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INVITED REVIEW

Male fertility

Male obesity and subfertility, is it really about increased adiposity?

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The prevalence of overweight and obesity in reproductive-aged men is increasing worldwide, with >70% of men >18 years classified as overweight or obese in some western nations. Male obesity is associated with male subfertility, impairing sex hormones, reducing sperm counts, increasing oxidative sperm DNA damage and changing the epigenetic status of sperm. These changes to sperm function as a result of obesity, are further associated with impaired embryo development, reduced live birth rates and increased miscarriage rates in humans. Animal models have suggested that these adverse reproductive effects can be transmitted to the offspring; suggesting that men's health at conception may affect the health of their children. In addition to higher adiposity, male obesity is associated with comorbidities, including metabolic syndrome, hypercholesterolemia, hyperleptinemia and a pro-inflammatory state, all which have independently been linked with male subfertility. Taken together, these findings suggest that the effects of male obesity on fertility are likely multifactorial, with associated comorbidities also influencing sperm, pregnancy and subsequent child health.

INTRODUCTION

Overweight/obesity is a global health problem that is reaching epidemic proportions with 2.1 billion adults classified as overweight or obese.[1] Since the 1970's the rates of overweight and obesity in reproductive-age men has nearly tripled, such that in westernized countries between 65% and 70% of adult men are now overweight or obese (Australia 68.2%, Canada 64.5%, USA 70.9% and UK 66.6%).[1] There is an increasing awareness that male overweight/obesity reduces sperm quality, and in particular alters the physical and molecular structure of sperm,[2–4] which is coincident with a growing number of couples requiring intra-cytoplasmic sperm injection (ICSI) for the treatment of male factor sperm defects.[5,6] In conjunction, obesity is also associated with a number of chronic states including metabolic syndrome, hyperlipidemia, cardiovascular disease and a pro-inflammatory state. Interestingly, metabolic syndrome, hyperlipidemia and a pro-inflammatory state are all independently linked with male subfertility.[7] It, therefore, remains to be determined how obesity elicits its effects on sperm, whether through higher levels of adiposity, an associated comorbidity or a combination of both. This review will provide an update on the current literature, as well as present some discussion around how comorbidities associated with obesity may be related to specific changes in sperm function and pregnancy health.

MALE OBESITY AND HYPOGONADISM

Examining the effect of obesity on the hormone regulation of spermatogenesis is underpinned by the hypothesis that the hypothalamic pituitary gonadal (HPG) axis is deregulated by obesity. Several studies document that increased male body mass index (BMI)

is associated with decreased plasma concentrations of sex hormone binding globulin (SHBG) testosterone, and a concomitant increased plasma concentration of estrogen.[8–18] Lower testosterone and higher estrogen concentrations have long been associated with subfertility and reduced sperm counts through disruption of the negative feedback loop of the HPG axis, and are, therefore, common clinical markers of male fertility.[19] The Sertoli cell is of particular interest in male subfertility as it is the only somatic cell in direct contact with the developing germ cell providing both physical and nutritional support. Adhesion of the developing germ cells to the Sertoli cells is dependent on testosterone, with a decrease in testosterone levels leading to retention and phagocytosis of mature spermatids, and reducing sperm counts.[20,21] Other hormones involved in the regulation of Sertoli cell function and spermatogenesis, such as follicle-stimulating hormone/ luteinizing hormone (LH) ratios, inhibin B and SHBG levels, have all been observed to be lower in males with high BMI.[2–4,8–10,14]

MALE OBESITY IMPAIRS SPERM FUNCTION

Sperm parameters

The effect of male obesity on sperm parameters (count, motility and morphology) has been well documented in human and animal models. There is a general consensus that in rodent models diet-induced male obesity reduces sperm motility, decreases sperm counts with increases in epididymal sperm transit time, and decreases the percentage of sperm with normal morphology.[22–30] However, there is currently one rodent study that found no effect of high-fat diet feeding for 16 weeks on sperm motility.[24] This may be explained by the minimal increase in serum cholesterol in the high-fat fed group, an intermediary factor

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which previously had been negatively correlated with sperm motility independent to the treatment group in a similar mouse model.[31] In addition, it should be noted that a number of these rodent studies reported significant reductions in serum testosterone levels and altered glucose homeostasis in the high-fat diet fed males, which could exacerbate the changes to sperm function observed.

Currently, the impact of male obesity on sperm count, motility and morphology in humans is controversial, with many contradicting studies. For example, reduced progressive motility is only reported in 13 out of 35 reports (**Table 1**), while the number reporting decreased sperm with normal morphology is reported in just 9 of 29 papers (**Table 1**). The discrepancies observed in the literature likely result from several limitations that are inherent in human studies. First, these studies can be confounded by lifestyle factors such as smoking, alcohol consumption and recreational drug use as well as co-factors such as metabolic syndrome, all which can impair sperm function. Second, as the majority of studies originate from fertility clinics where patient cohorts are frequently biased toward subfertile men, this may also confound findings. Thirdly, some studies rely on self-reporting of parameters, such as lifestyle factors and BMI, which can lead to inaccurate reporting. The most recent systematic review established that there was a J-shaped curve correlation with male BMI and abnormal sperm count, with overweight and obesity associated with higher rates of oligozoospermia (low sperm count) and azoospermia (no sperm) through evaluation of 21 studies.[32] This contradicted the previous systematic review in 2010 which stated no such effect.[2] The earlier systematic review drew its conclusions based on five studies, as data from other studies could not be consolidated, and therefore may have underestimated the effect of male overweight and obesity on sperm count.

Male obesity reactive oxygen species and dna integrity

In recent years it has become increasingly apparent that the content and structure of sperm DNA is equally, if not more important, than traditional WHO sperm parameters for determining the ability of sperm to generate a healthy pregnancy.[33] One of the main contributors for impairing sperm DNA structure is reactive oxygen species (ROS) which is commonly elevated in subfertile men.[34] Sperm is highly susceptible to ROS, as the majority of their antioxidant defense mechanisms are lost during the shedding of the cytoplasm at the final stages of spermiogenesis.[35,36] Sperm DNA integrity is vital for fertilization and embryonic development with a number of studies showing clear negative associations between sperm with high DNA damage and pregnancy outcomes.[37–41] In addition, high levels of seminal ROS have been linked with lower embryo implantation rates following assisted reproductive technologies.[42–44] There is a clear consensus in current literature that male obesity is associated with higher levels of sperm DNA damage (10 out of 12 studies show an increase), despite the use of various methodologies to measure sperm DNA integrity (i.e., TUNEL, COMET and SCSA).[10,11,14,18,45–53] This rise in sperm DNA damage, as a result of increased adiposity, has also been confirmed in rodent models of male obesity.[26–31]

Only five studies until date, one human[11] and four rodent,[27–29,31] have directly linked levels of sperm oxidative stress with male obesity, concluding that a positive association exists between increasing adiposity and higher sperm/seminal plasma ROS levels. Rodent models suggest increased oxidative stress may also be present in testicular tissue, with alterations in oxidative stress genes (*Sod*, *Gsh-px*, *Catalse*, *Nrf2*, *Cyp2e1*, *Cyp19a1*, *Tnf* and *Pparg*) observed in testes of high fat diet fed males.[29,30] Therefore, it appears that male obesity may be associated

Table 1: Discordance in studies with male obesity and its effect on WHO sperm parameters

Study	Concentration	Motility	Morphology
Strain *et al.*[147]	Decreased	No change	N/A
Jensen *et al.*[17]	Decreased	No change	Decreased
Magnusdottir *et al.*[148]	Decreased	Decreased	N/A
Fejes *et al.*[149]	Decreased	No change	No change
Koloszár *et al.*[150]	Decreased	N/A	N/A
Kort *et al.*[45]	Decreased*	Decreased*	Decreased*
Qin *et al.*[151]	No change	No change	No change
Hammoud *et al.*[152]	Decreased	Decreased	Decreased
Pauli *et al.*[13]	No change	No change	No change
Aggerholm *et al.*[8]	No change	No change	No change
Nicopoulou *et al.*[153]	Decreased	N/A	N/A
Hofny *et al.*[15]	Decreased	Decreased	Decreased
Stewart *et al.*[154]	Decreased	N/A	N/A
Chavarro *et al.*[14]	No change	No change	No change
Shayeb *et al.*[155]	No change	No change	Decreased
Koloszár *et al.*[150]	Decreased	N/A	N/A
Sekhavat and Moein *et al.*[156]	Decreased	Decreased	N/A
Paasch *et al.*[10]	Decreased	No change	Decreased
Tunc *et al.*[11]	Decreased	No change	No change
Rybar *et al.*[49]	No change	No change	No change
Bakos *et al.*[107]	Decreased	Decreased	No change
Kriegel *et al.*[157]	No change	No change	Decreased
Fariello *et al.*[48]	No change	Decreased	No change
Dupont *et al.*[46]	No change	Decreased	No change
Hajshafiha *et al.*[158]	Decreased	N/A	N/A
Sermondade *et al.*[56]	No change	No change	No change
Merhi *et al.*[108]	No change	No change	N/A
Thomas *et al.*[47]	No change	Decreased	Decreased
Pyttel *et al.*[159]	N/A	Decreased	N/A
Colaci *et al.*[110]	No change	No change	No change
Hammiche *et al.*[160]	Decreased	Decreased	N/A
Fariello *et al.*[48]	Decreased	Decreased	No change
Eskandar *et al.*[161]	No change	No change	No change
Shayeb *et al.*[155]	No change	No change	Decreased
Rybar *et al.*[49]	No change	No change	No change
La Vignera *et al.*[18]	No change	Decreased	No change
Thomsen *et al.*[50]	No change	No change	No change
Hadjkacem Loukil *et al.*[162]	No change	No change	No change
Eisenberg *et al.*[51]	Decreased#	No change	No change
Macdonald *et al.*[52]	No change	No change	No change
Leisegang *et al.*[53]	Decreased	No change	No change

*Significant for NMS=Volume × concentration × %motility × %morphology;
#Linear decline in sperm concentration in relation to increasing waist circumferences;
N/A sperm measure not assessed. NMS: normal motile sperm

with significant perturbations to oxidative state and DNA integrity of sperm, and have repercussions not only on sperm function, but on the resultant embryo.[54]

Sperm binding and mitochondrial function

Sperm parameters, including sperm binding and sperm mitochondrial health, are increasingly used for assessing functionality of sperm. Currently, only two human studies[55,56] and two rodent models[27,31] have measured sperm binding in relation to male obesity. Interestingly, the two human studies contradict each other, with one[55] showing decreased sperm binding to hyaluronan coated slides as BMI increased, and the other[56] reporting no change to sperm binding to human zona

pellucida as a result of increasing BMI. These discrepancies could be related to differences in the populations of men selected in each study (included all types and cause of infertility), or the methods used for determining sperm binding (HBT assay as assessed in only provides an indirect assessment sperm binding).[55] In contrast, the two studies to date using rodent models of male obesity found that mice fed a high-fat diet had reduced sperm binding to mouse oocytes, which was directly related to reduced sperm capacitation and fertilization.[27,31] These studies demonstrate a lack of coherence and the need for more controlled studies to establish a relationship between male obesity and sperm binding.

Three recent studies in humans have assessed the effects of male obesity on sperm mitochondrial function.[18,48,53] Two of the studies (JC-1 and mitochondrial C oxidize), found that sperm from male patients of higher BMI exhibited lower mitochondrial activity and lower mitochondrial membrane potential,[18,48] whereas Leisegang *et al*. additionally found that patients with higher BMI had increasing numbers of sperm with disturbed mitochondrial membrane potential. It is hypothesized that a cascade of events will occur due to these poor lifestyle behaviors beginning with impaired mitochondrial function and resulting in the generation of ROS and initiation of DNA damage.[57–59] Therefore, higher rates of DNA damage and alterations to sperm motility described in patients with increasing BMI may be related to dysfunctional sperm mitochondria.

SEMINAL PLASMA COMPOSITION

Growing evidence suggests that the seminal plasma may help modulate sperm function and their ability to interact with the female reproductive tract.[60,61] Consequently, any changes to seminal plasma composition as a result of obesity may interfere with these processes. Fructose levels are significantly higher in seminal plasma of overweight and obese men[62] with adiponectin, progranulin and alpha-glucosidase levels significantly lower.[47,62] Fructose is a carbohydrate transported into sperm and is one of its main energy sources.[63] Whether the high fructose levels in seminal plasma of obese males can explain the changes to sperm mitochondrial function remains to be determined. Alpha-glucosidase is an enzyme that is involved in sperm motility acquisition[64] and its decreased levels in seminal plasma of patients with high BMI may contribute to their reported motility defects. The role of adipokines (adiponectin and progranulin) in seminal plasma and on sperm function remains unclear. However, it is hypothesized that higher levels of adipocytes from increased epididymal fat associated with obesity may intensify secretion of these molecules into epididymal fluid, altering sperm function.[47]

DOES ADIPOSITY ALONE EXPLAIN THE OBSERVED EFFECTS?

While increasing BMI and adiposity have shown a clear influence on male sperm function and DNA integrity, it is unknown whether the direct mechanism is from increased adiposity or an associated comorbidity. Although BMI provides a useful population measure of overweight and obesity, as it is the same for both sexes and all ages of adults, it can only be considered a guide, as the correlation between the level of adiposity and BMI can vary between individuals and does not always take into account differences in muscle mass.[65] A number of studies assessing male obesity and fertility have used waist circumference in conjunction with BMI,[51,53,66] as waist circumference in humans has been determined to be a better indicator for cardiometabolic disease risk.[67] However, a benefit of animal models is that more extensive measures of adiposity can be obtained, which

usually includes measures of body composition using dual-energy X-ray absorptiometry,[31,68] as well as postmortem analysis of total adiposity, weight gain,[26,69,70] measurement of individual adipose depots in the central body cavity,[27,71] or a combination of these.[31] Furthermore, most animal studies assess metabolic parameters of the males that can provide additional information as to their metabolic and pro-inflammatory state.[27,31,71,72]

A recent rodent model of male obesity examined the impact of diet and/or exercise interventions on obesity-induced male subfertility. Despite the fact that improvements to sperm function (motility, DNA damage, ROS and mitochondrial function) were seen in all intervention groups,[31] interestingly only the exercise intervention group displayed improved sperm function even though their adiposity levels remained higher compared to controls.[31] This suggests that adiposity alone is not the sole determinant in the impaired sperm function ascribed to male obesity, with glucose regulation and free fatty acid (FFA) metabolism independent predictors of sperm function.[31] Furthermore in humans, male obesity has been associated with higher seminal plasma insulin and leptin levels with these same metabolites similarly deregulated in serum.[53] Both leptin and insulin have been shown to be present in human semen and are vital for sperm motility, capacitation and fertilization.[73,74] Hence, these observations support the hypothesis that increased adiposity may not be the sole driver for the impaired reproductive function of obese males. This may in part, explain some of the contradictions in the current literature around the direct effects of obesity on sperm parameters, as to date, the majority of human studies have not assessed metabolic parameters of the males.

Glucose and insulin

Hyperinsulinemia and hyperglycemia are common comorbidities in obese males and are also intermediary factors of obesity in many rodent studies.[23,31,72] Hyperinsulinemia and hyperglycemia have been shown to have an inhibitory effect on sperm quantity and quality (in the absence of obesity).[75,76] Common perturbations to sperm function in obesity such as decreased count, increased ROS and sperm DNA damage, are also prevalent in diabetic men.[76,77] High circulating levels of plasma insulin and insulin levels in seminal plasma of obese men[53] may contribute to perturbed sperm mitochondrial dysfunction as a result of obesity through alterations in cholesterol influx during capacitation and dysregulation of sperm energy homeostasis.[73,74] Furthermore, higher concentration of insulin reduces the production of SHBG, indirectly increasing the amount of active unbound estrogens and testosterone (not bound by SHBG) in the bloodstream.[78] Similarly, increased levels of circulating glucose have been shown to reduce the amount of LH released by the anterior pituitary in sheep,[79,80] which may further impair the HPG axis and altered sperm function seen in both diabetic and overweight/obese men. There is emerging evidence that low testosterone levels can also induce aspects of metabolic syndrome, and that obesity may not be the direct cause of reduced sperm counts seen in these men but rather a symptom of the same low testosterone.[81–83] In addition, high circulating levels of glucose in men with type I diabetes, independent of obesity, increases sperm oxidative stress and oxidative DNA adducts[84] via increased oxidative stress.[85] This highlights that not only can dysregulation of glucose and insulin perturb sperm function and hypogonadism, but also likely alters sperm DNA integrity. Thus, hyperinsulinemia and hyperglycaemia may be intermediates between male obesity and altered sperm function and may also be unrelated causes of altered sperm function, independent to BMI.

Lipids

High serum cholesterol levels are commonly seen in men with increased adiposity.[86] High serum cholesterol concentrations without a marked increase to body weight in a rabbit model has previously been reported to cause sperm dysfunction, with reduced sperm motility, count, morphology, capacitation and semen volume reported.[87] A large human cohort has also determined the negative impact of dyslipidemia on sperm function, assessing systematic lipid regulation in 501 men over two semen samples.[88] They found that higher levels of serum total cholesterol, free cholesterol and phospholipids were associated with a significantly lower percentage of sperm with an intact acrosome and smaller sperm heads after adjusting for BMI.[88] These changes to sperm are proposed to occur in the epididymis, where high levels of circulating cholesterols cause degradation in the proximal epididymis leading to sperm morphological abnormalities, decreased motility and premature acrosome reaction in a rodent model.[89] In addition, exposures to increasing levels of cholesterols as well as FFA in both human and animal sperm *in vitro*, caused higher levels of sperm oxidative stress,[90] which in itself can alter embryo and pregnancy outcomes.[42–44] Furthermore, seminal plasma saturated fatty acid levels in humans have been negatively related to sperm motility and count.[91] Further evidence implicating lipid dysregulation as a causative factor for impairing sperm function have been shown in intervention models. Olive oil supplements specifically designed to reduce plasma cholesterols in a rabbit model of high fat diet feeding demonstrated improvements to sperm motility capacitation and membrane integrity in those rabbits with restored cholesterol homeostasis,[92] and highlights the importance of cholesterol and lipid regulation for male fertility.

Leptin

Leptin plays an important role in regulating appetite and body weight, with leptin resistance resulting in elevated serum leptin concentrations commonly seen in overweight and obese men.[93] More recently a role for leptin was discovered in male fertility. Mammalian sperm contains the leptin receptor with seminal plasma containing leptin.[94] Leptin is required for sperm capacitation as it enhances cholesterol efflux and protein tyrosine phosphorylation, enabling mammalian sperm to modulate their metabolism, motility, and nitric oxide production with systematic leptin concentrations.[73,95,96] Seminal leptin concentrations are commonly elevated in subfertile men and have been negatively correlated with progressive sperm motility.[97,98] Additionally, systemic leptin concentrations in humans have been linked with perturbed testicular function through hormonal dysregulation of Leydig cell function and testosterone production.[99] In comparison, a mouse model of leptin dysregulation (*ob/ob* mice) that had a spontaneous mutation in their leptin gene became obese and were infertile.[100] However, the fertility of *ob/ob* males can be restored by leptin treatment, with leptin-treated *ob/ob* males having restored testicular weights and histology resulting in normal pregnancies and offspring upon mating.[100] Interestingly, recovery of fertility is not restored through weight loss,[100] again suggesting that metabolic state and not just adiposity is a determinate of fertility.

Inflammation

Increased visceral adiposity is associated with chronic inflammation (pro-inflammatory state), which is characterized by the production of abnormal adipocytokines, including tumor necrosis factors and interleukins.[93] A pro-inflammatory state induced by infection, environmental toxins, smoking or vasectomy reversals in men is associated with subfertility phenotypes similar to male obesity, including reduced sperm counts, sperm motility, sperm morphology,

increased anti-sperm antibodies, increased sperm ROS and DNA damage.[101] A link between male obesity and testes inflammation has been shown in a mouse model of obesity (through high-fat diet feeding) and type 2 diabetes (low dose streptozotocin).[102] Pro-inflammatory factors including endoplasmic reticulum stress chaperones and inhibitory κBβ were higher in testicular tissue of obese and diabetic mice.[102] These pro-inflammatory factors were associated with Leydig cell dysfunction. With a decrease in the levels of mRNA and steroidogenic acute regulatory protein, insulin receptor substrate, activated IκBβ and ER stress chaperone C/EBP homologous protein, likely attributed to their hypogonadism.[102] In addition, tumor necrosis factor-α, which is more abundant in obesity,[93] has been shown to lower human sperm membrane permeability to Ca^{2+}, and affect Ca^{2+} regulation in sperm cells *in vitro*, likely through higher levels of ROS and lipid peroxidation,[103] further suggesting a link between poor sperm function and a pro-inflammatory state.

CONSEQUENCES OF MALE OBESITY AT CONCEPTION

There is increasing evidence that male obesity not only negatively affects sperm function but is implicated in influencing pregnancy and offspring health. An overweight or obese male partner, with a female of normal BMI, has an increased infertility as compared with couples of normal weight.[104,105] A small number of *in vitro* fertilization (IVF)-based studies suggest similar outcomes, with obesity in males associated with reduced clinical pregnancy, decreased live birth rates and an increase in pregnancy-loss in couples.[16,106–110] In part, this effect appears to be due to reduced blastocyst development, sperm binding and fertilization rates during IVF, when the male partner is overweight or obese.[107,108,110,111] However, the same effect cannot always be seen with ICSI. Some reports show reduced pregnancy rates from obese males during ICSI cycles,[109,110] and yet three studies[50,106,112] saw no effect of male obesity on fertilization or live birth rates following ICSI. This suggests that the process of ICSI could potentially bypass the impairment of sperm function resulting from male obesity. More studies would be welcomed on this topic as limitations regarding sample size, cycle numbers, known female and male factor infertility, classification of groups (overweight + obese [BMI >25 kg m^{-2}] or just obese [BMI >30 kg m^{-2}]), inclusion of female BMI, smoking status, and the use of either IVF or ICSI, are potential confounders.

Rodent models of male obesity[23,69,70,113] enable the investigation of invasive molecular markers of embryo viability that are not possible to conduct in humans. When male mice were fed a high fat diet, embryos had extended 1st, 2nd, 3rd cleavage times, reduced cavitation and compaction,[69,70,113,114] as well as reduced cell numbers in the inner cell mass and trophectoderm in the late blastocysts.[69,70,113,114] These abnormalities caused functional reductions in outgrowth when embryos were plated onto a fibronectin cell layer.[69] It has been hypothesized that changes to embryo development and cell numbers are caused by alterations to mitochondrial function. The embryos produced by high-fat fed male rodents display reduced mitochondrial membrane potential, suggestive of uncoupling of the mitochondrial electron transport chain.[69] Additionally, they display higher rates of the cellular glycolysis with an increased rate of glucose uptake and lactate production.[70] The adverse mitochondrial phenotypes in the developing embryo have previously shown alterations to implantation and fetal growth.[115,116] The reported changes to embryo cell numbers, metabolism and function from rodent models of male obesity are the likely causes of reduced implantation and pregnancy rates seen in both human and animal models. Taken together, the animal models of obesity and human clinical data suggest that the male obesity has a negative effect

on embryo health, implantation, pregnancy establishment, and live birth outcomes.

It has been widely accepted that maternal BMI and glucose status preconception, during gestation, and during lactation, can program the resultant children for obesity and diabetes.[117] Recent studies now suggest that paternal health at conception can also impact on offspring health. Obese fathers are more likely to father an obese child[118] with altered IGF-2 methylation patterns in cord blood of their children.[119] Rodent models of male obesity have shown obesity, insulin resistance, glucose intolerance, pancreatic islet cell dysfunction, and changes in white adipose transcriptional profiles in female and male offspring throughout two generations.[71,72,120] These studies have provided some of the first evidence that paternal obesity at conception can program offspring to adverse metabolic health outcomes.

How metabolites and inflammation associated with obesity may contribute to paternal programming of pregnancy and offspring health.

A number of obesity-related comorbidities (as mentioned above), have also been shown to reduce pregnancy and offspring health, independent of obesity. For example, high circulation levels of glucose in men with type I diabetes resulted in reduced live birth rates,[121] and in a rodent model, reduced offspring growth and weight into adulthood.[122] Furthermore, a paternal pro-inflammatory state is also associated with reduced pregnancy rates and higher susceptibility to cancer and childhood malformations in subsequent offspring.[123,124]

A likely contributing factor is increased sperm DNA fragmentation, which is commonly elevated in obesity and its associated comorbidities. A two-step hypothesis for the development of DNA damage from environmental perturbations in human sperm has been proposed by Aitken and Curry,[57] Aitken and De Iuliis.[125] The first step describes defective chromatin remodeling during spermiogenesis, with sperm released from the lumen of the seminiferous tubules in an imperfect state (altered protamination as well as a number of other structural defects), creating a sperm cell that is vulnerable to attack. The second-step states that as a result of poor protamination, the sperm are vulnerable to DNA strand breaks, likely mediated through oxidative stress which increases sperm apoptosis and DNA fragmentation. Current literature implicates circulating metabolites and inflammation as causative agents in the imperfect spermiogenesis and further suggests that this involves perturbations to epigenetic status of sperm. We hypothesize that circulating metabolites and a pro-inflammatory state have a multifactorial approach and can alter (1) protamination and microRNA abundance during spermiogenesis, (2) change the epididymal microenvironment during sperm maturation as well as (3) directly target epigenetic marks of mature sperm (**Figure 1**).

Protamination and microRNA abundance during spermatogenesis
Sperm protamination is usually incomplete with around 1% of histones remaining in murine sperm[126] and up to 15% residual in human sperm.[127] There is evidence that the retention of these histones is not random, with key pluripotent genes necessary for early embryo development remaining histone bound.[128] The process of histone to protamine transition is reliant on histone acetylation and their regulators. Both histone deacetylases and acetylases,[129] with histone acetylation, are thought of as epigenetic markers capable of being transmitted to the oocyte during fertilization. Histone deacetylases can be regulated by metabolic state with a mouse model of obesity, high cholesterol and triglyceride concentrations shown to alter histone acetylation during spermiogenesis, changing the gene expression
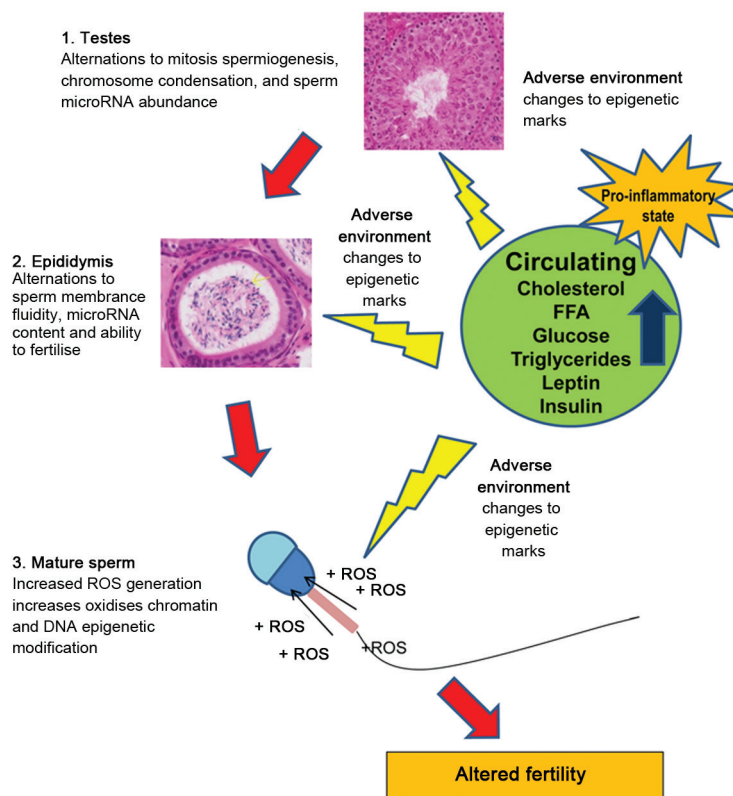

Figure 1: Hypothesis of how changes to circulating lipids, metabolites and a pro-inflammatory state associated with obesity may change the epigenetic status of sperm that ultimately cause subfertility.

and protein levels of SIRT6, a histone deacetylase.[24] Results showed increases in DNA damage in transitional spermatids, and in DNA damage and functional changes in mature sperm. Thus, changes to the regulation of histone deacetylases via alterations to serum lipids could result in perturbed or increased retention of histones and changes to histone acetylation, which could be inherited and alter offspring phenotypes. It is still to be determined whether these changes to histone deacetylase can be restored via interventions to reduce circulating lipids. However, preliminary studies which reversed high cholesterol levels via olive oil supplementation in rabbits showed improvements to sperm motility, capacitation and membrane integrity.[92]

MicroRNAs are important for the regulation of spermatogenesis and are present during the final stages of sperm maturation and during fertilization.[130] Evidence shows that the microRNA composition of spermatozoa can respond to environmental factors such as stress, and via this mechanism, make an important epigenetic contribution to the progeny persisting into future generations.[131] We previously reported dysregulation of microRNAs in the testes and mature sperm of rodent males fed a high-fat diet. This dysregulation of microRNAs altered levels of mRNA targets involved in molecular networks in embryonic development (pluripotency), metabolic disease (leptin/insulin signaling and carbohydrate/lipid metabolism), transcriptional regulation, RNA posttranslational modification and inflammation.[71] However, it was recently shown that microRNA can regulate metabolic and pro-inflammatory state, specifically playing an important role in the regulation of lipid and glucose homeostasis, and cytokine secretion.[132,133] Increases to serum cholesterol concentrations, glucose levels and interleukins are associated with changes to serum microRNAs.[134,135] As sperm microRNAs can alter the phenotype of the embryo and subsequent offspring,[136] changes to metabolic health and inflammatory states of males could result in abundance of sperm microRNAs. This may form the basis for altered sperm microRNAs in rodent models of obesity via high fat diet feeding.

Epididymal microenvironment

The epididymal endothelium transports proteins to the surface of sperm necessary for sperm maturation, which is proposed to occur through epididymosomes.[137] A recent study has shown that these epididymosomes can also transport microRNAs.[138] The epididymal endothelial ion transporters are operational between the circulating blood and epididymial lumen,[89,90] suggesting that increases to serum metabolites, pro-inflammatory state, or changes to the secretions of endothelium can alter sperm membrane fluidity, and therefore enhance or reduce susceptibility to damage. Hypercholesterolemia is able to alter the structure of epididymal endothelium and potential function,[89] while inflammation, as a result of epididymitis, may increase the influx of neutrophils and macrophages to the epididymial endothelium resulting in higher cytokine expression and apoptosis.[139] This indicates that changes to circulating metabolites and/or a pro-inflammatory state may alter epididymal endothelium function, modifying the epididymosomes content (i.e., protein and microRNA content) delivered to sperm.

Mature sperm

Both *in vivo* and *in vitro* studies have shown that increased exposure to cholesterol, FFAs, glucose insulin, and high levels of inflammatory cytokines alter sperm metabolism, reducing sperm motility and increasing sperm oxidative damage. Due to a lack of cytoplasmic scavenging enzymes and high levels of polyunsaturated fatty acids, sperm are highly susceptible to oxidative damage.[125] Increased levels of ROS are associated with changes to global methylation profiles of sperm.[140]

Further hypomethylation of imprinting genes and repeat elements in sperm are linked with reduced pregnancy rates.[141,142] Sperm harbor the oxidative form of 5-methylcytosine (5-hydroxymethylcytosine)[143] which is now considered the 6th DNA base[144] and important during the early stages of pronuclear formation.[145] 5-methylcytosine has been shown to oxidize to 5-hydroxymethylcytosine in the presence of ROS.[146] It is, therefore, plausible that obesity-related changes to circulating metabolites and a pro-inflammatory state may alter the methylation profiles of sperm via ROS, and be passed onto the newly fertilized embryo.

CONCLUSION

It is being increasingly documented that male overweight/obesity has a negative impact on sperm DNA quality and therefore on the subsequent pregnancy and offspring health. However, discordance in current literature about overweight/obesity effects on sperm parameters supports the hypothesis that increased adiposity may not be the sole driver of impaired reproductive function in obese males, with comorbidities also influencing reproductive health. This may in part, help explain some of the contradictions in the current literature as to date, the majority of human studies have not assessed the metabolic or inflammatory state of their patient population (i.e., glucose and insulin tolerance and C-reactive protein), typically relying on BMI as a marker for adiposity. In addition, the adiposity of the female partner is not always taken into account given the frequency of both partners being overweight or obese. As altered metabolic or pro-inflammatory states are independently linked with perturbed sperm function and can alter the epigenetic and chromatin state of sperm, we should be looking beyond BMI in the clinic and assess the entire metabolic and inflammatory profile of the patient, developing individual patient plans for the treatment of subfertility. Future studies assessing the impact of increased adiposity, with or without altered metabolic health in humans, will help to determine the impact increased adiposity has on sperm function. Whether this is a secondary phenotype to altered blood metabolites, and/or a pro-inflammatory state as a result of poor nutrition and lack of exercise, is still to be determined.

AUTHOR CONTRIBUTIONS

NOM and ML wrote and edited the manuscript.

COMPETING INTERESTS

All authors declare no competing financial interests.

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