REPRODUCTION
REVIEW

Obesity, energy balance and spermatogenesis

Pedro F Oliveira[1,2,3], Mário Sousa[1,2,4], Branca M Silva[5], Mariana P Monteiro[2,6,7] and Marco G Alves[2]

[1]Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal, [2]Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal, [3]i3S-Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal, [4]Centre for Reproductive Genetics Prof. Alberto Barros, Porto, Portugal, [5]CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilha, Portugal, [6]Department of Anatomy, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal and [7]Obesity & Bariatric Services and Centre for Obesity Research, University College of London Hospitals, University College of London, London, UK

Correspondence should be addressed to M G Alves; Email: alvesmarc@gmail.com

Abstract

Obesity has grown to pandemic proportions. It affects an increasing number of children, adolescents and young adults exposed to the silent comorbidities of this disorder for a longer period. Infertility has arisen as one important comorbidity associated with the energy dysfunction promoted by obesity. Spermatogenesis is a highly regulated process that is determined by specific energetic requirements. The reproductive potential of males relies on hormonal-dependent and -independent stimuli that control sperm quality. There are conflicting data concerning the impact of male overweight and obesity on sperm quality, as well as on the possible paternal-induced epigenetic trait inheritance of obesity. In addition, it remains a matter of debate whether massive weight loss induced by lifestyle interventions, drugs or bariatric surgery may or may not benefit obese men seeking fatherhood. Herein, we propose to discuss how energy balance may modulate hormonal signalling and sperm quality in overweight and obese men. We also discuss some molecular mechanisms that mediate obesity-related dysfunction in male reproductive system and how paternal obesity may lead to trait inheritance. Finally, we will discuss how lifestyle modifications and sustained weight loss, particularly the loss achieved by bariatric surgery, may revert some of the deleterious effects of obesity in men and their offspring.

Reproduction (2017) **153** R173–R185

Introduction

Nutritional habits have evolved towards an increasing consumption of processed foods rich in sugars and saturated fats, rendered as more attractive and affordable products over the past few decades. The number of overweight and obese individuals has increased concurrently with the establishment of those dietary and sedentary lifestyle habits, prompting obesity to a pandemic health problem. The World Health Organization has published relevant statistics suggesting that nearly 2 billion adults are already overweight and that over one-third of those are obese (Weight-Control Information Network 2012), while these numbers are expected to continue to grow. Alarmingly, the number of children, adolescent and young adults with metabolic disorders has also raised exponentially, which means that these individuals will have to face the disease for a long period of their life (Weight-Control Information Network 2012). Hence, some of the silent problems caused by overweight/obesity, such as male infertility, may become a serious health issue in the years to come.

High-calorie diets are more likely to induce an increase in body weight and lead to hyperglycaemia, hyperinsulinaemia and dyslipidaemia. This causes a chronic inflammatory state and a high metabolic rate necessary to sustain the body metabolic balance. Unfortunately, a high metabolic rate ends up with the formation of high levels of reactive oxygen species (ROS), which induce damages in DNA, proteins and compromises the integrity of cells' plasma membranes. These events are particularly important in the testicular environment as sperm cells are highly sensitive to oxidative stress (OS). Thus, it is not surprising that male obesity is associated with reduced pregnancy rates and increased pregnancy loss in couples subjected to medically assisted reproduction techniques (Keltz *et al.* 2010, Bakos *et al.* 2011*a*).

Disturbed energy homeostasis can lead to several metabolic disorders spanning from malnutrition to metabolic syndrome. These disorders have in common

ISSN 1470–1626 (paper) 1741–7899 (online)
DOI: 10.1530/REP-17-0018
Online version via www.reproduction-online.org

the capacity of inducing various male reproductive dysfunctions. Several integrators mediate the link between energy intake and reproduction. Though the relevance of most of these remains a matter of debate, the positive energy balance leading to severe endocrine dysfunctions is most probably the primary cause of obesity-induced male subfertility. Indeed, it has been proposed that the altered leptin, ghrelin and glucagon-like peptide-1 levels associated with obesity may play a pivotal role in the obesity-induced deleterious effects in male reproduction (for review Alves *et al.* 2016). The signals mediated by these hormones are pivotal for the control of satiety, regulating the energy intake as well as energy homeostasis. Notably, several of these hormones and their downstream signalling molecules regulate glucose and energy metabolism in peripheral organs, including the testis. The energy control in testis has been shown to be pivotal for spermatogenesis (for review Alves *et al.* 2014).

Multiple factors associated with obesity can compromise spermatogenesis, in particular, the accumulation of fat depots on the suprapubic and scrotal area, which leads to the increase of temperature in the testis. Accumulation of toxic substances and lipid-soluble endocrine disruptors in fat tissue may also amplify the deleterious effects induced by increased body weight (for review Rato *et al.* 2014). Defective spermatogenesis may result in multiple alterations in sperm parameters, defective capacitation and sperm binding, as well as sperm structural changes (particularly in chromatin). Alcohol intake is one of the lifestyle conditions that can interfere with several endocrine systems, including energy homeostasis as well as appetite regulation. In addition, the reproductive function can be highly affected by alcoholism (Kenna *et al.* 2012), which is able to induce morphological changes in testis and lower sperm quality (Eid *et al.* 2012, Ezzatabadipour *et al.* 2012). Experiments in rats showed that consumption of a 6% ethanol solution during 28 days resulted in a significant elevation of serum leptin (Pravdova *et al.* 2009). However, a cross-sectional study enrolling 820 subjects after acute coronary syndrome, coronary revascularisation or first ischaemic stroke, reported that drinking small amounts of alcohol was associated with decreased serum leptin concentrations with possible benefits in terms of cardiovascular risk (Mayer *et al.* 2010). In addition, chronic alcoholic individuals depict very low leptin concentrations (Santolaria *et al.* 2003) that have been attributed to the malnutrition that occurs in these patients with reduced fat tissue mass. Interestingly, the ghrelin system was suggested as a potential target to treat alcohol abuse as ghrelin antagonists showed effectiveness in reducing ethanol intake and preference without decreasing food intake (Gomez *et al.* 2015). Nevertheless, the mechanisms by which these drugs and hormones affect addiction remain unknown, and it will be important to evaluate their effect on male reproductive potential.

Obese men are also at increased risk to develop erectile dysfunction (Esposito *et al.* 2004), which compromises their reproductive health. Interestingly, it was reported that rats fed with a cafeteria diet presented lower number of ejaculations per hour (Lazaros *et al.* 2012), suggesting that energy intake could even alter the ejaculation pattern. Since then, several reports have shown that obesity and energy balance are important factors for male reproductive health. Nevertheless, the molecular mechanisms by which this happens remain a matter of debate. Herein, we discuss some of the putative mechanisms by which obesity and energy balance regulate spermatogenesis and thus influences male reproductive potential.

Energy balance, hormonal dysfunction and male reproduction

Overweight- and obesity-associated dysfunction of the hypothalamic–pituitary–testis (HPT) axis represents major causes for the observed alterations in spermatogenesis. Indeed, increased aromatisation of androgens steroids to oestrogens in adipose tissue in obese men were described a few decades ago; increased oestrogen levels are known to induce hypogonadotropic hypogonadism with the end result of decreasing total and free testosterone (Schneider *et al.* 1979). Anecdotally, treatment of an obese patient with the aromatase inhibitor anastrozole, led to decreased serum oestradiol levels, normalising luteinising hormone (LH) and testosterone levels, and restored spermatogenesis and fertility (Roth *et al.* 2008). This observation supports the therapeutic potential of aromatase in infertile obese men, though the use of these drugs for this aim in clinical practice needs to be supported by further evidence that can also grant future label recommendation. In addition, prepubertal onset followed by long-term excess body weight results in reduced Leydig cells number and increased levels of proinflammatory markers in the testis, suggesting that it is associated with chronic inflammation that may have a negative impact on steroidogenesis by Leydig cells (Wagner *et al.* 2016). Interestingly, it has long been established that excess of body weight, particularly when concomitant with hyperglycaemia, is associated with reduced endocrine activity in the testis and deleterious effects in the spermatogenesis (Hellman *et al.* 1963). HPT axis is very sensitive to energy balance, even to subtle changes, highlighting the relevance of a proper glucose homeostasis to sustain a regular spermatogenesis. Thus, gut and adipose-derived hormones (particularly ghrelin and leptin) have risen as crucial integrators of the link between energy balance,

food intake and male reproductive function (Fig. 1) (Alves *et al.* 2014).

High-fat diets lead to weight gain, increased circulating levels of leptin and oestradiol and additionally decreased levels of testosterone, in rats (Pauli *et al.* 2008, Vigueras-Villasenor *et al.* 2011). The ob/ob mice, a leptin-deficient obese rodent model, show impaired reproductive function (Swerdloff *et al.* 1976), reversible by intraperitoneal leptin replacement (Cleary *et al.* 2001). This emphasises the relevance of this hormone for male fertility. A study in leptin-deficient obese mice treated with sub- and physiological concentrations of leptin showed that spermatogenesis was improved in leptin-treated mice and that treatment with sub- to physiological concentrations dose dependently improves male reproductive function (Hoffmann *et al.* 2016). In fact, impaired spermatogenesis in infertile men was also shown to be associated with testicular expression of OB-R (Ishikawa *et al.* 2007). On the other hand, the excess of leptin production by adipose tissue was reported to have deleterious effects on sperm production and to induce apoptosis on germ cells (Isidori *et al.* 1999), which illustrates that this hormone may compromise male fertility via distinct mechanisms. For instance, leptin-induced steroidogenesis inhibition has already been reported (Tena-Sempere *et al.* 2001), and leptin was proposed as directly responsible for the signalling link between adipose tissue and altered steroidogenesis in the testis (Isidori *et al.* 1999).

Clinical studies also confirmed some of the actions reported for leptin in the male reproductive system of rodent models. A study with 122 obese men, of which 42 were fertile and 80 exhibited oligozoospermia, reported that obese oligozoospermic patients had a significant increase in the serum levels of follicle-stimulating hormone (FSH), LH, 17β-oestradiol (E2), prolactin (PRL) and leptin. In addition, serum leptin was positively correlated with abnormal sperm morphology and with altered serum levels of the other hormones (except E2), whereas being negatively correlated with sperm concentration, sperm motility and serum testosterone (Hofny *et al.* 2010). These results provide clear evidence that to some extent leptin mediates the link between obesity and infertility in men. A small study with 42 men further reported that obese men had decreased sperm concentration and vitality, as well as increased sperm DNA fragmentation. Additionally, seminal and serum leptin concentrations were correlated in those individuals, suggesting that leptin may have a deleterious effect on male reproductive function through an direct action on HPT axis or on specific sites (Leisegang *et al.* 2014). Indeed, the presence of Ob-R in testis, and particularly in testicular cells such as Leydig and Sertoli cells, also suggests that leptin may have a direct action on testicular cells (Fig. 1). For instance, the somatic testicular Sertoli cells responsible for the nutritional support of spermatogenesis express the Ob-R. Direct exposure to leptin leads to alterations in the metabolic support of spermatogenesis provided by Sertoli cells. At concentrations found in healthy lean men, leptin is able to upregulate the protein levels of GLUT2, whereas concentrations of leptin found in lean and obese patients increases the activity of LDH, the enzyme that catalyses the production of lactate necessary for the developing germ cells. Notably, all the concentrations of leptin decrease acetate production by Sertoli cells (Martins *et al.* 2015), which could be associated with alteration in the cells lipid droplet accumulation. Accumulation of cytoplasmic lipid droplets, which has been associated with the elimination of germ cell-derived apoptotic bodies by phagocytosis (Wang *et al.* 2006), was reported as one of the most striking deleterious effects of hyperthermia in Sertoli cells (Valles *et al.* 2014). Interestingly, accumulation

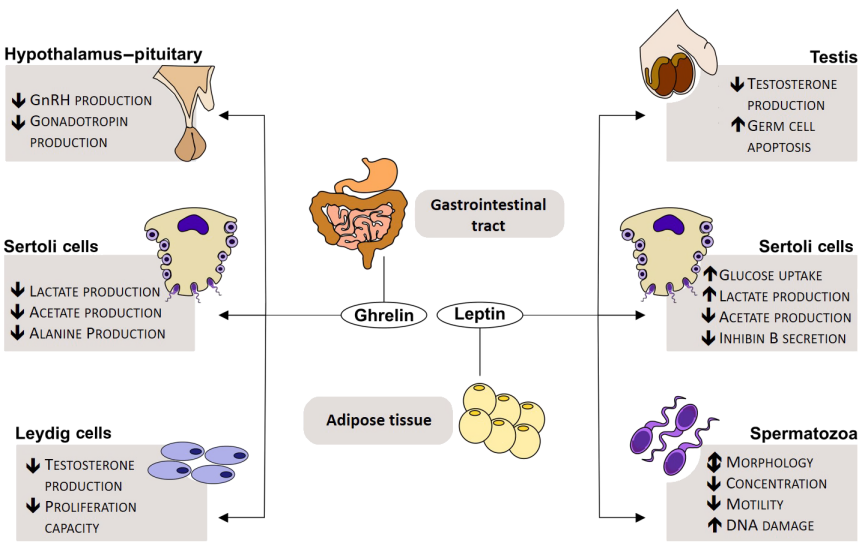

Figure 1 Effects of ghrelin and leptin on male reproductive tract and reproductive health. Ghrelin is produced in the gastrointestinal tract and impairs the testicular function by disrupting both the hypothalamic–pituitary–thyroid (HPT) axis, affecting Sertoli and Leydig cell functions. Leptin is produced by the adipose tissue, also impairs male reproductive function, by decreasing testosterone production, affecting Sertoli cell function, leading to a decline of sperm quality. GnRH, gonadotropin-releasing hormone; bidirectional arrows: alteration; down arrows: decrease; up arrows: increase.

of lipid droplets has also been described in testicular cells of Zucker rats (Young *et al.* 1982) suggesting that this may be a mechanism by which testicular cells respond to obese metabolic syndrome-induced testicular dysfunction. Indeed, in other cellular systems, such as intestinal epithelial cells (Fazolini *et al.* 2015), leptin activates the mTOR pathway triggering lipid droplet formation. mTOR controls Sertoli cells redox balance and metabolism (Jesus *et al.* 2016) and has been discussed as a central rheostat regulator of male reproductive potential (Jesus *et al.* 2017, Oliveira *et al.* 2017). Thus, it may be a key player in obesity-induced male subfertility/infertility by controlling lipid droplet formation in testicular cells, among other mechanisms. Interestingly, inhibin B, considered a marker for Sertoli cell function and spermatogenesis, is also decreased in obese males (Pauli *et al.* 2008). It has also been shown that leptin has a direct effect on spermatozoa (Jope *et al.* 2003), increasing sperm progressive and total motility as well as promoting the acrosome reaction (Li *et al.* 2009*a*). Moreover, *in vivo* exposure to higher levels of leptin decreases epididymal sperm count, whereas it increases the amount of abnormal sperm in rats (Haron *et al.* 2010). Thus, leptin seems to have divergent effects on maturing and ejaculated spermatozoa as well as direct or indirect effects on the male reproductive tract (through disturbances in HPT axis). Nevertheless, this is still a topic of intense discussion, and the molecular mechanisms by which these actions are mediated remain unknown. The role of other adipokines such as resistin, adiponectin, chemerin, omentin and visfatin on male reproductive tract and fertility are still obscure, although there are already some studies focusing on these molecular mediators (for review Dupont *et al.* 2015). For instance, resistin is expressed in the rat testis throughout testis postnatal development and is controlled by the coordinated action of hormones and other factors including gonadotropins, leptin and the nutritional status. Resistin is involved in the control of testicular testosterone secretion, and it was suggested that it may also be a rheostat of energy homeostasis that can influence reproduction (Nogueiras *et al.* 2004).

Ghrelin is a growth hormone-releasing peptide that controls energy metabolism in such a way that it mirrors the energy status of the individual. After food deprivation, its levels are increased and the plasma concentrations of ghrelin are usually negatively correlated with BMI (for review van der Lely *et al.* 2004). Interestingly, the levels of ghrelin present in the serum and in the testis of rats are identical (Catak *et al.* 2014). This suggests that this hormone may participate in the hormonal control of spermatogenesis or exert other direct effects on the male reproductive tract (Fig. 1). Systemic ghrelin administration reduces GnRH pulse frequency (Lebrethon *et al.* 2007) and ghrelin acts directly in the pituitary where it modulates gonadotrophin secretion (Fernandez-Fernandez *et al.* 2007). Thus, ghrelin may interfere with the reproductive success of males by acting on the hormonal signals that regulate spermatogenesis. In addition, the presence of its receptor, the growth hormone secretagogue receptor (GHS-R) in testicular cells, highlights that ghrelin may also act directly at those sites and promote a response. For instance, we have shown that human Sertoli cells express GHS-R and that ghrelin acts as an energy status sensor for the nutritional support of spermatogenesis by regulating the glycolytic and bioenergetic profiles of these cells (Martins *et al.* 2016). Moreover, ghrelin also emerged as a rheostat of endocrine and nonendocrine functions in Leydig cells, including interfering with testosterone production and cell proliferative capacity (Barreiro *et al.* 2004). Thus, ghrelin may regulate spermatogenesis in humans, though the exact molecular mechanisms by which it occurs, remain a matter of intense research.

Energy balance interferes with the complex hormonal signalling that integrates signals and regulates spermatogenesis. Several hormones respond to energy fluctuations and mediate direct and indirect effects in testicular cells. Thus, the lack of consensus concerning the effects of obesity in male reproduction, particularly in sperm quality, has been suggested to result from a minimum threshold of hormonal dysfunction necessary to induce significant alterations in the mechanisms responsible for those effects such as in sperm production and/or quality (MacDonald *et al.* 2010).

Sperm quality and obesity-induced male subfertility/infertility: clinical studies

There is clear evidence that energy unbalance and particularly obesity may have a negative impact on male reproductive potential (Fig. 2). Nevertheless, clinical studies and meta-analysis have not reached a consensus regarding the impact of obesity on sperm quality. In addition, data from studies performed in animal models lack confirmation in the human setting; thus, the discussion of results obtained using animal models of obesity must be cautious. As discussed previously, taking into consideration the effects of excess body weight in the male reproductive system, it was suggested that obesity decreases sperm quality. Indeed, data from 2035 men showed that obese men are more likely to have lower semen volume and fewer normal spermatozoa than men with normal body mass index (BMI) (Shayeb *et al.* 2011). A study with 166 men, including 38 severely obese, showed that BMI was negatively associated with sperm concentration, count, progressive motility and normal physiology. In addition, BMI was also negatively associated with the levels of total testosterone, SHBG, AMH and inhibin B (Andersen *et al.* 2015), thus suggesting a correlation between obesity, the levels of these hormones and sperm quality. Consequently, obese men have at least increased risk of low semen volume

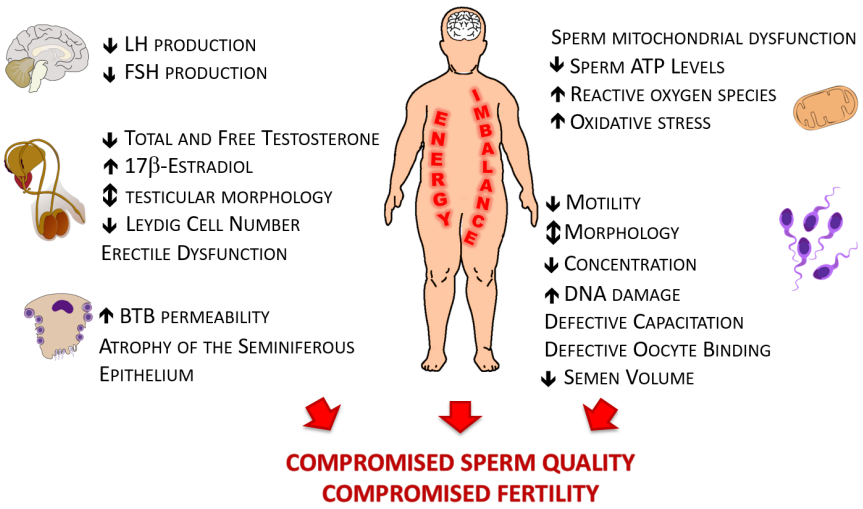

Figure 2 Major effects of obesity and energy imbalance on male reproductive tract and reproductive health. The energy imbalance impairs the testicular function by disrupting both the hypothalamic–pituitary–thyroid (HPT) axis, affecting steroidogenesis and spermatogenesis. Furthermore, it also impairs male reproductive function, declining sperm production and quality due to loss of blood–testis barrier permeability and to increased oxidative stress. Legend: BTB, blood–testis barrier; FSH, follicle-stimulating hormone; LH, luteinising hormone; bidirectional arrows: alteration; down arrows: decrease; up arrows: increase.

and decreased normal sperm morphology than men with normal weight, though these studies did not report any association between excess of weight and sperm concentration or motility. Worthy of note is that in men with moderate overweight, no sperm alterations were found, suggesting that there might be a threshold for the association between excess body weight and deleterious effect on male reproductive health.

A recently updated systematic review and meta-analysis concluded that excess body weight and obesity in men are associated with increased prevalence of azoospermia and oligozoospermia (Sermondade *et al.* 2013*b*). In addition, a meta-analysis reported a J-shaped association between BMI and the risk of abnormal sperm count (Sermondade *et al.* 2013*b*) though it was not determined if weight loss could revert this effect. A multi-institutional cohort of 4440 subfertile men was part of a study that performed a multivariate analysis and exposed a negative correlation between BMI and ejaculate volume and sperm characteristics including concentration, motility and morphology. In addition, the rates of azoospermia and oligospermia were also more prevalent in obese men than those in men with normal weight. The authors concluded that the BMI influences the hormonal profile and sperm characteristics, and thus, excess body weight may contribute to subfertility in men (Tunc *et al.* 2011). In another study in 970 patients seeking treatment for nonobstructive azoospermia, overweight men were shown to have worst pregnancy outcomes after microdissection testicular sperm extraction than those with normal weight. In addition, no men with BMI over 43 kg/m^2 were able to contribute to a successful pregnancy (Ramasamy *et al.* 2013).

Colaci and coworkers (Colaci *et al.* 2012) reported that couples with obese male partners have 84% lower odds ratio for birth than couples with normal-weight men. In addition, this study also highlighted that male obesity has a possible deleterious effect on the chances of achieving live birth after intracytoplasmic sperm injection (ICSI) treatment. However, a recent study enrolling 8490 couples who underwent *in vitro* fertilisation (IVF) or ICSI showed no significant effect of the male partner BMI on live birth rate (Zhu *et al.* 2015), which is in agreement with the findings of a prospective study in 721 couples undergoing infertility treatment (Schliep *et al.* 2015). Nonetheless, this study suggested that the BMI of the father influences the sex ratio of the offspring by raising the probability to give birth to male singletons with increased BMI (Zhu *et al.* 2015). The controversial data derived from these studies illustrate the need to identify the mechanisms by which overweight or obesity interfere with male fertility before reaching a consensus.

Differences in dietary habits and intake of macro and micronutrients can potentially interfere with sperm membrane composition (thus influencing sperm quality), which may explain the discrepancies found between studies. A study conducted in 144 men from the general population aimed to examine the association of circulating fatty acids and BMI, with spermatozoa fatty acids composition and semen characteristics. The authors concluded that the amount of docosahexaenoic acid (DHA, 22:5, ω-3) was positively correlated with sperm quality, particularly sperm count, concentration, vitality, progressive motility and normal sperm morphology. In addition, DHA was negatively associated with DNA fragmentation index. On the other hand, palmitic acid (C16:0) and linoleic acid (C18:2; ω-6) levels in sperm depicted a positive and negative correlation with sperm count respectively. It is of note that BMI was negatively associated with DHA and palmitic acid levels in sperm (Andersen *et al.* 2016), revealing that BMI may alter some important sperm features by influencing sperm fatty acid composition. The fact that there is a relationship between serum fatty acids and sperm fatty acids composition suggests that BMI affects the spermatozoa fatty acid composition by a direct action in the testis, although individual variations in diet composition and

obesity may influence the interpretation of the results. Unfortunately, there is a lack of mechanistic studies to understand why overweight men yield worsened pregnancy outcomes than normal-weight men. In this matter, animal studies have shed some light.

Few studies have focused on male BMI (Sarais *et al.* 2016), although several retrospective and meta-analyses have been conducted to unveil the effects of female BMI on the number and quality of retrieved oocytes, fertilisation rate, incidences of ongoing pregnancy and live births among other factors affecting couples undergoing IVF. A retrospective study carried out in patients seeking infertility treatment who received IVF or ICSI treatment including a total of 8490 couples concluded that live birth success of overweight and obese groups was similar to the normal-weight group, although increased male BMI augmented the probability of giving birth to male singletons (Zhu *et al.* 2015). Nevertheless, most of these studies have several confounding factors including those associated with the mother. For instance, there may be an effect of high maternal BMI on the semen quality of the offspring (Ramlau-Hansen *et al.* 2007). Thus, there is a need for further studies that could add novel insights into these topics.

Molecular mechanisms that mediate obesity-related reproductive dysfunction in males

There are numerous animal models to study obesity. Several rely on diet-induced fat accumulation attained with the use of high-fat or high-energy diets to mimic the dietary habits of modern societies, a major contributor for the obesity pandemic. Although most of the previous studies aiming to disclose the effects of obesity or energy balance on male reproduction focused on sperm and lacked a mechanistic perspective, a common feature reported in animals with diet-induced or chemically induced obesity, is the decrease in gonadal volume and weight while body weight increases. Monosodium glutamate-induced obesity in rats decreases testosterone and FSH plasma levels and sperm counts. In addition, testis, epididymis, prostate and seminal vesicle weights, as well as the seminiferous tubular diameter are also decreased in these animals (Fernandes *et al.* 2012). A recent study showed that feeding rats with high-fat diet during 4 weeks reduces sperm concentration and motility. Interestingly, those animals presented impaired mitochondrial respiration, with decreased ATP levels and augmented OS. When evaluating the spermatozoa of these animals, pyruvate dehydrogenase and lactate dehydrogenase activities, as well as citrate synthase were significantly diminished (Ferramosca *et al.* 2016). Notably, high-fat diet feeding in rats did not alter mitochondrial complex I activity in sperm significantly, whereas that of all other complexes decreased significantly. Thus, these results suggest uncoupling

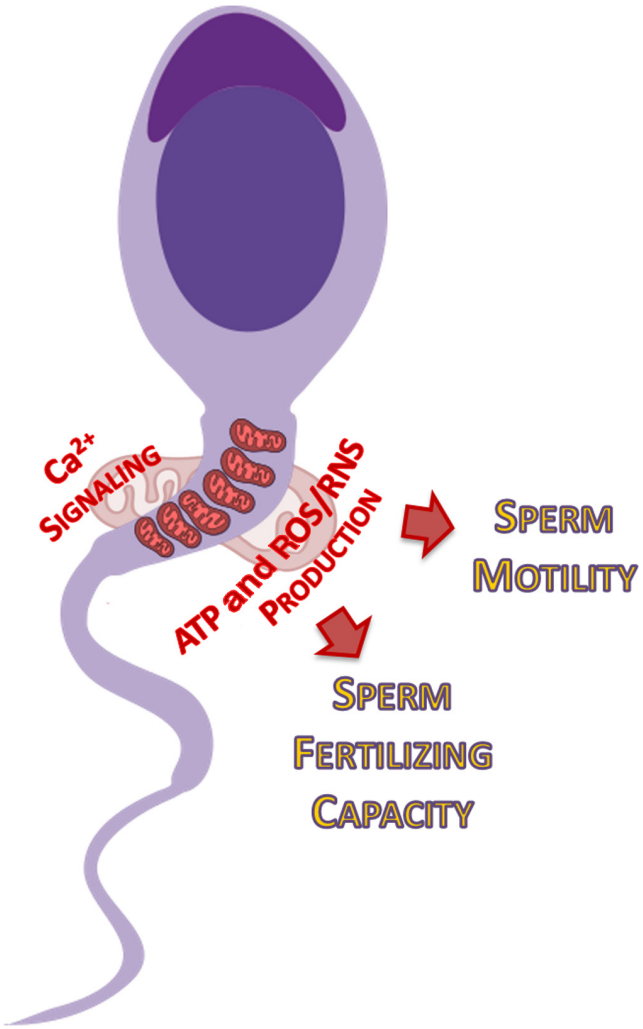

Figure 3 Diagram illustrating mitochondria relevance in sperm midpice. Mitochondria regulate calcium signalling as well as production of ATP. In addition, since sperm midpiece has a high quantity of active mitochondria, it is also a place where reactive oxygen species are produced. These processes mediated by mitochondria are essential for sperm motility and sperm fertilising activity.

of mitochondrial respiration with ATP production in sperm of rats fed with high-fat diet (Ferramosca *et al.* 2016), which is not surprising since mitochondria are key players in sperm physiology and function, known to be involved in sperm motility and fertilising capacity, among other processes (Fig. 3).

Interestingly, a clinical study with 651 couples undergoing fertility treatment showed that sperm mitochondrial activity was lower in overweight men who also presented higher ROS and DNA fragmentation. This illustrates that mitochondrial dysfunction may be responsible for the lower sperm quality frequently reported in men with higher BMI (Yang *et al.* 2016). Also, semen analysis in 81 men seeking for fertility clinic care reported that BMI is correlated with ROS production,

sperm DNA damage, markers of inflammation and alterations in reproductive hormones. Interestingly, there was no association between increased ROS and DNA integrity or sperm motility (Tunc *et al.* 2011), suggesting that elevated ROS may affect other molecular mechanisms that end up by compromising male reproductive health.

ROS modulate essential functions of sperm including capacitation, hyperactivation and acrosomal reaction (Fig. 3). However, unbalanced ROS results in the oxidation of sperm membranes, which are mainly composed of polyunsaturated fatty acids, loss of mitochondrial function (Wang *et al.* 2003) and also DNA fragmentation (Kodama *et al.* 1997). Indeed, it was reported that BMI is positively correlated with DNA fragmentation index in sperm (Kort *et al.* 2006). Several studies reported that obese men have lower total sperm counts and higher DNA damage than normal-weight men do (Chavarro *et al.* 2010, Dupont *et al.* 2013). Another study went far beyond and showed in 305 males that increased BMI is associated not only with decreased progressive motility and increased DNA fragmentation but also with decreased sperm mitochondrial activity (Fariello *et al.* 2012). This study provided clear evidence that sperm mitochondria activity may be sensitive to the BMI and correlated with the subfertility/infertility reported in overweight/obese individuals.

Besides mitochondrial activity, other hypotheses have been raised to explain subfertility/infertility in overweight and obese men. For instance, the morphological changes in testicular cells observed in those individuals were attributed to the increased permeability of the blood–testis barrier (BTB). The BTB, composed by adjacent Sertoli cells, assures the unique biochemical milieu required to the occurrence of spermatogenesis (for review Stanton 2016). In fact, male mice fed with a high-fat diet (HFD) for 10 weeks developed obesity and presented an increase in sperm abnormalities with a marked decrease on sperm motility, which were suggested to occur due to severe disruption in BTB and atrophy of the seminiferous epithelia (Fan *et al.* 2015). Actually, sperm alterations and testicular cells morphological changes were accompanied by interruption of BTB and severe decline of key components of the barrier.

Obese mice resulting from high-fat diet feeding also have decreased fertilisation ability, with altered binding capacity to the zona pellucida (Bakos *et al.* 2011*b*), which could be reverted by resuming a normal diet or exercise (Palmer *et al.* 2012). However, no association between the BMI and sperm–zona pellucida-binding ability was reported in 306 men from couples diagnosed with primary idiopathic or mild male factor infertility (Sermondade *et al.* 2013*a*).

One of the major confounders of the studies aiming to address the effects of excess adiposity in male reproductive function is the lack of phenotypical characterisation of obesity. BMI is only a crude measure of adiposity that in spite of its widespread use in the clinical setting harbours several limitations and provides no information on the relative body distribution or type of adipose tissue (brown, beige or white), which are of utmost relevance. To further expose the importance of adipose tissue composition, brown adipose tissue (BAT) transplantation can improve several metabolic parameters including glucose homeostasis and insulin sensitivity (Gunawardana & Piston 2012, Stanford *et al.* 2013). In addition, a recent report showed that BAT from male donor mice transplanted into age- and sex-matched recipient mice fed with high-fat diet reduced body fat and epididymal fat mass. In addition, triglycerides content in epididymis and in the testis decreased. Notably, sperm motility was restored and the fertility impairment caused by diet-induced obesity was ameliorated (Liu *et al.* 2016). Thus, differences in regional distribution and composition of adipose tissue may be responsible for some of the apparently conflicting results concerning the influence of obesity in sperm quality. Further studies will be needed to understand how sperm quality is influenced by energy unbalance and the molecular mechanisms affected by obesity that may end-up in subfertility or infertility in overweight or obese men.

Paternal obesity and food-induced trait inheritance

Maternal hyperglycaemia or obesity during gestation or lactation are well-known risk factors for the development reprogramming that induces obesity and diabetes in the offspring (O'Reilly & Reynolds 2013, Isganaitis *et al.* 2014). However, recent evidence suggests that the paternal metabolic profile at conception also influences the metabolic profile of the offspring, with obese men being more likely to father obese children (Li *et al.* 2009*b*). These detrimental effects upon the offspring's metabolic health induced by paternal obesity (Fullston *et al.* 2013, Ng *et al.* 2014) suggest that information is being transmitted via sperm. The classic dogma was that spermatozoa are transcriptionally inactive. However, it is now clear that mature spermatozoa contain high amounts of RNAs and non-coding RNAs though their potential role is not fully understood (Ostermeier *et al.* 2004, Miller *et al.* 2005). Emerging evidence suggests that microRNA composition in spermatozoa responds to environmental and lifestyle factors. For instance, the transgenerational inheritance of traumatic stress was shown to be mediated by sperm microRNAs (Gapp *et al.* 2014). Interestingly, the altered phenotype passes to the offspring when mice are crossed with healthy partners. This shows that food-induced trait inheritance may be mediated by RNA signalling (Grandjean *et al.* 2015).

It is very interesting to note that in a study with 651 couples undergoing fertility treatment, males with a BMI higher than 28 kg/m^2 had a lower fertilisation,

good-quality embryo and clinical pregnancy rates than males with a normal BMI (Yang *et al*. 2016). In addition, individuals with higher BMI presented more ROS and sperm DNA fragmentation rate than normal-weight ones (Yang *et al*. 2016). Testis transcriptome of obese and healthy mice showed that a specific mRNA (miR19b) might be responsible for diet-induced metabolic alterations that influence the phenotype of the offspring. Paternal high-fat-induced obesity was also reported to promote an intergenerational transmission of obesity. This transmission is effected through altered sperm epigenetics signatures associated with phenotypes that include obesity and metabolic characteristics associated with its comorbidities (e.g. insulin resistance, impaired glucose tolerance) at different degrees (Fullston *et al*. 2013), illustrating that obesity induces crucial fingerprints in sperm that may be responsible for health problems in the offspring. microRNAs are regulated by the metabolic phenotype of the individual and are involved in glucose homeostasis (Fernandez-Hernando *et al*. 2013). Indeed, paternal type 2 diabetes is known to program certain metabolic phenotypes in the offspring (Meigs *et al*. 2000, Wei *et al*. 2014). Thus, it is likely that metabolic diseases, such as obesity and type 2 diabetes mellitus, induce transgenerational signatures transmitted via sperm. Recently, a study identified a distinct small non-coding RNAs profile in sperm from obese when compared with lean men. This shows that spermatozoa from obese men carry a distinct epigenetic fingerprint (Donkin *et al*. 2016). Nevertheless, the epigenetic signature in obese men remains largely unknown, although it was reported that the metabolic changes may also epigenetically influence the somatic tissues of the offspring. It was also recently shown that transgenerational sperm epigenetic alterations subsequently alter the development of the testicular somatic Sertoli cell. The altered epigenome and transcriptome in Sertoli cell was correlated with the onset of adult infertility (Guerrero-Bosagna *et al*. 2013). Thus, somatic testicular cells may be key drivers of acquired epigenetic obesity inheritance.

A study with F1, F2 and F3 derived from F0 mattings of obese mice with prediabetes suggested a non-genetic inheritance of obesity that could occur through the action of sperm non-coding RNAs. It also suggested that there are some fingerprints of the paternal exposure to obesity that may be passed through generations. Notably, it was also suggested that dietary intervention can promote a metabolic reprogramming, but the propensity for metabolic dysfunction still be maintained in the offspring, which might well be associated with the dramatic rise in the incidence of metabolic disorders (Cropley *et al*. 2016). Remarkably, mice fed with high-fat diet originate offspring with latent metabolic defects (e.g. impaired glucose homeostasis, insulin resistance and dyslipidaemia) that can be evaded when the mice avoid high-fat diets (Li *et al*. 2013). These findings suggest that improving the paternal metabolic health would improve fertility and lead to improved metabolic health of the offspring. Thus, it is crucial to understand the molecular mechanisms by which these latent metabolic defects are installed and passed through generations.

Can weight loss by lifestyle intervention or bariatric surgery improve male reproductive health?

In 2012, Palmer and coworkers in diet-induced male C57BL6 mice subjected to diet and exercise concluded that diet alone can reduce adiposity and serum cholesterol levels although only ameliorated glucose tolerance when combined with exercise. In addition, both diet and exercise improved sperm quality by reducing sperm DNA damage, production of ROS and mitochondrial membrane potential, suggesting an association between systemic metabolic status and sperm quality and function (Palmer *et al*. 2012). Interestingly, sperm parameters of 20 morbidly obese men were determined at baseline, after exercise during 4 months prior to a bariatric surgery intervention and again 24 months later. Despite a significant BMI reduction, amelioration of hormonal dysfunction and improvement of the quality of sexual function, there were no differences concerning sperm parameters after 4 months of exercise or 20 months after surgery (Reis *et al*. 2012). Using a mouse model, diet or exercise interventions for 8 weeks in obese founder males resulted in metabolic benefits to the offspring. In addition, the most striking improvement resulted from the exercise-only intervention despite the authors reporting higher adiposity than those subjected to diet (McPherson *et al*. 2015), which illustrates that the positive benefits of exercise may surpass the deleterious effect of increased adiposity. This hypothesis is still under investigation and should not be overlooked. In the search to improve male fertility in obese men, several dietary or natural compounds were tested. For instance, resveratrol in a dose of 30 µmol/L used *in vitro* was shown to improve sperm motility after 30 min, as well as seminal plasma zinc concentration and spermatozoa acrosin reaction in semen samples of obese men with astenospermia (Cui *et al*. 2016). In rats with high-fat diet-induced obesity, treatment with 100 mg/kg metformin during 8 weeks improved semen parameters, increased testicular weight, reduced testicular apoptosis, restored hormonal homeostasis and improved metabolic characteristics when compared with those not treated with this antidiabetic drug (Yan *et al*. 2015). The search for viable ways to improve the reproductive health of overweight/obese men is still overlooked, but these studies provide evidence that it is possible to revert some of the deleterious effects promoted by energy unbalance.

The use of bariatric surgery for treatment of severe obesity has disclosed some important cues concerning energy balance and male reproduction. Secondary

infertility was reported by di Frega and coworkers (di Frega *et al.* 2005) in six patients that underwent Roux-en-Y gastric bypass. The authors suggested that the secondary azoospermia with complete spermatogenic arrest that was detected could be due to the nutritional imbalance and toxic insult resulting from the weight loss, rather than the hormonal dysfunction. Two male patients were tested for fertility treatment in two consecutive cycles before and after bariatric surgery and a marked reduction in sperm parameters between 12 and 18 months after surgery was noted (Lazaros *et al.* 2012). This led to the suggestion that bariatric surgery may impact negatively on spermatogenesis in the first months after surgery. Indeed, severe worsening in semen parameters was reported in 3 patients who underwent bariatric surgery 3 months earlier (Sermondade *et al.* 2012), also indicating that the first months after surgery may have deleterious effects on male reproductive potential. However, 1 patient had the condition reverted after 24 months, and clinical pregnancies were obtained in the other patients (Sermondade *et al.* 2012), illustrating that, after an initial period of severe weight loss and metabolic dysfunction, the male reproductive health may be improved after bariatric surgery. In a more recent prospective study of 46 male patients that underwent sleeve gastrectomy, testosterone levels were shown to increase 12 months after surgery. Interestingly, sperm concentration in men with azoospermia and oligospermia was also increased after surgery suggesting that weight loss may improve sperm quality (El Bardisi *et al.* 2016).

Indeed, there are other studies presenting evidence that bariatric surgery may have a long-term positive impact on the male reproductive health. In a small randomised trial with 20 morbidly obese men for 24 months, the authors showed that surgery-induced weight loss reverted some of the hormonal dysfunctions previously reported in obese men and increased erectile function quality (Reis *et al.* 2010). Also, a study conducted in 17 obese men that underwent a ringed vertical gastroplasty showed that serum inhibin B levels were elevated in most of those men and there was a weak but significant inverse correlation with BMI (Globerman *et al.* 2005). Thus, these studies suggest that bariatric surgery may improve the reproductive health of obese men. However, the conflicting literature on the impact of bariatric surgery and massive weight loss on male fertility may be due to the existence of a threshold of balance between positive effects, such as the hormonal, psychological and sexual effects, and the negative effects arising from nutritional depletion and the weight loss induced release of lipophilic toxic substances. It is also worthy of note that there are some studies based on the effects of obesity, excess energy intake and sperm parameters. Notwithstanding, few studies have explored the effects of low body weight or poor energy intake on male reproductive health. A paradigm that should be addressed, if we take into account the current increase in the search for low-energy diets or dietary supplements that may deplete energy resources.

Concluding remarks

Males with excess body weight due to a surplus of adipose tissue have reduced sperm, low binding capacity, low fertilisation ability and difficulties to undergo capacitation and acrosome reaction, which suggests that sperm quality may be impaired (Fig. 2). Nevertheless, the mechanisms by which a positive energy balance promotes dysfunction on these sperm functions remain a matter of debate. Conflicting results derived from different studies could be due to several causes. An important contributor is the fact that data from meta-analysis and systematic reviews are often derived from a mixture of male individuals with a great heterogeneity in their reproductive status (fertile vs subfertile). In addition, there are differences in the way each laboratory determines sperm quality, and the biological significance of the sperm differences in these studies are often neglected and not put in perspective, as a mild diminution of sperm quality is very important for a subfertile couple, whereas in couples with normal fertility, it can be neglected. Furthermore, research has not taken into account other habits that may be even more deleterious for sperm quality than energy unbalance, such as smoking or ethanol intake. Besides, most of the studies rely on self-reporting of habits or lifestyle, which is actually prone to inaccuracy. Routine semen analysis is not a reliable method to determine the fertilising potential of spermatozoa and do not include the prediction of the changes that occur in the female reproductive tract before fertilisation. Thus, clinicians and researchers discuss that newer tests to predict the success of fertilisation are needed (Franken & Oehninger 2012, Wang & Swerdloff 2014). Indeed, current tests do not study spermatozoa ability to reach and fertilise the oocyte. After ejaculation, the spermatozoa has to travel across the female reproductive tract, hyperactivate, undergo the very controlled process of acrosome reaction, penetrate the cumulus and zona pellucida and finally fuse with and fertilise the oocyte. Thus, factors related to the female reproductive tract and control of the events spanning from ejaculation until fertilisation need evaluation. However, this is not currently performed due to controversy and lack of strong methodology. Therefore, the semen analysis used to evaluate the male reproductive health lacks the ability to retrieve very relevant information that compromises the evaluation of the obesity effects on male fertility.

We must also take into consideration that most studies use only BMI as a measure of adiposity. However, BMI is a measure of weight in relation to height and does not always reflect the percentage of fat. In fact, men with

increased muscle mass have a high BMI, which is not usually associated with decreased sperm quality. A more detailed characterisation of the adiposity degree and relative body fat distribution, as well as the reproductive hormone and metabolic profiles should be determined as much as possible before inferring their effects in sperm quality, as these may introduce a complex network of effects.

Future preventive interventions may include a weight loss recommendation for overweight or obese men during the window of pregnancy planning. Recent evidence shows that obesity may induce molecular changes in sperm conveyed into transgenerational epigenetic inheritance. Those changes can be mediated by modified RNA levels, DNA methylation, protamination and histone acetylation. They may end-up in deleterious effects in the offspring. Increased energy intake is a key driver for the obesity crisis. However, the latest findings suggest that transgenerational epigenetic inheritance is associated with the offspring's metabolic profile. Taken in consideration that obesity compromises male fertility, it is also pivotal to unveil the molecular mechanisms responsible for inherited subfertility/infertility. Finally, the effect of significant weight loss attained through low energy intake or surgical treatment of obesity should not be overlooked. Infertility and subfertility are silent comorbidities associated with energy balance. Policymakers should seriously consider this matter as a priority as it may explain the decrease in sperm quality and birth rates we are witnessing. Moreover, assisted reproductive technologies are very expensive and should not to be used indiscriminately. A therapeutic approach towards obesity-related male subfertility and/or infertility is mandatory, but it has to be implemented with an integrative approach including weight loss, epigenetic contribution, energy balance and a window of opportunity for treatment. In recent years, there is also an increased interest in developing and optimising new compounds that control the levels of hormones dysregulated during obesity. For instance, inhibition of ghrelin O-acyltransferase (GOAT), which catalyses an essential octanoylation step in ghrelin maturation, has been described as a potential avenue for controlling ghrelin signalling and new small-molecule GOAT inhibitors have already been synthesised (McGovern *et al.* 2016, McGovern-Gooch *et al.* 2017). It will be crucial to test how those new compounds may affect the HPT axis and also their direct effect on testicular cells. All these issues will be a major challenge for the years to come.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

This work was supported by the Portuguese Foundation for Science and Technology: M G Alves (IFCT2015 and PTDC/BIM-MET/4712/2014); P F Oliveira (IFCT2015 and PTDC/BBB-BQB/1368/2014); CICS-UBI (Pest-C/SAU/UI0709/2014) and UMIB (Pest-OE/SAU/UI0215/2014); co-funded by FEDER funds through the POCI/COMPETE 2020. The authors are thankful to MSN, BA, for professional English proofreading and linguistic suggestions.

References

Alves MG, Dias TR, Silva BM & Oliveira PF 2014 Metabolic cooperation in testis as a pharmacological target: from disease to contraception. *Current Molecular Pharmacology* **7** 83–95. (doi:10.2174/1874467208666150126153830)

Alves MG, Jesus TT, Sousa M, Goldberg E, Silva BM & Oliveira PF 2016 Male fertility and obesity: are ghrelin, leptin and glucagon-like peptide-1 pharmacologically relevant? *Current Pharmaceutical Design* **22** 783–791. (doi:10.2174/1381612822666151209151550)

Andersen JM, Herning H, Aschim EL, Hjelmesaeth J, Mala T, Hanevik HI, Bungum M, Haugen TB & Witczak O 2015 Body mass index is associated with impaired semen characteristics and reduced levels of anti-mullerian hormone across a wide weight range. *PLoS ONE* **10** e0130210. (doi:10.1371/journal.pone.0130210)

Andersen JM, Ronning PO, Herning H, Bekken SD, Haugen TB & Witczak O 2016 Fatty acid composition of spermatozoa is associated with BMI and with semen quality. *Andrology* **4** 857–865. (doi:10.1111/andr.12227)

Bakos HW, Henshaw RC, Mitchell M & Lane M 2011*a* Paternal body mass index is associated with decreased blastocyst development and reduced live birth rates following assisted reproductive technology. *Fertility and Sterility* **95** 1700–1704. (doi:10.1016/j.fertnstert.2010.11.044)

Bakos HW, Mitchell M, Setchell BP & Lane M 2011*b* The effect of paternal diet-induced obesity on sperm function and fertilization in a mouse model. *International Journal of Andrology* **34** 402–410. (doi:10.1111/j.1365-2605.2010.01092.x)

Barreiro ML, Gaytan F, Castellano JM, Suominen JS, Roa J, Gaytan M, Aguilar E, Dieguez C, Toppari J & Tena-Sempere M 2004 Ghrelin inhibits the proliferative activity of immature Leydig cells in vivo and regulates stem cell factor messenger ribonucleic acid expression in rat testis. *Endocrinology* **145** 4825–4834. (doi:10.1210/en.2004-0732)

Catak Z, Aydin S, Sahin I, Kuloglu T, Aksoy A & Dagli AF 2014 Regulatory neuropeptides (ghrelin, obestatin and nesfatin-1) levels in serum and reproductive tissues of female and male rats with fructose-induced metabolic syndrome. *Neuropeptides* **48** 167–177. (doi:10.1016/j.npep.2014.04.002)

Chavarro JE, Toth TL, Wright DL, Meeker JD & Hauser R 2010 Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertility and Sterility* **93** 2222–2231. (doi:10.1016/j.fertnstert.2009.01.100)

Cleary MP, Bergstrom HM, Dodge TL, Getzin SC, Jacobson MK & Phillips FC 2001 Restoration of fertility in young obese (Lep(ob) Lep(ob)) male mice with low dose recombinant mouse leptin treatment. *International Journal of Obesity and Related Metabolic Disorders* **25** 95–97. (doi:10.1038/sj.ijo.0801525)

Colaci DS, Afeiche M, Gaskins AJ, Wright DL, Toth TL, Tanrikut C, Hauser R & Chavarro JE 2012 Men's body mass index in relation to embryo quality and clinical outcomes in couples undergoing in vitro fertilization. *Fertility and Sterility* **98** 1193.e1191–1199.e1191. (doi:10.1016/j.fertnstert.2012.07.1102)

Cropley JE, Eaton SA, Aiken A, Young PE, Giannoulatou E, Ho JW, Buckland ME, Keam SP, Hutvagner G, Humphreys DT *et al*. 2016 Male-lineage transmission of an acquired metabolic phenotype induced by grandpaternal obesity. *Molecular Metabolism* **5** 699–708. (doi:10.1016/j.molmet.2016.06.008)

Cui X, Jing X, Wu X & Yan M 2016 Protective effect of resveratrol on spermatozoa function in male infertility induced by excess weight and obesity. *Molecular Medicine Reports* **14** 4659–4665. (doi:10.3892/mmr.2016.5840)

di Frega AS, Dale B, Di Matteo L & Wilding M 2005 Secondary male factor infertility after Roux-en-Y gastric bypass for morbid obesity: case report. *Human Reproduction* **20** 997–998. (doi:10.1093/humrep/deh707)

Donkin I, Versteyhe S, Ingerslev LR, Qian K, Mechta M, Nordkap L, Mortensen B, Appel EV, Jorgensen N, Kristiansen VB *et al*. 2016 Obesity and bariatric surgery drive epigenetic variation of spermatozoa in humans. *Cell Metabolism* **23** 369–378. (doi:10.1016/j.cmet.2015.11.004)

Dupont C, Faure C, Sermondade N, Boubaya M, Eustache F, Clement P, Briot P, Berthaut I, Levy V, Cedrin-Durnerin I *et al*. 2013 Obesity leads to higher risk of sperm DNA damage in infertile patients. *Asian Journal of Andrology* **15** 622–625. (doi:10.1038/aja.2013.65)

Dupont J, Pollet-Villard X, Reverchon M, Mellouk N & Levy R 2015 Adipokines in human reproduction. *Hormone Molecular Biology and Clinical Investigation* **24** 11–24. (doi:10.1515/hmbci-2015-0034)

Eid N, Ito Y & Otsuki Y 2012 Enhanced mitophagy in Sertoli cells of ethanol-treated rats: morphological evidence and clinical relevance. *Journal of Molecular Histology* **43** 71–80. (doi:10.1007/s10735-011-9372-0)

El Bardisi H, Majzoub A, Arafa M, AlMalki A, Al Said S, Khalafalla K, Jabbour G, Basha M, Al Ansari A & Sabanegh E Jr 2016 Effect of bariatric surgery on semen parameters and sex hormone concentrations: a prospective study. *Reproductive BioMedicine Online* **33** 606–611. (doi:10.1016/j.rbmo.2016.08.008)

Esposito K, Giugliano F, Di Palo C, Giugliano G, Marfella R, D'Andrea F, D'Armiento M & Giugliano D 2004 Effect of lifestyle changes on erectile dysfunction in obese men: a randomized controlled trial. *JAMA* **291** 2978–2984. (doi:10.1001/jama.291.24.2978)

Ezzatabadipour M, Azizollahi S, Sarvazad A, Mirkahnooj Z, Mahdinia Z & Nematollahi-Mahani SN 2012 Effects of concurrent chronic administration of alcohol and nicotine on rat sperm parameters. *Andrologia* **44** 330–336. (doi:10.1111/j.1439-0272.2012.01284.x)

Fan Y, Liu Y, Xue K, Gu G, Fan W, Xu Y & Ding Z 2015 Diet-induced obesity in male C57BL/6 mice decreases fertility as a consequence of disrupted blood-testis barrier. *PLoS ONE* **10** e0120775. (doi:10.1371/journal.pone.0120775)

Fariello RM, Pariz JR, Spaine DM, Cedenho AP, Bertolla RP & Fraietta R 2012 Association between obesity and alteration of sperm DNA integrity and mitochondrial activity. *BJU International* **110** 863–867. (doi:10.1111/j.1464-410X.2011.10813.x)

Fazolini NP, Cruz AL, Werneck MB, Viola JP, Maya-Monteiro CM & Bozza PT 2015 Leptin activation of mTOR pathway in intestinal epithelial cell triggers lipid droplet formation, cytokine production and increased cell proliferation. *Cell Cycle* **14** 2667–2676. (doi:10.1080/15384101.2015.1041684)

Fernandes GS, Arena AC, Campos KE, Volpato GT, Anselmo-Franci JA, Damasceno DC & Kempinas WG 2012 Glutamate-induced obesity leads to decreased sperm reserves and acceleration of transit time in the epididymis of adult male rats. *Reproductive Biology and Endocrinology* **10** 105. (doi:10.1186/1477-7827-10-105)

Fernandez-Fernandez R, Tena-Sempere M, Roa J, Castellano JM, Navarro VM, Aguilar E & Pinilla L 2007 Direct stimulatory effect of ghrelin on pituitary release of LH through a nitric oxide-dependent mechanism that is modulated by estrogen. *Reproduction* **133** 1223–1232. (doi:10.1530/REP-06-0227)

Fernandez-Hernando C, Ramirez CM, Goedeke L & Suarez Y 2013 MicroRNAs in metabolic disease. *Arteriosclerosis, Thrombosis, and Vascular Biology* **33** 178–185. (doi:10.1161/ATVBAHA.112.300144)

Ferramosca A, Conte A, Moscatelli N & Zara V 2016 A high-fat diet negatively affects rat sperm mitochondrial respiration. *Andrology* **4** 520–525. (doi:10.1111/andr.12182)

Franken DR & Oehninger S 2012 Semen analysis and sperm function testing. *Asian Journal of Andrology* **14** 6–13. (doi:10.1038/aja.2011.58)

Fullston T, Ohlsson Teague EM, Palmer NO, DeBlasio MJ, Mitchell M, Corbett M, Print CG, Owens JA & Lane M 2013 Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content. *FASEB Journal* **27** 4226–4243. (doi:10.1096/fj.12-224048)

Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J, Farinelli L, Miska E & Mansuy IM 2014 Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. *Nature Neuroscience* **17** 667–669. (doi:10.1038/nn.3695)

Globerman H, Shen-Orr Z, Karnieli E, Aloni Y & Charuzi I 2005 Inhibin B in men with severe obesity and after weight reduction following gastroplasty. *Endocrine Research* **31** 17–26. (doi:10.1080/07435800500228971)

Gomez JL, Cunningham CL, Finn DA, Young EA, Helpenstell LK, Schuette LM, Fidler TL, Kosten TA & Ryabinin AE 2015 Differential effects of ghrelin antagonists on alcohol drinking and reinforcement in mouse and rat models of alcohol dependence. *Neuropharmacology* **97** 182–193. (doi:10.1016/j.neuropharm.2015.05.026)

Grandjean V, Fourré S, De Abreu DAF, Derieppe M-A, Remy J-J & Rassoulzadegan M 2015 RNA-mediated paternal heredity of diet-induced obesity and metabolic disorders. *Scientific Reports* **5**. (doi:10.1038/srep18193)

Guerrero-Bosagna C, Savenkova M, Haque MM, Nilsson E & Skinner MK 2013 Environmentally induced epigenetic transgenerational inheritance of altered Sertoli cell transcriptome and epigenome: molecular etiology of male infertility. *PLoS ONE* **8** e59922. (doi:10.1371/journal.pone.0059922)

Gunawardana SC & Piston DW 2012 Reversal of type 1 diabetes in mice by brown adipose tissue transplant. *Diabetes* **61** 674–682. (doi:10.2337/db11-0510)

Haron MN, D'Souza UJ, Jaafar H, Zakaria R & Singh HJ 2010 Exogenous leptin administration decreases sperm count and increases the fraction of abnormal sperm in adult rats. *Fertility and Sterility* **93** 322–324. (doi:10.1016/j.fertnstert.2009.07.995)

Hellman B, Jacobsson L & Taeljedal IB 1963 Endocrine activity of the testis in obese-hyperglycaemic mice. *Acta Endocrinologica* **44** 20–26. (doi:10.1530/acta.0.0440020)

Hoffmann A, Manjowk GM, Wagner IV, Kloting N, Ebert T, Jessnitzer B, Lossner U, Stukenborg JB, Bluher M, Stumvoll M *et al*. 2016 Leptin within the subphysiological to physiological range dose dependently improves male reproductive function in an obesity mouse model. *Endocrinology* **157** 2461–2468. (doi:10.1210/en.2015-1966)

Hofny ER, Ali ME, Abdel-Hafez HZ, Kamal Eel D, Mohamed EE, Abd El-Azeem HG & Mostafa T 2010 Semen parameters and hormonal profile in obese fertile and infertile males. *Fertility and Sterility* **94** 581–584. (doi:10.1016/j.fertnstert.2009.03.085)

Isganaitis E, Woo M, Ma H, Chen M, Kong W, Lytras A, Sales V, Decoste-Lopez J, Lee KJ, Leatherwood C *et al*. 2014 Developmental programming by maternal insulin resistance: hyperinsulinemia, glucose intolerance, and dysregulated lipid metabolism in male offspring of insulin-resistant mice. *Diabetes* **63** 688–700. (doi:10.2337/db13-0558)

Ishikawa T, Fujioka H, Ishimura T, Takenaka A & Fujisawa M 2007 Expression of leptin and leptin receptor in the testis of fertile and infertile patients. *Andrologia* **39** 22–27. (doi:10.1111/j.1439-0272.2006.00754.x)

Isidori AM, Caprio M, Strollo F, Moretti C, Frajese G, Isidori A & Fabbri A 1999 Leptin and androgens in male obesity: evidence for leptin contribution to reduced androgen levels. *Journal of Clinical Endocrinology and Metabolism* **84** 3673–3680. (doi:10.1210/jc.84.10.3673)

Jesus TT, Oliveira PF, Silva J, Barros A, Ferreira R, Sousa M, Cheng CY, Silva BM & Alves MG 2016 Mammalian target of rapamycin controls glucose consumption and redox balance in human Sertoli cells. *Fertility and Sterility* **105** 825.e823–833.e823. (doi:10.1016/j.fertnstert.2015.11.032)

Jesus TT, Oliveira PF, Sousa M, Cheng CY & Alves MG 2017 Mammalian target of rapamycin (mTOR): a central regulator of male fertility? *Critical Reviews in Biochemistry and Molecular Biology* 1–22. (doi:10.1080/10409238.2017.1279120)

Jope T, Lammert A, Kratzsch J, Paasch U & Glander HJ 2003 Leptin and leptin receptor in human seminal plasma and in human spermatozoa. *International Journal of Andrology* **26** 335–341. (doi:10.1111/j.1365-2605.2003.00434.x)

Keltz J, Zapantis A, Jindal SK, Lieman HJ, Santoro N & Polotsky AJ 2010 Overweight men: clinical pregnancy after ART is decreased in IVF but

not in ICSI cycles. *Journal of Assisted Reproduction and Genetics* **27** 539–544. (doi:10.1007/s10815-010-9439-y)

Kenna GA, Swift RM, Hillemacher T & Leggio L 2012 The relationship of appetitive, reproductive and posterior pituitary hormones to alcoholism and craving in humans. *Neuropsychology Review* **22** 211–228. (doi:10.1007/s11065-012-9209-y)

Kodama H, Yamaguchi R, Fukuda J, Kasai H & Tanaka T 1997 Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertility and Sterility* **68** 519–524. (doi:10.1016/S0015-0282(97)00236-7)

Kort HI, Massey JB, Elsner CW, Mitchell-Leef D, Shapiro DB, Witt MA & Roudebush WE 2006 Impact of body mass index values on sperm quantity and quality. *Journal of Andrology* **27** 450–452. (doi:10.2164/jandrol.05124)

Lazaros L, Hatzi E, Markoula S, Takenaka A, Sofikitis N, Zikopoulos K & Georgiou I 2012 Dramatic reduction in sperm parameters following bariatric surgery: report of two cases. *Andrologia* **44** 428–432. (doi:10.1111/j.1439-0272.2012.01300.x)

Lebrethon MC, Aganina A, Fournier M, Gerard A, Parent AS & Bourguignon JP 2007 Effects of in vivo and in vitro administration of ghrelin, leptin and neuropeptide mediators on pulsatile gonadotrophin-releasing hormone secretion from male rat hypothalamus before and after puberty. *Journal of Neuroendocrinology* **19** 181–188. (doi:10.1111/j.1365-2826.2006.01518.x)

Leisegang K, Bouic PJ, Menkveld R & Henkel RR 2014 Obesity is associated with increased seminal insulin and leptin alongside reduced fertility parameters in a controlled male cohort. *Reproductive Biology and Endocrinology* **12** 34. (doi:10.1186/1477-7827-12-34)

Li HW, Chiu PC, Cheung MP, Yeung WS & O WS 2009*a* Effect of leptin on motility, capacitation and acrosome reaction of human spermatozoa. *International Journal of Andrology* **32** 687–694. (doi:10.1111/j.1365-2605.2008.00931.x)

Li L, Law C, Lo Conte R & Power C 2009*b* Intergenerational influences on childhood body mass index: the effect of parental body mass index trajectories. *American Journal of Clinical Nutrition* **89** 551–557. (doi:10.3945/ajcn.2008.26759)

Li CC, Young PE, Maloney CA, Eaton SA, Cowley MJ, Buckland ME, Preiss T, Henstridge DC, Cooney GJ, Febbraio MA *et al*. 2013 Maternal obesity and diabetes induces latent metabolic defects and widespread epigenetic changes in isogenic mice. *Epigenetics* **8** 602–611. (doi:10.4161/epi.24656)

Liu H, Liu X, Wang L & Sheng N 2016 Brown adipose tissue transplantation ameliorates male fertility impairment caused by diet-induced obesity. *Obesity Research and Clinical Practice* Epub ahead of print. (doi:10.1016/j.orcp.2016.06.001)

MacDonald AA, Herbison GP, Showell M & Farquhar CM 2010 The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. *Human Reproduction Update* **16** 293–311. (doi:10.1093/humupd/dmp047)

Martins AD, Moreira AC, Sa R, Monteiro MP, Sousa M, Carvalho RA, Silva BM, Oliveira PF & Alves MG 2015 Leptin modulates human Sertoli cells acetate production and glycolytic profile: a novel mechanism of obesity-induced male infertility? *Biochimica et Biophysica Acta* **1852** 1824–1832. (doi:10.1016/j.bbadis.2015.06.005)

Martins AD, Sa R, Monteiro MP, Barros A, Sousa M, Carvalho RA, Silva BM, Oliveira PF & Alves MG 2016 Ghrelin acts as energy status sensor of male reproduction by modulating Sertoli cells glycolytic metabolism and mitochondrial bioenergetics. *Molecular and Cellular Endocrinology* **434** 199–209. (doi:10.1016/j.mce.2016.07.008)

Mayer O Jr, Filipovsky J, Galovcova M, Bruthans J, Hrbkova J, Vrzalova J, Cifkova R & Simon J 2010 An inverse association between serum leptin concentration and reported alcohol intake in patients with manifest vascular disease. *European Journal of Clinical Nutrition* **64** 1350–1357. (doi:10.1038/ejcn.2010.153)

McGovern KR, Darling JE & Hougland JL 2016 Progress in small molecule and biologic therapeutics targeting ghrelin signaling. *Mini Reviews in Medicinal Chemistry* **16** 465–480. (doi:10.2174/1389557515666150722101329)

McGovern-Gooch KR, Mahajani NS, Garagozzo A, Schramm AJ, Hannah LG, Sieburg MA, Chisholm JD & Hougland JL 2017 Synthetic triterpenoid inhibition of human ghrelin O-acyltransferase: the involvement of a functionally required cysteine provides mechanistic insight into ghrelin acylation. *Biochemistry* **56** 919–931. (doi:10.1021/acs.biochem.6b01008)

McPherson NO, Owens JA, Fullston T & Lane M 2015 Preconception diet or exercise intervention in obese fathers normalizes sperm microRNA profile and metabolic syndrome in female offspring. *American Journal of Physiology-Endocrinology and Metabolism* **308** E805–E821. (doi:10.1152/ajpendo.00013.2015)

Meigs JB, Cupples LA & Wilson PW 2000 Parental transmission of type 2 diabetes: the Framingham Offspring Study. *Diabetes* **49** 2201–2207. (doi:10.2337/diabetes.49.12.2201)

Miller D, Ostermeier GC & Krawetz SA 2005 The controversy, potential and roles of spermatozoal RNA. *Trends in Molecular Medicine* **11** 156–163. (doi:10.1016/j.molmed.2005.02.006)

Ng SF, Lin RC, Maloney CA, Youngson NA, Owens JA & Morris MJ 2014 Paternal high-fat diet consumption induces common changes in the transcriptomes of retroperitoneal adipose and pancreatic islet tissues in female rat offspring. *FASEB Journal* **28** 1830–1841. (doi:10.1096/fj.13-244046)

Nogueiras R, Barreiro ML, Caminos JE, Gaytan F, Suominen JS, Navarro VM, Casanueva FF, Aguilar E, Toppari J, Dieguez C *et al*. 2004 Novel expression of resistin in rat testis: functional role and regulation by nutritional status and hormonal factors. *Journal of Cell Science* **117** 3247–3257. (doi:10.1242/jcs.01196)

O'Reilly JR & Reynolds RM 2013 The risk of maternal obesity to the long-term health of the offspring. *Clinical Endocrinology* **78** 9–16. (doi:10.1111/cen.12055)

Oliveira PF, Cheng CY & Alves MG 2017 Emerging role for mammalian target of rapamycin in male fertility. *Trends in Endocrinology and Metabolism* **28** 165–167. (doi:10.1016/j.tem.2016.12.004)

Ostermeier GC, Miller D, Huntriss JD, Diamond MP & Krawetz SA 2004 Reproductive biology: delivering spermatozoan RNA to the oocyte. *Nature* **429** 154. (doi:10.1038/429154a)

Palmer NO, Bakos HW, Owens JA, Setchell BP & Lane M 2012 Diet and exercise in an obese mouse fed a high-fat diet improve metabolic health and reverse perturbed sperm function. *American Journal of Physiology: Endocrinology and Metabolism* **302** E768–E780. (doi:10.1152/ajpendo.00401.2011)

Pauli EM, Legro RS, Demers LM, Kunselman AR, Dodson WC & Lee PA 2008 Diminished paternity and gonadal function with increasing obesity in men. *Fertility and Sterility* **90** 346–351. (doi:10.1016/j.fertnstert.2007.06.046)

Pravdova E, Macho L & Fickova M 2009 Alcohol intake modifies leptin, adiponectin and resistin serum levels and their mRNA expressions in adipose tissue of rats. *Endocrine Regulations* **43** 117–125. (doi:10.4149/endo_2009_03_117)

Ramasamy R, Bryson C, Reifsnyder JE, Neri Q, Palermo GD & Schlegel PN 2013 Overweight men with nonobstructive azoospermia have worse pregnancy outcomes after microdissection testicular sperm extraction. *Fertility and Sterility* **99** 372–376. (doi:10.1016/j.fertnstert.2012.10.025)

Ramlau-Hansen CH, Nohr EA, Thulstrup AM, Bonde JP, Storgaard L & Olsen J 2007 Is maternal obesity related to semen quality in the male offspring? A pilot study. *Human Reproduction* **22** 2758–2762. (doi:10.1093/humrep/dem219)

Rato L, Alves MG, Cavaco JE & Oliveira PF 2014 High-energy diets: a threat for male fertility? *Obesity Reviews* **15** 996–1007. (doi:10.1111/obr.12226)

Reis LO, Favaro WJ, Barreiro GC, de Oliveira LC, Chaim EA, Fregonesi A & Ferreira U 2010 Erectile dysfunction and hormonal imbalance in morbidly obese male is reversed after gastric bypass surgery: a prospective randomized controlled trial. *International Journal of Andrology* **33** 736–744. (doi:10.1111/j.1365-2605.2009.01017.x)

Reis LO, Zani EL, Saad RD, Chaim EA, de Oliveira LC & Fregonesi A 2012 Bariatric surgery does not interfere with sperm quality—a preliminary long-term study. *Reproductive Sciences* **19** 1057–1062. (doi:10.1177/1933719112440747)

Roth MY, Amory JK & Page ST 2008 Treatment of male infertility secondary to morbid obesity. *Nature Clinical Practice: Endocrinology and Metabolism* **4** 415–419. (doi:10.1038/ncpendmet0844)

Santolaria F, Perez-Cejas A, Aleman MR, Gonzalez-Reimers E, Milena A, de la Vega MJ, Martinez-Riera A & Gomez-Rodriguez MA 2003

Low serum leptin levels and malnutrition in chronic alcohol misusers hospitalized by somatic complications. *Alcohol and Alcoholism* **38** 60–66. (doi:10.1093/alcalc/agg015)

Sarais V, Pagliardini L, Rebonato G, Papaleo E, Candiani M & Vigano P 2016 A comprehensive analysis of body mass index effect on in vitro fertilization outcomes. *Nutrients* **8** 109. (doi:10.3390/nu8030109)

Schliep KC, Mumford SL, Ahrens KA, Hotaling JM, Carrell DT, Link M, Hinkle SN, Kissell K, Porucznik CA & Hammoud AO 2015 Effect of male and female body mass index on pregnancy and live birth success after in vitro fertilization. *Fertility and Sterility* **103** 388–395. (doi:10.1016/j.fertnstert.2014.10.048)

Schneider G, Kirschner MA, Berkowitz R & Ertel NH 1979 Increased estrogen production in obese men. *Journal of Clinical Endocrinology and Metabolism* **48** 633–638. (doi:10.1210/jcem-48-4-633)

Sermondade N, Massin N, Boitrelle F, Pfeffer J, Eustache F, Sifer C, Czernichow S & Levy R 2012 Sperm parameters and male fertility after bariatric surgery: three case series. *Reproductive BioMedicine Online* **24** 206–210. (doi:10.1016/j.rbmo.2011.10.014)

Sermondade N, Dupont C, Faure C, Boubaya M, Cedrin-Durnerin I, Chavatte-Palmer P, Sifer C & Levy R 2013*a* Body mass index is not associated with sperm-zona pellucida binding ability in subfertile males. *Asian Journal of Andrology* **15** 626–629. (doi:10.1038/aja.2013.10)

Sermondade N, Faure C, Fezeu L, Shayeb AG, Bonde JP, Jensen TK, Van Wely M, Cao J, Martini AC, Eskandar M *et al*. 2013*b* BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. *Human Reproduction Update* **19** 221–231. (doi:10.1093/humupd/dms050)

Shayeb AG, Harrild K, Mathers E & Bhattacharya S 2011 An exploration of the association between male body mass index and semen quality. *Reproductive BioMedicine Online* **23** 717–723. (doi:10.1016/j.rbmo.2011.07.018)

Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM, Markan KR, Nakano K, Hirshman MF, Tseng YH *et al*. 2013 Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. *Journal of Clinical Investigation* **123** 215–223. (doi:10.1172/JCI62308)

Stanton PG 2016 Regulation of the blood-testis barrier. *Seminars in Cell and Developmental Biology* **59** 166–173. (doi:10.1016/j.semcdb.2016.06.018)

Swerdloff RS, Batt RA & Bray GA 1976 Reproductive hormonal function in the genetically obese (ob/ob) mouse. *Endocrinology* **98** 1359–1364. (doi:10.1210/endo-98-6-1359)

Tena-Sempere M, Manna PR, Zhang FP, Pinilla L, Gonzalez LC, Dieguez C, Huhtaniemi I & Aguilar E 2001 Molecular mechanisms of leptin action in adult rat testis: potential targets for leptin-induced inhibition of steroidogenesis and pattern of leptin receptor messenger ribonucleic acid expression. *Journal of Endocrinology* **170** 413–423. (doi:10.1677/joe.0.1700413)

Tunc O, Bakos HW & Tremellen K 2011 Impact of body mass index on seminal oxidative stress. *Andrologia* **43** 121–128. (doi:10.1111/j.1439-0272.2009.01032.x)

Valles AS, Aveldano MI & Furland NE 2014 Altered lipid homeostasis in Sertoli cells stressed by mild hyperthermia. *PLoS ONE* **9** e91127. (doi:10.1371/journal.pone.0091127)

van der Lely AJ, Tschop M, Heiman ML & Ghigo E 2004 Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocrine Reviews* **25** 426–457. (doi:10.1210/er.2002-0029)

Vigueras-Villasenor RM, Rojas-Castaneda JC, Chavez-Saldana M, Gutierrez-Perez O, Garcia-Cruz ME, Cuevas-Alpuche O, Reyes-Romero MM & Zambrano E 2011 Alterations in the spermatic function generated by obesity in rats. *Acta Histochemica* **113** 214–220. (doi:10.1016/j.acthis.2009.10.004)

Wagner IV, Kloting N, Atanassova N, Savchuk I, Sprote C, Kiess W, Soder O & Svechnikov K 2016 Prepubertal onset of obesity negatively impacts on testicular steroidogenesis in rats. *Molecular and Cellular Endocrinology* **437** 154–162. (doi:10.1016/j.mce.2016.08.027)

Wang C & Swerdloff RS 2014 Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. *Fertility and Sterility* **102** 1502–1507. (doi:10.1016/j.fertnstert.2014.10.021)

Wang X, Sharma RK, Gupta A, George V, Thomas AJ, Falcone T & Agarwal A 2003 Alterations in mitochondria membrane potential and oxidative stress in infertile men: a prospective observational study. *Fertility and Sterility* **80** (Supplement 2) 844–850. (doi:10.1016/S0015-0282(03)00983-X)

Wang H, Wang H, Xiong W, Chen Y, Ma Q, Ma J, Ge Y & Han D 2006 Evaluation on the phagocytosis of apoptotic spermatogenic cells by Sertoli cells in vitro through detecting lipid droplet formation by Oil Red O staining. *Reproduction* **132** 485–492. (doi:10.1530/rep.1.01213)

Wei Y, Yang CR, Wei YP, Zhao ZA, Hou Y, Schatten H & Sun QY 2014 Paternally induced transgenerational inheritance of susceptibility to diabetes in mammals. *PNAS* **111** 1873–1878. (doi:10.1073/pnas.1321195111)

Weight-Control Information Network 2012 *Overweight and Obesity Statistics*. WIN 2010. https://www.niddk.nih.gov/health-information/health-statistics/Pages/overweight-obesity-statistics.aspx (assessed January 2017).

Yan WJ, Mu Y, Yu N, Yi TL, Zhang Y, Pang XL, Cheng D & Yang J 2015 Protective effects of metformin on reproductive function in obese male rats induced by high-fat diet. *Journal of Assisted Reproduction and Genetics* **32** 1097–1104. (doi:10.1007/s10815-015-0506-2)

Yang Q, Zhao F, Hu L, Bai R, Zhang N, Yao G & Sun Y 2016 Effect of paternal overweight or obesity on IVF treatment outcomes and the possible mechanisms involved. *Scientific Reports* **6** 29787. (doi:10.1038/srep29787)

Young RA, Frink R & Longcope C 1982 Serum testosterone and gonadotropins in the genetically obese male Zucker rat. *Endocrinology* **111** 977–981. (doi:10.1210/endo-111-3-977)

Zhu J, Tang W, Mao J, Li J, Zhuang X, Liu P & Qiao J (2015). Effect of male body mass index on live-birth sex ratio of singletons after assisted reproduction technology. *Fertility and Sterility* **104** 1406–1410 .e1401–1402. (doi:10.1016/j.fertnstert.2015.08.017)

Received 9 January 2017
First decision 30 January 2017
Revised manuscript received 4 March 2017
Accepted 9 March 2017