

Impact of obesity on male fertility, sperm function and molecular composition

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Male obesity in reproductive-age men has nearly tripled in the past 30 years and coincides with an increase in male infertility worldwide. There is now emerging evidence that male obesity impacts negatively on male reproductive potential not only reducing sperm quality, but in particular altering the physical and molecular structure of germ cells in the testes and ultimately mature sperm. Recent data has shown that male obesity also impairs offspring metabolic and reproductive health suggesting that paternal health cues are transmitted to the next generation with the mediator mostly likely occurring via the sperm. Interestingly the molecular profile of germ cells in the testes and sperm from obese males is altered with changes to epigenetic modifiers. The increasing prevalence of male obesity calls for better public health awareness at the time of conception, with a better understanding of the molecular mechanism involved during spermatogenesis required along with the potential of interventions in reversing these deleterious effects. This review will focus on how male obesity affects fertility and sperm quality with a focus on proposed mechanisms and the potential reversibility of these adverse effects.

Introduction

Obesity is a global health problem that is reaching epidemic proportions with 1.6 billion adults classified as overweight and an extra 400 million adults classified as obese.¹ It accounts for 7.5% of the total burden of disease² costing approximately \$21 billion dollars each year³ in Australia. Using Australia as an example of a westernised society, since the 1970s the rates of obesity in reproductive-age men has nearly tripled.⁴ This obesity is coincident with an increase in male infertility as evidenced by the increase in couples seeking artificial reproductive technologies (ART) especially intracytoplasmic sperm injection (ICSI).^{5,6} There is increasing awareness that male obesity reduces sperm quality, in particular altering the physical and molecular structure of germ cells in the testes and mature sperm for a review see refs.⁷⁻⁹ Furthermore, there is increasing evidence that paternal health cues can be passed to the next generation with male age associated with an increase in autistic spectrum disorders¹⁰ and

environmental exposures associated with increases in incidences of childhood disease.^{11,12} Alarming, there is now evidence in animal models that paternal obesity increases the susceptibility to obesity and diabetes in offspring, suggesting a possible mechanism in the amplification of these chronic diseases.^{13,14} Therefore, this review will focus on how male obesity affects fertility and sperm quality with a focus on proposed mechanisms for this altered spermatogenesis and the potential reversibility of these adverse effects.

Male Obesity Negatively Impacts Fertilization and Pregnancy

In the last 5–10 years it has been demonstrated that maternal obesity is associated with changes to the oocyte that negatively impact embryo development, which reduces subsequent pregnancy establishment after in vitro fertilization.¹⁵⁻¹⁷ Only recently in the last 2–3 years has the impact of an obese male partner on embryo development and pregnancy been assessed. Currently, there is mounting evidence that male obesity may be equally implicated in reducing fertility and embryo health. Couples with an overweight or obese male partner, with a female of normal body mass index (BMI), have increased odds ratio for increased time to conceive compared with couples with normal weight male partners.^{18,19} A limited number of clinical studies suggest similar outcomes. With obesity in males associated with decreased pregnancy rates and an increase of pregnancy loss in couples undergoing ART (Fig. 1).²⁰⁻²² In part, this effect appears to be due to reduced blastocyst development, sperm binding and fertilization rates during in vitro fertilization (IVF), when the male partner is overweight or obese.^{20,23} However, more studies would be welcomed on this topic as limitations regarding sample size, cycle numbers, known factor infertility and the use of either IVF or ICSI are potential cofounders. This is suggested in the Keltz et al.²² study where they did not see the same changes to fertilization and embryo development when sperm were injected directly into the oocyte suggesting that the process of ICSI was by passing some impairment of the sperm to bind and fertilize. Although, this is not surprising as animal models of obesity have shown that the capacitation status and sperm binding ability of high fat diet mice were impaired compared with controls^{24,25} suggesting that post ejaculation maturation was altered and can be bypassed by ICSI. These embryology based findings which have established that male obesity at the time of conception impairs

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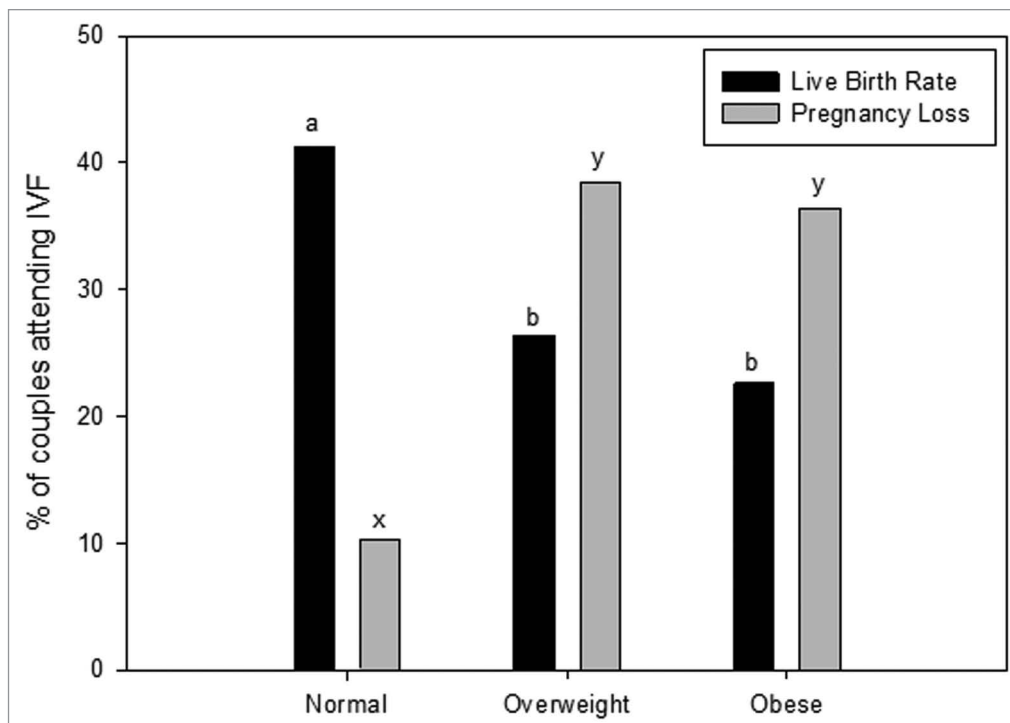


Figure 1. The effect of male obesity on pregnancy success in couples undergoing assisted reproductive technologies. Data taken from ref. 20 from 305 couples undergoing assisted reproductive technologies. BMI classification ranges, Normal (18.5 – 24.9 kg/m²), Overweight (25.0 – 29.9 kg/m²) and Obese (≥ 30 kg/m²). Data was analyzed through a multivariate analysis including both paternal and maternal BMI. Different letters denote significance at $p < 0.05$.

embryo health, therefore reducing implantation and live birth rates are paralleled by animal models of male obesity.^{26,27} This highly suggests a functional change to the molecular makeup of sperm that impacts directly on both sperm function but also on subsequent embryo development.

Paternal Obesity and Programming the Health of the Next Generation

It is widely accepted that nutritional challenges during gestation (including maternal obesity) program molecular changes in the developing fetus that result in increased susceptibility to adult chronic diseases for a review see refs.^{28,29} However less is known about the influence of paternal obesity on childhood and adult health. Epidemiology studies have concluded that obese fathers are more likely to father an obese child.^{30,31} Although, it must be noted that the extent of the individual contributions of genetic, epigenetic and environmental cannot be separated in these association studies due to the common raising environment of both father and child. In light of this limitation, animal models of paternal obesity have been developed, which have more directly demonstrated marked changes to both the metabolic and reproductive health of subsequent offspring.^{13,14,32} Data from a rat model of diet induced obesity and reduced glucose tolerance demonstrated that paternal obesity compromised pancreatic function through altered gene transcription and islet cell dysfunction in female offspring.¹³ Subsequently, a mouse model of diet induced obesity without reduced glucose tolerance showed that paternal

obesity compromised both first and second generation metabolic and reproductive health, with the female offspring additionally having increased fat mass demonstrating the first direct evidence of transmission of obesity.^{14,32} Significantly, F1 offspring had compromised gamete health with increased oxidative stress noted in sperm of male offspring and changes to oocyte mitochondrial function in female offspring.¹⁴ Taken together these data suggest that paternal obesity at the time of conception has a marked effect on offspring health therefore, directly implicating the sperm as the mediator for these changes, likely through a molecular mechanism that is transmitted to the resultant embryo and offspring (Fig. 2).

Male Obesity on Traditional Sperm Parameters

There are several studies that have investigated the impact of male obesity on the traditional sperm parameters mandated by the world health organization (WHO), namely sperm concentration, sperm motility and sperm morphology (summarized in Table 1). There is some evidence that male obesity reduces sperm concentration with 15 out of 23 recent studies showing this (Table 1). In contrast, there are many contradicting reports with regard to sperm motility (with 7/19 showing decreased motility) and morphology (7/16 showing decreased normal forms) and it is currently unclear if male obesity has an impact on these parameters (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 (*ie* smoking, alcohol consumption and recreational drug use) and co-pathologies, which can themselves impair sperm function. Second, the majority of studies originate from fertility clinics, where patient cohorts are frequently biased toward, sub-fertile men, which may also confound findings. Third, some studies rely on self-reporting of parameters such as lifestyle factors and BMI, which can lead to under reporting.

Due to these difficulties in interpreting data from human studies, rodent models of male obesity have now been established to assess the impact of male obesity on sperm function, however it is necessary to be aware of the differences between species. These studies have demonstrated that males fed a high fat diet to induce obesity had reduced sperm motility and a decrease in percentage of sperm with normal morphology,^{24,27,33-35} however it should be noted that a number of these studies had significant reductions in testosterone^{24,25} and altered glucose homeostasis²⁴ in their high fat diet groups which could be contributing to the results. Although there is some contention in the literature with regard to the effect male obesity has on traditional WHO sperm parameters, the changes reported indicate that the sperm are indeed compromised on more subtle levels.

Male Obesity on Sperm DNA Integrity and Oxidative Stress

While traditional WHO sperm parameters (sperm concentration and motility) are important measures of male fertility it is becoming increasingly apparent that the molecular structure and content of the sperm is equally important to the ability of a sperm to generate a healthy term pregnancy. Sperm DNA integrity is important for successful fertilization and normal embryonic development, as evidenced by sperm with poor DNA integrity being negatively correlated with successful pregnancies.³⁶⁻⁴⁰ Furthermore, sperm oxidative stress correlated with decreased sperm motility, increased sperm DNA damage, decreased acrosome reaction and lower embryo implantation rates following IVF.⁴¹⁻⁴³ Numerous human studies as well as an animal study have determined that a relationship between obesity and reduced sperm DNA integrity exists, despite the use of a variety of different methodologies to measure sperm DNA integrity (TUNEL, COMET, SCSA, etc).^{33,44-48} Only two studies, one human⁴⁹ and one rodent^{25,33} have directly linked levels of sperm oxidative stress with male BMI. Both studies concluded that a positive association between increasing BMI and increased sperm oxidative stress exists. In

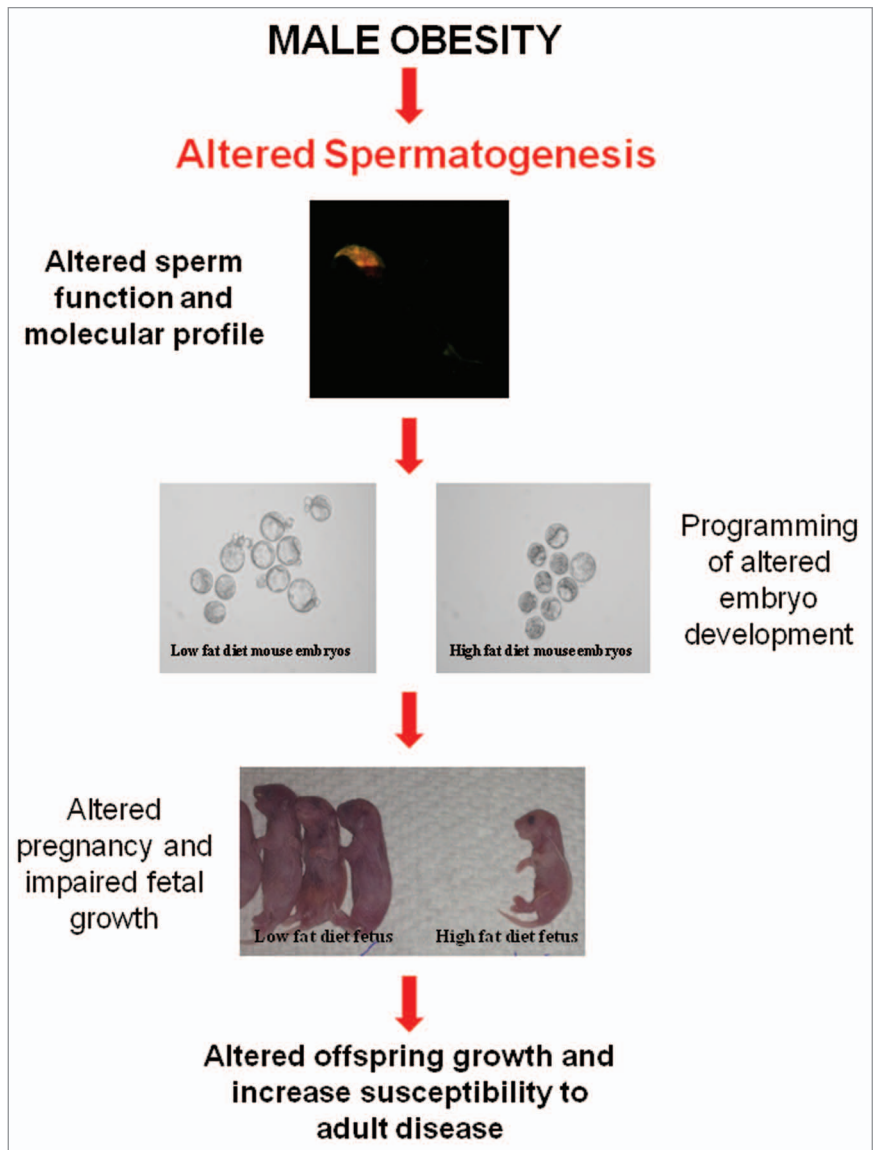


Figure 2. Hypothesis for the effect of male obesity on spermatogenesis and how it impacts offspring health. Paternal obesity in rodents has been shown to negatively impact the metabolic¹³ and reproductive health¹⁴ of offspring. Sperm are the likely mediator for altering the developmental profile of the embryo,^{20,26} fetes^{20,26} and then resultant offspring.^{13,14,30,31} This change is likely to be molecular in nature⁹⁹ and resulting from impaired spermatogenesis as a result of the obesity phenotype most likely occurring through changes to acetylation,³⁵ methylation or non-coding RNA status of sperm .

summary, there are conflicting reports about the interaction of male obesity with traditional WHO sperm parameters, but it is becoming clearer that male obesity is associated with significant changes to the molecular composition of sperm which has implications for its function but also for the resultant embryo.

Male Obesity and Altered Hormone Profiles

Spermatogenesis is a highly complex and selective processes whereby sperm are continually produced from the onset of puberty until death for a review see refs.^{50,51} This highly specialized process is under strict control from sex steroids, which in

Table 1. Summary of the studies investigating paternal obesity and their effect on basic sperm parameters

	Concentration	Motility	Morphology
Strain et al. ¹⁴⁶	Decreased	No change	n/a
Jensen et al. ⁵⁸	Decreased	No change	Decreased
Magnusdottir et al. ¹⁴⁷	Decreased	Decreased	n/a
Fejes et al. ¹⁴⁸	Decreased	No change	No change
Koloszar et al. ¹⁴⁹	Decreased	n/a	n/a
Kort et al. ⁴⁵	Decreased *	Decreased *	Decreased *
Qin et al. ¹⁵⁰	No change	No change	No change
Hammoud et al. ¹⁵¹	Decreased	Decreased	Decreased
Pauli et al. ⁵⁶	No change	No change	No change
Aggerholm et al. ⁵²	No change	No change	No change
Nicopoulou et al. ¹⁵²	Decreased	n/a	n/a
Hofny et al. ¹⁵³	Decreased	Decreased	Decreased
Stewart et al. ¹⁵⁴	Decreased	n/a	n/a
Chavarro et al. ¹⁵⁵	No change	No change	No change
Shayeb et al. ¹⁵⁶	No change	No change	Decreased
Koloszar et al. ¹⁴⁹	Decreased	n/a	n/a
Sekhvat et al. ¹⁵⁷	Decreased	Decreased	n/a
Paasch et al. ⁵⁴	Decreased	No change	Decreased
Tunc et al. ⁴⁹	Decreased	No change	No change
Rybar et al. ¹⁵⁸	No change	No change	No change
Bakos et al. ²⁰	Decreased	Decreased	No change
Kriegel et al. ⁴⁶	No change	No change	Decreased
Fariello et al. ⁴⁸	No change	Decreased	No change

*Significant for Normal Motile Sperm (NMS) = volume*concentration*% motility*%morphology.

turn are regulated by the hypothalamus, pituitary and Leydig and Sertoli cells located in the testes for a review see refs.^{50,51} Examining the effect of obesity on this process is underpinned by the hypothesis that the hypothalamic pituitary gonadal (HPG) axis is deregulated by obesity.

Several studies document that increased male BMI is associated with reduced plasma concentrations of SHBG and therefore testosterone and a concomitant increased plasma concentration of estrogen.^{21,44,49,52-58} Decreased testosterone and increased estrogen have long been associated with sub fertility and reduced sperm counts by disrupting the negative feedback loop of the HPG axis and are therefore common markers of fertility for a review see ref.⁵⁹ The Sertoli cell is the only somatic cell in direct contact with the developing germ cells by providing both physical and nutritional support and are therefore are of interest in male infertility. Adhesion of Sertoli cells to the developing germ cells is dependent on testosterone, with decreased testosterone leading to retention and phagocytosis of mature spermatids and therefore reducing sperm counts.^{60,61} Other hormones involved in the regulation of Sertoli cell function and spermatogenesis, such as FSH/LH ratios, inhibin B and Sex Hormone Binding Globulin levels have all been observed to be decreased in males with increased BMI for a review see refs.⁷⁻⁹ With mouse knockout models including both loss of FSH or FSH receptor associated

with decreased testis weight, and sperm output due to a reduction in Sertoli cell numbers.^{62,63} Therefore it remains plausible that the decreased sperm counts observed in male obesity are at least in part a result of changes to the HPG axis through testosterone and estrogen and likely reduced Sertoli cell function.

Obesity, Metabolic Syndrome and Fertility

Hyperinsulinemia and hyperglycemia is a common occurrence in obese individuals and are constant confounding factors in many rodent studies of male obesity.^{13,24,27} Hyperinsulinemia and hyperglycemia have been shown to have an inhibitory effect on sperm quantity and quality and therefore could be attributing to the reduced fertility seen in obese men for a review see refs.^{64,65} With commonly altered makers of sperm function such as decreased count, increased reactive oxygen species and sperm DNA damage also seen in diabetic patients for a review see refs.^{65,66} High circulating levels of insulin is suggested as one possible mechanism for the above effects with increased insulin reducing the production of SHBG in the liver thereby indirectly increasing the amount of active unbound estrogens and testosterone (not bound by SHBG) in the blood stream.⁶⁷ The decreased levels of SHBG to sustain homeostatic levels of testosterone could contribute to the decreased levels of testosterone and decreased sperm counts seen in these patients. Increasing levels of circulating glucose have also been shown to reduce the amount of LH released by the anterior pituitary in sheep^{68,69} and therefore could contribute to the impaired HPG axis and altered sperm parameters seen in diabetic and overweight and obese men. Additionally, there is emerging evidence that low testosterone levels can also induce aspects of metabolic syndrome and therefore obesity may not be the direct cause of reduced sperm counts seen in these men but a symptom of the same low testosterone.⁷⁰⁻⁷²

Interaction of Adipose Tissue with Hormonal Regulation

A working hypothesis proposed that elevated estrogens in obese men may in part result from the increased mass of white adipose tissue. White adipose tissue is responsible for aromatase activity and adipose derived hormones and adipokines, which are elevated in obese men.⁷³ Aromatase cytochrome P450 enzyme, is produced by many tissues including adipose tissue and testicular Leydig cells, and in men activity converts testosterone to estrogens.^{74,75} Due to obesity increased influx of white adipose tissue it is suggested that elevated estrogen concentrations may result from an increased conversion of androgens to estrogens by white adipose tissue therefore contributing to the increased plasma estrogen levels seen.⁷⁶⁻⁷⁸ Another key hormone produced by white adipose tissue is leptin, which plays a pivotal role in the regulation of energy intake and expenditure for a review see refs.^{79,80} Leptin mainly targets receptors in the hypothalamus by counteracting the effects of neuropeptide Y. However leptin receptors have recently been discovered in ovaries and testes, functioning to regulate the HPG axis.⁸¹⁻⁸⁵ Specifically, increased levels of leptin significantly decreased the production of testosterone from

Leydig cells.⁸⁶ Taken together this suggests that elevated leptin levels commonly found in obese males⁸⁷ could alter the HPG axis, thus contributing to the decreased testosterone production observed.

Interaction of Adipose Tissue on Testicular Temperature

One side effect of obesity that may potentially contribute to altered sperm production/parameters is raised gonadal heat resulting from increased scrotal adiposity. The process of spermatogenesis is highly sensitive to heat, with optimal temperature ranging between 34–35°C in humans.⁸⁸ Increased testicular heat is associated with reduced sperm motility, increased sperm DNA damage and increased sperm oxidative stress.⁸⁹⁻⁹¹ Changes to testicular temperature can occur via a number of mechanisms such as physical disorders (*ie* varicoceles), increased scrotal adiposity or environmental disturbances (*ie* prolonged bike riding) and are associated with reduced sperm function and sub fertility.⁹²⁻⁹⁸ It is therefore not surprising that increased testicular heat caused by increased adiposity in obesity has been proposed as a possible mechanism. It is noteworthy that increased sperm DNA damage and oxidative stress are commonly impaired in obese patients and that a single study which investigated the surgical removal of scrotal fat reported an improvement in sperm parameters.⁹⁵

Impact of Male Obesity on Molecular Aspects of Spermatogenesis

Recent data, which has shown that paternal health cues are transmitted to the next generation most likely via the sperm, has resulted in a renewed interest into the molecular function of sperm⁹⁹ and has helped lead to our current hypothesis (Fig. 2). The mechanisms inducing changes to sperm molecular composition are yet to be determined in obese individuals. However, several studies examining transgenerational effects^{13,14} have proposed epigenetic modifications to the sperm through changes to non-coding RNA content, methylation and acetylation status which are changed in obese individuals for a review see refs.^{99,100} Additional reports suggest that the proteomic profiles of sperm also differ between obese and non obese men.⁴⁶ It is now becoming increasingly accepted that the environment that the founder generation is exposed to impacts the phenotype of subsequent generations with the term 'transgenerational epigenetic inheritance' coined to reflect this phenomena.^{101,102} Rodent models of male diet induced obesity document impaired metabolic and reproductive phenotypes in F1 offspring^{13,14,32} and therefore suggest that transgenerational epigenetic inheritance is involved.

Methylation. Methylation of DNA and histones is dynamic during spermatogenesis and is vital for the normal processes of spermatogenesis and fundamental for a successful pregnancy. Changes to sperm methylation is required and essential for X chromosome inactivation during meiosis and for the establishment of paternally imprinted genes in sperm.^{103,104} Generally, hypermethylated DNA at promoter regions inhibits gene expression by excluding transcription factor binding. In contrast,

hypomethylation generally allows increased access of transcription factors to the DNA and increases gene expression. It is estimated that 96% of the genomic CpGs in sperm DNA are usually methylated, although there are site specific variations of methylation in mature sperm.¹⁰⁵

Analysis throughout human spermatogenesis has determined that DNA methyltransferase proteins (DNMT 1, 3A, 3B) are present during the spermatogenic cycle as knockout studies result in changes to sperm methylation and in some cases sperm function.¹⁰⁶ The stage specific changes in nuclear localization of these three proteins during spermatogenesis coincides with the establishment of the methylation imprints in the spermatogonia. Subsequent maintenance of these imprints occurs throughout the remainder of spermatogenesis suggesting methylation imprints are key molecular events during spermatogenesis.¹⁰⁷

There is some evidence that the methylation status of sperm DNA is associated with sub-fertility. Hypomethylation of imprinted genes and repeat elements in sperm have been linked with reduced pregnancy success and correlate with increased sperm DNA damage in males undergoing fertility treatment.¹⁰⁸⁻¹¹¹ Additionally, altered levels of methylation in the promoter regions of genes such as MTHFR are associated with decreased sperm function. Further, imprinted regions such as H19 and ALU repeat elements are more likely to be hypomethylated in subfertile men.^{110,111}

Environmental exposures have also been linked with changes to methylation status of sperm. Toxins such as exposure to 5-aza-2'-deoxycytidine, tamoxifen and chemotherapy agents disturb the de novo methylation activity in sperm as shown in animal models.¹¹²⁻¹¹⁴ This aberrant methylation was observed at imprinted regions such as *Igf2* and *H19*. Subsequently this lead to a disruption of the DNA methylation reprogramming of the male pronucleus, which in turn increases post implantation pregnancy loss.¹¹²⁻¹¹⁴ Moreover, excessive alcohol consumption in men been associated with site-specific hypomethylation in sperm,¹¹⁵ a finding confirmed in animal models.¹¹⁶ Excessive alcohol consumption impacts negatively on offspring prenatal growth and also alters the methylation status of offspring DNA.^{117,118}

To date there is little information as to the impact of obesity on the methylation status of DNA originating from male germ cells, however obesity has been shown to alter the methylation status of DNA originating from other tissues for a review see ref.¹¹⁹ Whether the metabolic and reproductive changes observed in offspring as well as reduced fertilization and increased pregnancy loss induced from paternal obesity results from alterations to de novo methylation patterns of developmental genes in the male germ line is yet to be determined.

Acetylation. Histone acetylation is vital for spermatogenesis to proceed and is necessary and essential for the removal of histones so they can be replaced by protamines during spermiogenesis. Furthermore, histone acetylation is essential to relax chromatin structure that allows for the repair of the DNA double and single strand breaks that result.¹²⁰ Protamination is required to enable the tight packing of the DNA that occurs within the sperm head, which aids in the protection against DNA damage in the absence of normal cellular defenses that

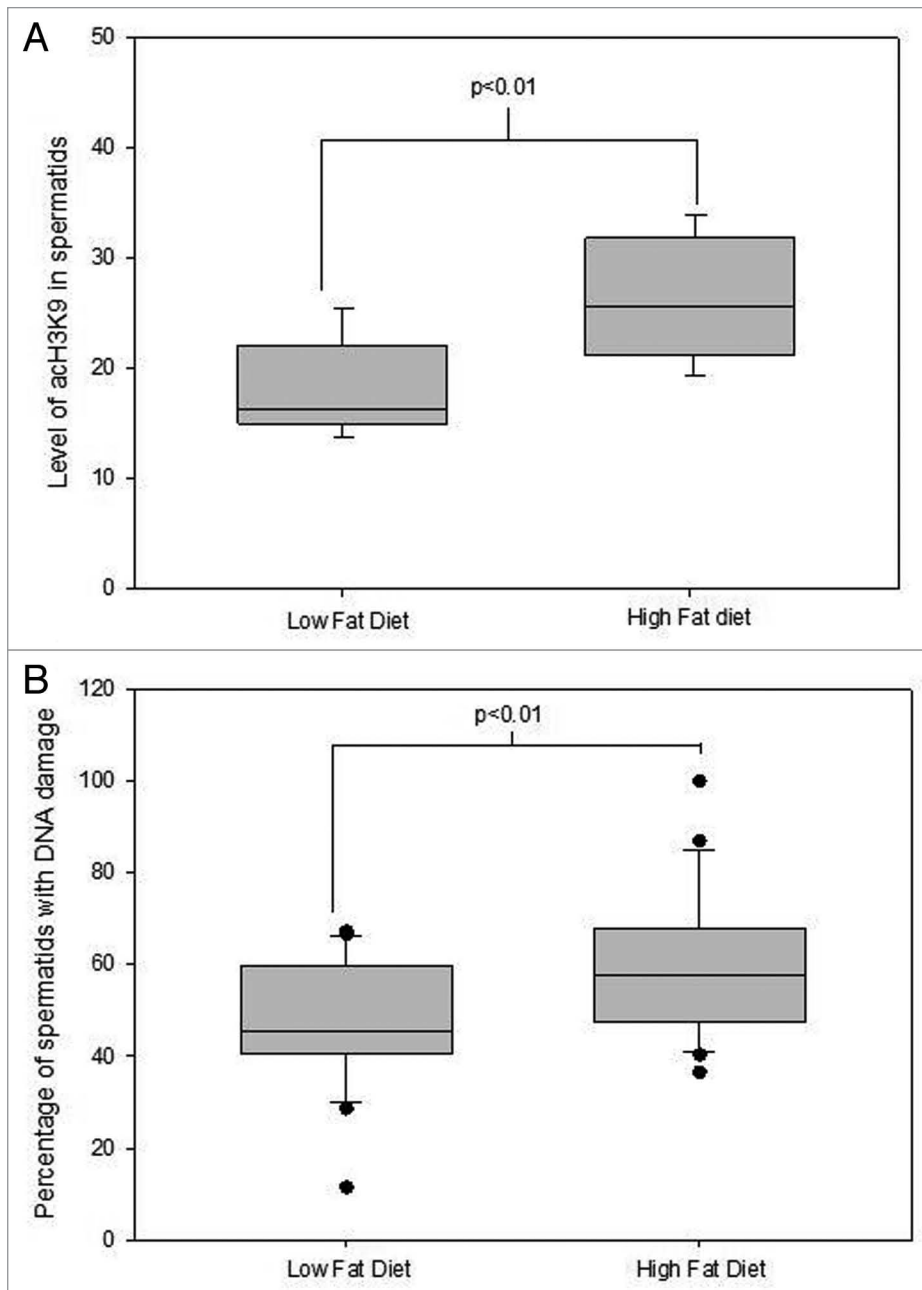


Figure 3. The effect of diet induced obesity in C57BL6 mice on Acetylation and DNA damage levels in spermatids during protamination. Data taken from.³⁵ Data was analyzed through a univariate general linear model with replicate fitted as a covariate and mouse ID as a random factor. Correlation data was determined by a Pearson's Rho. (A) The effect of diet induced obesity in mice on acetylation levels of H3K9 in elongating spermatids representative of > 120 spermatids from at least 5 mice per treatment group. (B) The effect of diet induced obesity in mice on DNA damage levels in elongating spermatids representative of > 4000 spermatids from at least 5 mice per treatment group. There was a negative correlation found between acetylation levels of H3K9 and DNA damage levels in round spermatids $R^2 = 0.60$, $p = 0.023$ $n = 8$ animals.

are greatly diminished by the shedding of the cytoplasm during epididymial transport. The histone to protamine transition is incomplete with roughly 1% of histones remaining in mature murine sperm.¹²¹ Curiously, up to 15% of histones are retained in human mature sperm.¹²²

acetylation represent a potential epigenetic basis for the programming observed in resultant embryos and sired by obese males.¹⁰⁶

RNA and small non-coding RNA. The long held dogma was that sperm were transcriptionally and translationally silent and that the small amounts of RNA contained were thought

There is evidence that the retention of these histones during protamination is not random with key pluripotency regulating genes remaining histone bound (*ie Nanog, Oct4 and Sprouty*).¹²³ Therefore these loci are capable of normal somatic cell histone modifications.¹²³ Thus, alterations to histone acetylation at such loci due to environmental cues could result in epigenetic modifications to sperm that might form the basis of paternal programming of offspring.

The N-terminus of histones is a key region attracting post translational modifications such as acetylation. Acetylated histones in mature sperm are thought to represent epigenetic marks capable of transmission to the oocyte during fertilization and regulate gene expression in early embryogenesis. Given that key pluripotency genes retain histones it is proposed that this is in readiness for immediate activation of expression of these genes post fertilization.¹²³ Hyperacetylation of H4 and H3 is required for normal spermiogenesis and for appropriate replacement of histones by protamines during spermiogenesis. Studies exploring the roles of histone deacetylases (HDAC) found that germ cells treated with HDAC inhibitors, result in premature hyperacetylation of late round spermatids.¹²⁴⁻¹²⁶ The functional consequence of this early hyperacetylation is still to be fully understood, however studies indicate that an increased rate of DNA damage occurs as the result.^{125,127} Interestingly, male mice fed a high fat diet similarly displayed altered acetylation status in late round spermatids which also correlated with increased DNA damage in the germ cells³⁵ (Fig. 3). Alterations to sperm histone acetylation correlates with poor protamination, which in turn positively correlates with increased DNA damage in mature sperm and therefore potentially contributes to poor sperm parameters observed in obese males.¹²⁸⁻

¹³⁰ Taken together, alterations to histone

to be remnants left over from spermatogenesis.¹³¹ However, it is now evident that mature sperm contain a regulated suite of both mRNA and other non-coding RNA that are suggested to be important for normal fertilization and subsequent embryonic development, with active transcription and translation occurring in the sperm's mitochondria.¹³²⁻¹³⁵ Although it is not yet clear what the precise role these RNAs play, it has been empirically proven that these RNA can cause phenotypic change in resultant offspring after injection into oocytes, albeit at amounts that far exceed biological concentrations.¹³⁶ While to date there is little known about mRNA abundance in sperm from obese males, one rodent model of obesity and diabetes has shown significant differences in several mRNA within testes compared with lean controls.²⁷

Mature sperm also contain significant levels of small non-coding RNAs including silencing RNAs (siRNAs), microRNAs (miRNAs) and in a recent study piwi-interacting RNA (piRNAs).¹³⁷ Small non coding RNAs are 20–22 nucleotides (nt) in length and contain an abundance of stop codons and generally lack open reading frames.¹³⁸ They regulate at the level of both transcription and translation via control of chromatin organization, mRNA stability and protein synthesis. Interestingly, microRNAs also regulate methylation in several tissues.¹³⁹ Indeed, hypomethylation of repeat elements in the male germline has been associated with an increase in miR-29, which is predicted to downregulate DNMT3a, a protein necessary for establishing genomic methylation.¹⁴⁰

It is apparent that these RNAs have a role in the oocyte during fertilization and in embryo development, fetal survival and offspring phenotype. Alteration of microRNA abundance in the male pronucleus of recently fertilised zygotes produce offspring of phenotypes of variable severity depending on the ratios of microRNAs injected.¹⁴¹ One preliminary study reported that the microRNA profile is altered in sperm as a result of male obesity in rodents.¹⁴² However, the impact of these changes on fertilization and embryo health remains to be determined.

Reversibility

While it is becoming clearer that male obesity has negative impacts on fertility, sperm function and long-term impacts on the health burden of the offspring It is equally clear that simple interventions such as changes to diet and/or exercise can reverse both the disease state and the offspring outcomes. There is emerging evidence that intake of selenium enriched probiotics by obese rodents improves both their metabolic health and fertility

measures (sperm count and motility).¹⁴³ Furthermore, our recent studies of diet and exercise interventions in an obese mouse model have determined that sperm function is correlated with the metabolic health of the individual.²⁴ Improvements in metabolic health such as a return of plasma concentrations of glucose, insulin and cholesterol to normal levels result in improvements in sperm motility and morphology, concomitant with improvements to molecular composition such as reductions in oxidative stress and reduced DNA damage.²⁴ To date there is little information about the impact of diet/exercise intervention in obese men with regard to semen parameters in the human. The largest study to date examined 43 obese men during a 14 week residential weight loss program and demonstrated significant improvements to both total sperm count and sperm morphology in men who lost the greatest amounts of weight.¹⁴⁴ However, a recent case report of three patients who underwent bariatric surgery to achieve drastic weight loss demonstrated that sperm parameters worsened.¹⁴⁵ These parameters remained poorer two months post surgery in all patients and only one patient had minimal improvements after two years.¹⁴⁵ However, the impact of nutritional deficiencies that might persist even after surgical intervention, and metabolic health of these men were not studied. The full potential of diet and exercise interventions to restore the fertility of obese men and improve embryo and offspring outcomes are yet to be fully characterized.

Conclusion

There is emerging evidence that male obesity negatively impacts fertility through changes to hormone levels, as well as direct changes to sperm function and sperm molecular composition. Data from animal models implicate the nutritional status of the father as setting the developmental trajectory of resultant offspring. Both male and female offspring born to fathers with sub-optimal nutrition have a constellation of metabolic and reproductive health pathologies. Nutritionally induced alterations to both the physical and molecular composition of sperm evidently implicates it as the mediator of these impacts on both the father's fertility and the health of the next generation, sparking renewed research interest in spermatogenesis and the detrimental effects of obesity. Additionally with the recent animal studies showing that simple diet and exercise interventions can be used to reverse the damaging effects of obesity on sperm function, understanding the impacts will be important for the development of public health messages for men considering fatherhood.

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