dissociation of adrenarche and gonadarche in diabetes mellitus. *Clin Endocrinol (Oxf)* 1984;**20**:717–724.

Cooper TG, Yeung CH, Nashan D, Jockenhovel F, Nieschlag E. Improvement in the assessment of human epididymal function by the use of inhibitors in the assay of alpha-glucosidase in seminal plasma. *Int J Androl* 1990;**13**:297–305.

De Young L, Yu D, Bateman RM, Brock GB. Oxidative stress and antioxidant therapy: their impact in diabetes-associated erectile dysfunction. *J Androl* 2004;**25**: 830–836.

Diabetes C, Complications Trial /Epidemiology of Diabetes I, Complications Research G, Lachin JM, White NH, Hainsworth DP, Sun W, Cleary PA, Nathan DM. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. *Diabetes* 2015;**64**:631–642.

Dias TR, Alves MG, Rato L, Casal S, Silva BM, Oliveira PF. White tea intake prevents prediabetes-induced metabolic dysfunctions in testis and epididymis preserving sperm quality. *J Nutr Biochem* 2016;**37**:83–93.

Dias VL, Rajpert-De Meyts E, McLachlan R, Loveland KL. Analysis of activin/TGFB-signaling modulators within the normal and dysfunctional adult human testis reveals evidence of altered signaling capacity in a subset of seminomas. *Reproduction* 2009;**138**:801–811.

Dichmann DS, Miller CP, Jensen J, Scott Heller R, Serup P. Expression and misexpression of members of the FGF and TGFbeta families of growth factors in the developing mouse pancreas. *Dev Dyn* 2003;**226**:663–674.

Donmez YB, Kizilay G, Topcu-Tarladacalisir Y. MAPK immunoreactivity in streptozotocin-induced diabetic rat testis. *Acta Cir Bras* 2014;**29**:644–650.

Eisenberg ML, Chen Z, Ye A, Buck Louis GM. Relationship between physical occupational exposures and health on semen quality: data from the Longitudinal Investigation of Fertility and the Environment (LIFE) Study. *Fertil Steril* 2015;**103**: 1271–1277.

Favaro WJ, Padovani CR, Cagnon VH. Ultrastructural and proliferative features of the ventral lobe of the prostate in non-obese diabetic mice (NOD) following androgen and estrogen replacement associated to insulin therapy. *Tissue Cell* 2009;**41**:119–132.

Fedder J, Kaspersen MD, Brandslund I, Hojgaard A. Retrograde ejaculation and sexual dysfunction in men with diabetes mellitus: a prospective, controlled study. *Andrology* 2013;**1**:602–606.

Forrester HB, de Kretser DM, Leong T, Hagekyriakou J, Sprung CN. Follistatin attenuates radiation-induced fibrosis in a murine model. *PLoS One* 2017;**12**: e0173788.

Franko A, von Kleist-Retzow JC, Bose M, Sanchez-Lasheras C, Brodesser S, Krut O, Kunz WS, Wiedermann D, Hoehn M, Stohr O *et al*. Complete failure of insulin-transmitted signaling, but not obesity-induced insulin resistance, impairs respiratory chain function in muscle. *J Mol Med (Berl)* 2012;**90**:1145–1160.

Frenkel GP, Homonnai ZT, Drasnin N, Sofer A, Kaplan R, Kraicer PF. Fertility of the streptozotocin-diabetic male rat. *Andrologia* 1978;**10**:127–136.

Friedman JE, Ishizuka T, Liu S, Farrell CJ, Bedol D, Koletsky RJ, Kaung HL, Ernsberger P. Reduced insulin receptor signaling in the obese spontaneously hypertensive Koletsky rat. *Am J Physiol* 1997;**273**:E1014–E1023.

Galardo MN, Riera MF, Pellizzari EH, Chemes HE, Venara MC, Cigorraga SB, Meroni SB. Regulation of expression of Sertoli cell glucose transporters 1 and 3 by FSH, IL1 beta, and bFGF at two different time-points in pubertal development. *Cell Tissue Res* 2008;**334**:295–304.

Garcia-Diez LC, Corrales Hernandez JJ, Hernandez-Diaz J, Pedraz MJ, Miralles JM. Semen characteristics and diabetes mellitus: significance of insulin in male infertility. *Arch Androl* 1991;**26**:119–128.

Gomez O, Ballester B, Romero A, Arnal E, Almansa I, Miranda M, Mesonero JE, Terrado J. Expression and regulation of insulin and the glucose transporter GLUT8 in the testes of diabetic rats. *Horm Metab Res* 2009;**41**:343–349.

Greene LF, Kelalis PP, Weeks RE. Retrograde Ejaculation of Semen Due to Diabetic Neuropathy; Report of 4 Cases. *Fertil Steril* 1963;**14**:617–625.

Griffeth RJ, Bianda V, Nef S. The emerging role of insulin-like growth factors in testis development and function. *Basic Clin Androl* 2014;**24**:12.

Griswold MD, Merryweather J. Insulin stimulates the incorporation of 32Pi into ribonucleic acid in cultured sertoli cells. *Endocrinology* 1982;**111**:661–667.

Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, Macisaac RJ, Clarke S, Zajac JD, Jerums G. Low testosterone levels are common and associated with insulin resistance in men with diabetes. *J Clin Endocrinol Metab* 2008;**93**:1834–1840.

Grunewald S, Said TM, Paasch U, Glander HJ, Agarwal A. Relationship between sperm apoptosis signalling and oocyte penetration capacity. *Int J Androl* 2008;**31**: 325–330.

Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 2014;**103**:137–149.

Gumieniczek A, Wilk M. Nitrosative stress and glutathione redox system in four different tissues of alloxan-induced hyperglycemic animals. *Toxicol Mech Methods* 2009;**19**:302–307.

Hashimoto O, Funaba M. Activin in glucose metabolism. *Vitam Horm* 2011;**85**:217–234.

Hassan AA, Hassouna MM, Taketo T, Gagnon C, Elhilali MM. The effect of diabetes on sexual behavior and reproductive tract function in male rats. *J Urol* 1993;**149**:148–154.

Hay N. Reprogramming glucose metabolism in cancer: can it be exploited for cancer therapy? *Nat Rev Cancer* 2016;**16**:635–649.

Holstein A, Patzer O, Tiemann T, Vortherms J, Kovacs P. Number and sex ratio of children and impact of parental diabetes in individuals with Type 1 diabetes. *Diabet Med* 2012;**29**:1268–1271.

Hombrebueno JR, Chen M, Penalva RG, Xu H. Loss of synaptic connectivity, particularly in second order neurons is a key feature of diabetic retinal neuropathy in the Ins2Akita mouse. *PLoS One* 2014;**9**:e97970.

Horvath EM, Magenheim R, Kugler E, Vacz G, Szigethy A, Levardi F, Kollai M, Szabo C, Lacza Z. Nitrative stress and poly(ADP-ribose) polymerase activation in healthy and gestational diabetic pregnancies. *Diabetologia* 2009;**52**: 1935–1943.

Hutson JC. Altered biochemical responses by rat Sertoli cells and peritubular cells cultured under simulated diabetic conditions. *Diab tologia* 1984;**26**:155–158.

Hutson JC, Stocco DM, Campbell GT, Wagoner J. Sertoli cell function in diabetic, insulin-treated diabetic, and semi-starved rats. *Diabetes* 1983;**32**:112–116.

Jutte NH, Jansen R, Grootegoed JA, Rommerts FF, van der Molen HJ. FSH stimulation of the production of pyruvate and lactate by rat Sertoli cells may be involved in hormonal regulation of spermatogenesis. *J Reprod Fertil* 1983;**68**:219–226.

Kam J, Tsang VH, Chalasani V. Retrograde ejaculation: a rare presenting symptom of type 1 diabetes mellitus. *Urol Case Rep* 2017;**10**:9–10.

Kasturi SS, Tannir J, Brannigan RE. The metabolic syndrome and male infertility. *J Androl* 2008;**29**:251–259.

Khaneshi F, Nasrolahi O, Azizi S, Nejati V. Sesame effects on testicular damage in streptozotocin-induced diabetes rats. *Avicenna J Phytomed* 2013;**3**:347–355.

Kianifard D, Sadrkhanlou RA, Hasanzadeh S. The ultrastructural changes of the sertoli and leydig cells following streptozotocin induced diabetes. *Iran J Basic Med Sci* 2012;**15**:623–635.

Kilarkaje N, Al-Hussaini H, Al-Bader MM. Diabetes-induced DNA damage and apoptosis are associated with poly (ADP ribose) polymerase 1 inhibition in the rat testis. *Eur J Pharmacol* 2014;**737**:29–40.

Kim ST, Moley KH. Paternal effect on embryo quality in diabetic mice is related to poor sperm quality and associated with decreased glucose transporter expression. *Reproduction* 2008;**136**:313–322.

Kiss L, Szabo C. The pathogenesis of diabetic complications: the role of DNA injury and poly(ADP-ribose) polymerase activation in peroxynitrite-mediated cytotoxicity. *Mem Inst Oswaldo Cruz* 2005;**100**:29–37.

Koh PO. Streptozotocin-induced diabetes increases apoptosis through JNK phosphorylation and Bax activation in rat testes. *J Vet Med Sci* 2007a;**69**:969–971.

Koh PO. Streptozotocin-induced diabetes increases the interaction of Bad/Bcl-XL and decreases the binding of pBad/14-3-3 in rat testis. *Life Sci* 2007b;**81**: 1079–1084.

Kokk K, Verajankorva E, Wu XK, Tapfer H, Poldoja E, Pollanen P. Immunohistochemical detection of glucose transporters class I subfamily in the mouse, rat and human testis. *Medicina (Kaunas)* 2004;**40**:156–160.

Korejo NA, Wei QW, Shah AH, Shi FX. Effects of concomitant diabetes mellitus and hyperthyroidism on testicular and epididymal histoarchitecture and steroidogenesis in male animals. *J Zhejiang Univ Sci B* 2016;**17**:850–863.

Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: a review of literature. *J Hum Reprod Sci* 2015;**8**:191–196.

Kurbatov D, Russo GI, Galstyan GR, Rozhivanov R, Lepetukhin A, Dubsky S, Shwartz YG, Cimino S, Morgia G, Sansalone S. Correction of retrograde ejaculation in patients with diabetes mellitus using endourethral collagen injection: preliminary results. *J Sex Med* 2015;**12**:2126–2129.

La Vignera S, Calogero AE, Condorelli R, Lanzafame F, Giammusso B, Vicari E. Andrological characterization of the patient with diabetes mellitus. *Minerva Endocrinologica* 2009;**34**:1–9.

La Vignera S, Condorelli RA, Di Mauro M, Lo Presti D, Mongioi LM, Russo G, Calogero AE. Reproductive function in male patients with type 1 diabetes mellitus. *Andrology* 2015;**3**:1082–1087.

La Vignera S, Condorelli RA, Vicari E, Lotti F, Favilla V, Morgia G, Maggi M, Calogero AE. Seminal vesicles and diabetic neuropathy: ultrasound evaluation after prolonged treatment with a selective phosphodiesterase-5 inhibitor. *Andrology* 2013;**1**:245–250.

Leal AM, Oliveira MC, Couri CE, Moraes DA, Stracieri AB, Pieroni F, Soares RD, Barros GN, Simoes BP, Foss MC *et al*. Testicular function in patients with type 1 diabetes treated with high-dose CY and autologous hematopoietic SCT. *Bone Marrow Transplant* 2012;**47**:467–468.

Li S, Guo S, He F, Zhang M, He J, Yan Y, Ding Y, Zhang J, Liu J, Guo H *et al*. Prevalence of diabetes mellitus and impaired fasting glucose, associated with risk factors in rural Kazakh adults in Xinjiang, China. *Int J Environ Res Public Health* 2015;**12**:554–565.

Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidemiol* 2009;**62**:e1–e34.

Lo CS, Shi Y, Chang SY, Abdo S, Chenier I, Filep JG, Ingelfinger JR, Zhang SL, Chan JS. Overexpression of heterogeneous nuclear ribonucleoprotein F stimulates renal Ace-2 gene expression and prevents TGF-beta1-induced kidney injury in a mouse model of diabetes. *Diabetologia* 2015;**58**:2443–2454.

Long CA, Boulom V, Albadawi H, Tsai S, Yoo HJ, Oklu R, Goldman MH, Watkins MT. Poly-ADP-ribose-polymerase inhibition ameliorates hind limb ischemia reperfusion injury in a murine model of type 2 diabetes. *Ann Surg* 2013;**258**:1087–1095.

Lopez-Alvarenga JC, Zarinan T, Olivares A, Gonzalez-Barranco J, Veldhuis JD, Ulloa-Aguirre A. Poorly controlled type I diabetes mellitus in young men selectively suppresses luteinizing hormone secretory burst mass. *J Clin Endocrinol Metab* 2002;**87**:5507–5515.

Lotti F, Corona G, Castellini G, Maseroli E, Fino MG, Cozzolino M, Maggi M. Semen quality impairment is associated with sexual dysfunction according to its severity. *Hum Reprod* 2016;**31**:2668–2680.

Loveland KL, Hedger MP. 7 - Activins and inhibins in Sertoli cell biology: Implications for testis development and function A2 - Griswold. In: *Michael D. Sertoli Cell Biology*, 2nd edn. Oxford: Academic Press, 2015;201–232.

Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. *Annu Rev Cell Dev Biol* 2011;**27**:441–464.

MacLean JA II, Hu Z, Welborn JP, Song HW, Rao MK, Wayne CM, Wilkinson MF. The RHOX homeodomain proteins regulate the expression of insulin and other metabolic regulators in the testis. *J Biol Chem* 2013;**288**:34809–34825.

Mallidis C, Agbaje I, O'Neill J, McClure N. The influence of type I diabetes mellitus on spermatogenic gene expression. *Fertil Steril* 2009a;**92**:2085–2087.

Mallidis C, Agbaje I, Rogers D, Glenn J, McCullough S, Atkinson AB, Steger K, Stitt A, McClure N. Distribution of the receptor for advanced glycation end products in the human male reproductive tract: prevalence in men with diabetes mellitus. *Hum Reprod* 2007a;**22**:2169–2177.

Mallidis C, Agbaje IM, Rogers DA, Glenn JV, Pringle R, Atkinson AB, Steger K, Stitt AW, McClure N. Advanced glycation end products accumulate in the reproductive tract of men with diabetes. *Int J Androl* 2009b;**32**:295–305.

Mallidis C, Green BD, Rogers D, Agbaje IM, Hollis J, Migaud M, Amigues E, McClure N, Browne RA. Metabolic profile changes in the testes of mice with streptozotocin-induced type I diabetes mellitus. *Int J Androl* 2007b;**32**:156–165.

Mangoli E, Talebi AR, Anvari M, Pourentezari M. Effects of experimentally-induced diabetes on sperm parameters and chromatin quality in mice. *Iran J Reprod Med* 2013;**11**:53–60.

Maremanda KP, Khan S, Jena GB. Role of Zinc Supplementation in Testicular and Epididymal Damages in Diabetic Rat: Involvement of Nrf2, SOD1, and GPX5. *Biol Trace Elem Res* 2016;**173**:452–464.

Maresch CC, Stute DC, Ludlow H, Hammes HP, de Kretser DM, Hedger MP, Linn T. Hyperglycemia is associated with reduced testicular function and activin dysregulation in the Ins2Akita+/- mouse model of type I diabetes. *Mol Cell Endocrinol* 2017;**446**:91–101.

Maric C, Forsblom C, Thorn L, Waden J, Groop PH, FinnDiane Study G. Association between testosterone, estradiol and sex hormone binding globulin levels in men with type I diabetes with nephropathy. *Steroids* 2010;**75**:772–778.

Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Med* 2012;**9**:e1001356.

Mendell JR, Sahenk Z, Al-Zaidy S, Rodino-Klapac LR, Lowes LP, Alfano LN, Berry K, Miller N, Yalvac M, Dvorchik I *et al*. Follistatin gene therapy for sporadic inclusion body myositis improves functional outcomes. *Mol Ther* 2017;**25**:870–879.

Meyer-Ficca ML, Ihara M, Lonchar JD, Meistrich ML, Austin CA, Min W, Wang ZQ, Meyer RG. Poly(ADP-ribose) metabolism is essential for proper nucleoprotein exchange during mouse spermiogenesis. *Biol Reprod* 2011;**84**:218–228.

Mohasseb M, Ebied S, Yehia MA, Hussein N. Testicular oxidative damage and role of combined antioxidant supplementation in experimental diabetic rats. *J Physiol Biochem* 2011;**67**:185–194.

Morgante G, Tosti C, Orvieto R, Musacchio MC, Piomboni P, De Leo V. Metformin improves semen characteristics of oligo-terato-asthenozoospermic men with metabolic syndrome. *Fertil Steril* 2011;**95**:2150–2152.

Morrison JF, Dhanasekaran S, Sheen R, Frampton CM, Mensah-Brown E. The effect of streptozotocin-induced diabetes on the rat seminal vesicle: a possible pathophysiological basis for disorders of ejaculation. *Ann N Y Acad Sci* 2006;**1084**:267–279.

Moss JL, Crosnoe LE, Kim ED. Effect of rejuvenation hormones on spermatogenesis. *Fertil Steril* 2013;**99**:1814–1820.

Mueckler M, Thorens B. The SLC2 (GLUT) family of membrane transporters. *Mol Aspects Med* 2013;**34**:121–138.

Murray FT, Cameron DF, Orth JM. Gonadal dysfunction in the spontaneously diabetic BB rat. *Metabolism* 1983;**32**:141–147.

Murray FT, Cameron DF, Vogel RB, Thomas RG, Wyss HU, Zauner CW. The pituitary-testicular axis at rest and during moderate exercise in males with diabetes mellitus and normal sexual function. *J Androl* 1988;**9**:197–206.

Murray FT, Orth J, Gunsalus G, Weisz J, Li JB, Jefferson LS, Musto NA, Bardin CW. The pituitary-testicular axis in the streptozotocin diabetic male rat: evidence for gonadotroph, Sertoli cell and Leydig cell dysfunction. *Int J Androl* 1981;**4**:265–280.

Nakamura T, Sugino K, Titani K, Sugino H. Follistatin, an activin-binding protein, associates with heparan sulfate chains of proteoglycans on follicular granulosa cells. *J Biol Chem* 1991;**266**:19432–19437.

Nakayama Y, Yamamoto T, Abe SI. IGF-I, IGF-II and insulin promote differentiation of spermatogonia to primary spermatocytes in organ culture of newt testes. *Int J Dev Biol* 1999;**43**:343–347.

Navarro-Casado L, Juncos-Tobarra MA, Chafer-Rudilla M, de Onzono LI, Blazquez-Cabrera JA, Miralles-Garcia JM. Effect of experimental diabetes and STZ on male fertility capacity. Study in rats. *J Androl* 2010;**31**:584–592.

Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP *et al*. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000;**404**:787–790.

Niven MJ, Hitman GA, Badenoch DF. A study of spermatozoal motility in type I diabetes mellitus. *Diabet Med* 1995;**12**:921–924.

O'Neill J, Czerwiec A, Agbaje I, Glenn J, Stitt A, McClure N, Mallidis C. Differences in mouse models of diabetes mellitus in studies of male reproduction. *Int J Androl* 2010;**33**:709–716.

Ofstad AP, Gullestad L, Orvik E, Aakhus S, Endresen K, Ueland T, Aukrust P, Fagerland MW, Birkeland KI, Johansen OE. Interleukin-6 and activin A are independently associated with cardiovascular events and mortality in type 2 diabetes: the prospective Asker and Baerum Cardiovascular Diabetes (ABCD) cohort study. *Cardiovasc Diabetol* 2013;**12**:126.

Ogawa K, Abe K, Kurosawa N, Kurohmaru M, Sugino H, Takahashi M, Hayashi Y. Expression of alpha, beta A and beta B subunits of inhibin or activin and follistatin in rat pancreatic islets. *FEBS Lett* 1993;**319**:217–220.

Oliveira PF, Alves MG, Rato L, Laurentino S, Silva J, Sa R, Barros A, Sousa M, Carvalho RA, Cavaco JE *et al*. Effect of insulin deprivation on metabolism and metabolism-associated gene transcript levels of in vitro cultured human Sertoli cells. *Biochim Biophys Acta* 2012;**1820**:84–89.

Ozbek E, Otunctemur A, Dursun M, Sahin S, Besiroglu H, Koklu I, Erkoc M, Danis E, Bozkurt M. Diabetes mellitus and HbA1c levels associated with high grade prostate cancer. *Asian Pac J Cancer Prev* 2014;**15**:2555–2558.

Paasch U, Heidenreich F, Pursche T, Kuhlisch E, Kettner K, Grunewald S, Kratzsch J, Dittmar G, Glander HJ, Hoflack B et al. Identification of increased amounts of eppin protein complex components in sperm cells of diabetic and obese individuals by difference gel electrophoresis. *Mol Cell Proteomics* 2011;**10**:M110 007187.

Padron RS, Dambay A, Suarez R, Mas J. Semen analyses in adolescent diabetic patients. *Acta Diabetol Lat* 1984;**21**:115–121.

Palmeira CM, Santos DL, Seica R, Moreno AJ, Santos MS. Enhanced mitochondrial testicular antioxidant capacity in Goto-Kakizaki diabetic rats: role of coenzyme Q. *Am J Physiol Cell Physiol* 2001;**281**:C1023–C1028.

Perrard-Sapori MH, Chatelain PC, Rogemond N, Saez JM. Modulation of Leydig cell functions by culture with Sertoli cells or with Sertoli cell-conditioned medium: effect of insulin, somatomedin-C and FSH. *Mol Cell Endocrinol* 1987;**50**:193–201.

Pitetti JL, Calvel P, Zimmermann C, Conne B, Papaioannou MD, Aubry F, Cederroth CR, Urner F, Fumel B, Crausaz M et al. An essential role for insulin and IGF1 receptors in regulating sertoli cell proliferation, testis size, and FSH action in mice. *Mol Endocrinol* 2013;**27**:814–827.

Pontes DA, Fernandes GS, Piffer RC, Gerardin DC, Pereira OC, Kempinas WG. Ejaculatory dysfunction in streptozotocin-induced diabetic rats: the role of testosterone. *Pharmacol Rep* 2011;**63**:130–138.

Porto EM, Dos Santos SA, Ribeiro LM, Lacorte LM, Rinaldi JC, Justulin LA Jr, Felisbino SL. Lobe variation effects of experimental diabetes and insulin replacement on rat prostate. *Microsc Res Tech* 2011;**74**:1040–1048.

Rato L, Alves MG, Cavaco JE, Oliveira PF. High-energy diets: a threat for male fertility? *Obesity Reviews* 2014a;**15**:996–1007.

Rato L, Alves MG, Dias TR, Cavaco JE, Oliveira PF. Testicular metabolic reprogramming in neonatal streptozotocin-induced type 2 diabetic rats impairs glycolytic flux and promotes glycogen synthesis. *J Diabetes Res* 2015a;**2015**:973142.

Rato L, Alves MG, Dias TR, Lopes G, Cavaco JE, Socorro S, Oliveira PF. High-energy diets may induce a pre-diabetic state altering testicular glycolytic metabolic profile and male reproductive parameters. *Andrology* 2013;**1**:495–504.

Rato L, Alves MG, Duarte AI, Santos MS, Moreira PI, Cavaco JE, Oliveira PF. Testosterone deficiency induced by progressive stages of diabetes mellitus impairs glucose metabolism and favors glycogenesis in mature rat Sertoli cells. *Int J Biochem Cell Biol* 2015b;**66**:1–10.

Rato L, Duarte AI, Tomas GD, Santos MS, Moreira PI, Socorro S, Cavaco JE, Alves MG, Oliveira PF. Pre-diabetes alters testicular PGC1-alpha/SIRT3 axis modulating mitochondrial bioenergetics and oxidative stress. *Biochimica et Biophysica Acta* 2014b;**1837**:335–344.

Ribeiro DL, Marques SF, Alberti S, Spadella CT, Manzato AJ, Taboga SR, Dizeyi N, Abrahamsson PA, Goes RM. Malignant lesions in the ventral prostate of alloxan-induced diabetic rats. *Int J Exp Pathol* 2008;**89**:276–283.

Ricci G, Catizone A, Esposito R, Pisanti FA, Vietri MT, Galdieri M. Diabetic rat testes: morphological and functional alterations. *Andrologia* 2009;**41**:361–368.

Riera MF, Galardo MN, Pellizzari EH, Meroni SB, Cigorraga SB. Molecular mechanisms involved in Sertoli cell adaptation to glucose deprivation. *Am J Physiol Endocrinol Metab* 2009;**297**:E907–E914.

Robertson DM, Klein R, de Vos FL, McLachlan RI, Wettenhall RE, Hearn MT, Burger HG, de Kretser DM. The isolation of polypeptides with FSH suppressing activity from bovine follicular fluid which are structurally different to inhibin. *Biochem Biophys Res Commun* 1987;**149**:744–749.

Robinson R, Fritz IB. Metabolism of glucose by Sertoli cells in culture. *Biol Reprod* 1981;**24**:1032–1041.

Rocha A, Iniguez G, Godoy C, Gaete X, Lopez P, Loreti N, Campo S, Rey RA, Codner E. Testicular function during adolescence in boys with type 1 diabetes mellitus (T1D): absence of hypogonadism and differences in endocrine profile at the beginning and end of puberty. *Pediatr Diabetes* 2014;**15**:198–205.

Roessner C, Paasch U, Kratzsch J, Glander HJ, Grunewald S. Sperm apoptosis signalling in diabetic men. *Reprod Biomed Online* 2012;**25**:292–299.

Sarma AV, Hotaling J, Dunn RL, Cleary PA, Braffett BH, Kim C, Martin C, Herman W, Gatcomb P, Jacobson AM et al. Poor glycemic control is associated with reduced prostate specific antigen concentrations in men with type 1 diabetes. *J Urol* 2015;**193**:786–793.

Scarano WR, Messias AG, Oliva SU, Klinefelter GR, Kempinas WG. Sexual behaviour, sperm quantity and quality after short-term streptozotocin-induced hyperglycaemia in rats. *Int J Androl* 2006;**29**:482–488.

Schiavi RC, Fisher C, Quadland M, Glover A. Nocturnal penile tumescent evaluation of erectile function in insulin-dependent diabetic men. *Diabetologia* 1985; **28**:90–94.

Schmidt RE, Green KG, Snipes LL, Feng D. Neuritic dystrophy and neuronopathy in Akita (Ins2(Akita)) diabetic mouse sympathetic ganglia. *Exp Neurol* 2009;**216**: 207–218.

Schoeller EL, Albanna G, Frolova AI, Moley KH. Insulin rescues impaired spermatogenesis via the hypothalamic-pituitary-gonadal axis in Akita diabetic mice and restores male fertility. *Diabetes* 2012;**61**:1869–1878.

Schoeller EL, Chi M, Drury A, Bertschinger A, Esakky P, Moley KH. Leptin monotherapy rescues spermatogenesis in male Akita type 1 diabetic mice. *Endocrinology* 2014;**155**:2781–2786.

Sebai H, Selmi S, Rtibi K, Gharbi N, Sakly M. Protective Effect of Lavandula stoechas and Rosmarinus officinalis essential oils against reproductive damage and oxidative stress in alloxan-induced diabetic rats. *J Med Food* 2015;**18**:241–249.

Seethalakshmi L, Menon M, Diamond D. The effect of streptozotocin-induced diabetes on the neuroendocrine-male reproductive tract axis of the adult rat. *J Urol* 1987;**138**:190–194.

Sharaf AA, el-Din AK, Hamdy MA, Hafeiz AA. Effect of ascorbic acid on oxygen consumption, glycolysis and lipid metabolism of diabetic rat testis. *Ascorbic acid and diabetes, I. J Clin Chem Clin Biochem* 1978;**16**:651–655.

Shayakhmetova GM, Bondarenko LB, Matvienko AV, Kovalenko VM. Correlation between spermatogenesis disorders and rat testes CYP2E1 mRNA contents under experimental alcoholism or type I diabetes. *Adv Med Sci* 2014;**59**: 183–189.

Shi GJ, Zheng J, Wu J, Qiao HQ, Chang Q, Niu Y, Sun T, Li YX, Yu JQ. Beneficial effects of Lycium barbarum polysaccharide on spermatogenesis by improving antioxidant activity and inhibiting apoptosis in streptozotocin-induced diabetic male mice. *Food Funct* 2017;**8**:1215–1226.

Shrikhande G, Khaodhiar L, Scali S, Lima C, Hubbard M, Dudley K, Ganda O, Ferran C, Veves A. Valsartan improves resting skin blood flow in type 2 diabetic patients and reduces poly(adenosine diphosphate-ribose) polymerase activation. *J Vasc Surg* 2006;**43**:760–770. discussion 770-761.

Shrilatha B, Muralidhara. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: its progression and genotoxic consequences. *Reprod Toxicol* 2007;**23**:578–587.

Shrilatha B, Muralidhara. Occurrence of oxidative impairments, response of antioxidant defences and associated biochemical perturbations in male reproductive milieu in the Streptozotocin-diabetic rat. *Int J Androl* 2007;**30**: 508–518.

Shrivastav P, Swann J, Jeremy JY, Thompson C, Shaw RW, Dandona P. Sperm function and structure and seminal plasma prostanoid concentrations in men with IDDM. *Diabetes Care* 1989;**12**:742–744.

Silink M. Childhood diabetes: a global perspective. *Horm Res* 2002;**57**:1–5.

Singh S, Malini T, Rengarajan S, Balasubramanian K. Impact of experimental diabetes and insulin replacement on epididymal secretory products and sperm maturation in albino rats. *J Cell Biochem* 2009;**108**:1094–1101.

Sjoberg L, Pitkaniemi J, Haapala L, Kaaja R, Tuomilehto J. Fertility in people with childhood-onset type 1 diabetes. *Diabetologia* 2013;**56**:78–81.

Skinner MK, Griswold MD. Secretion of testicular transferrin by cultured Sertoli cells is regulated by hormones and retinoids. *Biol Reprod* 1982;**27**:211–221.

Soudamani S, Malini T, Balasubramanian K. Effects of streptozotocin-diabetes and insulin replacement on the epididymis of prepubertal rats: histological and histomorphometric studies. *Endocr Res* 2005;**31**:81–98.

Szabat M, Johnson JD, Piret JM. Reciprocal modulation of adult beta cell maturity by activin A and follistatin. *Diabetologia* 2010;**53**:1680–1689.

Tabak O, Gelisgen R, Erman H, Erdenen F, Muderrisoglu C, Aral H, Uzun H. Oxidative lipid, protein, and DNA damage as oxidative stress markers in vascular complications of diabetes mellitus. *Clin Invest Med* 2011;**34**:E163–E171.

Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* 2006;**7**:85–96.

Trindade AA, Simoes AC, Silva RJ, Macedo CS, Spadella CT. Long term evaluation of morphometric and ultrastructural changes of testes of alloxan-induced diabetic rats. *Acta Cir Bras* 2013;**28**:256–265.

Tsounapi P, Honda M, Dimitriadis F, Kawamoto B, Hikita K, Muraoka K, Saito M, Sofikitis N, Takenaka A. Impact of antioxidants on seminal vesicles function and fertilizing potential in diabetic rats. *Asian J Androl* 2017a;**19**:639–646.

Tsounapi P, Honda M, Dimitriadis F, Shimizu S, Shiomi T, Hikita K, Saito M, Tomita S, Sofikitis N, Takenaka A. Antioxidant treatment ameliorates diabetes-induced dysfunction of the vas deferens in a rat model. *Andrologia* 2017b;1–11. doi:10.1111/and.12795. [Epub ahead of print].

Ueno N, Ling N, Ying SY, Esch F, Shimasaki S, Guillemin R. Isolation and partial characterization of follistatin: a single-chain Mr 35,000 monomeric protein that inhibits the release of follicle-stimulating hormone. *Proc Natl Acad Sci U S A* 1987; **84**:8282–8286.

Ulisse S, Jannini EA, Pepe M, De Matteis S, D'Armiento M. Thyroid hormone stimulates glucose transport and GLUT1 mRNA in rat Sertoli cells. *Mol Cell Endocrinol* 1992;**87**:131–137.

van Dam EW, Dekker JM, Lentjes EG, Romijn FP, Smulders YM, Post WJ, Romijn JA, Krans HM. Steroids in adult men with type 1 diabetes: a tendency to hypogonadism. *Diabetes Care* 2003;**26**:1812–1818.

Venegas-Pino DE, Wang PW, Stoute HK, Singh-Pickersgill NA, Hong BY, Khan MI, Shi Y, Werstuck GH. Sex-Specific Differences in an ApoE(-/-):Ins2(+ /Akita) Mouse Model of Accelerated Atherosclerosis. *Am J Pathol* 2016;**186**:67–77.

Ventimiglia E, Capogrosso P, Boeri L, Serino A, Colicchia M, Ippolito S, Scano R, Papaleo E, Damiano R, Montorsi F *et al*. Infertility as a proxy of general male health: results of a cross-sectional survey. *Fertil Steril* 2015;**104**:48–55.

Verma R, Haldar C. Photoperiodic modulation of thyroid hormone receptor (TR-alpha), deiodinase-2 (Dio-2) and glucose transporters (GLUT 1 and GLUT 4) expression in testis of adult golden hamster, Mesocricetus auratus. *J Photochem Photobiol B* 2016;**165**:351–358.

Vigneri R, Goldfine ID, Frittitta L. Insulin, insulin receptors, and cancer. *J Endocrinol Invest* 2016;**39**:1365–1376.

Vignozzi L, Rastrelli G, Corona G, Gacci M, Forti G, Maggi M. Benign prostatic hyperplasia: a new metabolic disease? *J Endocrinol Invest* 2014;**37**:313–322.

Wessells H, Penson DF, Cleary P, Rutledge BN, Lachin JM, McVary KT, Schade DS, Sarma AV, Diabetes C, Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Effect of intensive glycemic therapy on erectile function in men with type 1 diabetes. *J Urol* 2011;**185**:1828–1834.

Wiebe JC, Santana A, Medina-Rodriguez N, Hernandez M, Novoa J, Mauricio D, Wagner AM, T1Dgc. Fertility is reduced in women and in men with type 1 diabetes: results from the Type 1 Diabetes Genetics Consortium (T1DGC). *Diabetologia* 2014;**57**:2501–2504.

Wijayarathna R, de Kretser DM. Activins in reproductive biology and beyond. *Hum Reprod Update* 2016;**22**:342–357. https://doi.org/10.1093/humupd/dmv058.

Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004;**27**: 1047–1053.

World Health Organisation. *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation*. WHO Press, 2006.

World Health Organization. *Global report on diabetes*. Switzerland: WHO Press, 2016.

Writing Team for the Diabetes C, Complications Trial/Epidemiology of Diabetes I, and Complications Research G. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA* 2002;**287**:2563–2569.

Wu H, Chen Y, Winnall WR, Phillips DJ, Hedger MP. Regulation of activin A release from murine bone marrow-derived neutrophil precursors by tumour necrosis factor-alpha and insulin. *Cytokine* 2013;**61**:199–204.

Wu H, Wu M, Chen Y, Allan CA, Phillips DJ, Hedger MP. Correlation between blood activin levels and clinical parameters of type 2 diabetes. *Exp Diabetes Res* 2012;**2012**:410579.

Yan WJ, Mu Y, Yu N, Yi TL, Zhang Y, Pang XL, Cheng D, Yang J. Protective effects of metformin on reproductive function in obese male rats induced by high-fat diet. *J Assist Reprod Genet* 2015;**32**:1097–1104.

Yasuda H, Inoue K, Shibata H, Takeuchi T, Eto Y, Hasegawa Y, Sekine N, Totsuka Y, Mine T, Ogata E *et al*. Existence of activin-A in A- and D-cells of rat pancreatic islet. *Endocrinology* 1993;**133**:624–630.

Yigitturk G, Acara AC, Erbas O, Oltulu F, Yavasoglu NUK, Uysal A, Yavasoglu A. The antioxidant role of agomelatine and gallic acid on oxidative stress in STZ induced type I diabetic rat testes. *Biomed Pharmacother* 2017;**87**:240–246.

Yonezawa A, Ebiko M, Yoshizumi M, Ise SN, Watanabe C, Mizoguchi H, Iwasaki M, Kimura Y, Sakurada S. Effects of insulin replacement on ejaculatory dysfunction in streptozotocin-induced diabetic rats. *Int J Urol* 2009;**16**:208–211.

Young JC, Wakitani S, Loveland KL. TGF-beta superfamily signaling in testis formation and early male germline development. *Semin Cell Dev Biol* 2015;**45**:94–103.

Yu T, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. *Proc Natl Acad Sci USA* 2006;**103**:2653–2658.

Zhao Y, Tan Y, Dai J, Li B, Guo L, Cui J, Wang G, Shi X, Zhang X, Mellen N *et al*. Exacerbation of diabetes-induced testicular apoptosis by zinc deficiency is most likely associated with oxidative stress, p38 MAPK activation, and p53 activation in mice. *Toxicol Lett* 2011;**200**:100–106.

Zierler K. Whole body glucose metabolism. *Am J Physiol* 1999;**276**:E409–E426.

Zysk JR, Bushway AA, Whistler RL, Carlton WW. Temporary sterility produced in male mice by 5-thio-D-glucose. *J Reprod Fertil* 1975;**45**:69–72.