Male infertility, characterized by hypogonadism, decreased semen quality or ejaculatory dysfunction, accounts for approximately 20% of infertility cases. Obesity and metabolic dysfunction have been identified, among other causal factors, to contribute to male infertility. In the context of the Western world’s ‘obesity epidemic’, this article discusses three main biological mechanisms linking obesity to impaired male reproductive function: hypogonadism, testicular heat stress/hypoxia-induced apoptosis and endocrine disruption by ‘obesogens’. Among these, obesity-induced hypogonadism is undoubtedly the most clinically significant and is easily assessed. Rapidly expanding areas of research in this area include leptin modulation of kisspeptins and hypothalamic–pituitary–testicular hormone pathways, and roles of other adipocytokines in male infertility, as well as the impact of exposure to obesogens on the quality of semen.

Keywords: endocrine disrupters • estrogen • hypogonadism • leptin • obesity • obesogens • semen

Mechanisms of obesity-induced male infertility


Approximately 30–40% of infertility cases can be attributed to problems with the male partner [1]. Obesity and related concomitant metabolic abnormalities are among the proposed causes of male infertility [2]. Metabolic syndrome has been characterized as a constellation of disorders, including Type 2 diabetes, coronary heart disease, obesity with visceral abdominal fat distribution, dyslipidemia, hypertension and impaired glucose metabolism/insulin resistance [3–5]. In the context of the ‘obesity epidemic’ in the Western world, this paper discusses three main biological mechanisms linking obesity to impaired male reproductive function. These mechanisms include hypogonadism, testicular heat-stress-/hypoxia-induced apoptosis and endocrine disruption by ‘obesogens’.

Spermatogenesis & the assessment of male fertility

Testicular function is regulated by the hypothalamic–pituitary–testicular (HPT) axis (Figure 1). Gonadotropin-releasing hormone (GnRH) is released by hypothalamic neurons and stimulates the release of pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [2], both of which play major roles in the regulation of testicular steroidogenesis and spermatogenesis, respectively (Figure 2). The mature spermatozoa (sperm) are polarized cells, each consisting of a head, midpiece and tail (Figure 3). These structural entities of sperm are used as landmarks for morphological categorization. Testicular injury, disease or impairments of the HPT axis can produce abnormalities of spermatogenesis with consequences, including the production of fewer, abnormal or underdeveloped sperm, characterized by morphological defects or reduced motility. Further modifications of biochemical and kinetic properties of sperm take place in the female reproductive tract; a process known as capacitation, which includes the acquisition of hyperactive motility (Figure 4) [6,7]. Once bound and penetrating the egg zona pellucida, sperm undergo the zona pellucida-induced acrosome reaction (Figure 3), an acrosomal exocytosis event that involves the release of hydrolytic acrosomal enzymes, followed by fusion of one of these acrosome-reacted sperm with the egg plasma membrane and sperm incorporation into the egg (fertilization).

The determination of spermatozoa concentration, morphology and motility remains the primary clinical tool for the assessment of male infertility [8]. Reproductive hormone profiles (free and total testosterone, estradiol, LH and FSH) in addition to semen parameters are typically used to assess male reproductive function. Development of clinical assays to delineate new parameters that better reflect sperm fertilizing competence are needed.
Adipogenesis & the assessment of obesity

Adipocytes are the main cellular constituents of the adipose tissue and play an important role in regulating triglyceride and free fatty acid levels. Adipocytes are derived from multipotent stem cells that differentiate into preadipocytes and, subsequently, mature adipocytes [9]. Estrogen is a positive regulator of adipogenesis, stimulating preadipocyte proliferation and growth of mature adipocytes [10]. By contrast, adipocyte differentiation and maturation is negatively regulated by androgens such that high androgen levels both drive differentiation of the multipotent stem cells toward myogenesis, thereby inhibiting adipogenesis [11], and inhibit adipogenic differentiation of existing preadipocytes [12]. Adipose tissue has endocrine function and synthesizes chemical messengers, known as adipokines or adipocyte-derived hormones, in addition to the aromatization of androgens to estrogens. Basal aromatase activity decreases during adipocyte maturation with tenfold less aromatase activity in mature adipocytes compared to preadipocytes. Aromatase activity is also site dependent with higher activity in preadipocytes in subcutaneous tissue compared with omental cells [13].

The obese male is generally characterized as having greater than 25% body fat of total body mass with a BMI in excess of 30 kg/m² [14]. BMI is used as the chief indicator of obesity, with stratified BMI categories as follows: 18.5–24.9 kg/m² (normal), 25 kg/m² and above (overweight) and 30 kg/m² and above (obese). Other more accurate methods to assess obesity include measurement of skinfold thickness [15], hydrostatic weighing, dual-energy x-ray absorptiometry (originally designed to measure bone mineral density, this technology differentiates between absorption of x-rays in bone vs soft tissue) or whole-body adipose tissue computed tomography (CT) and MRI combined with waist:hip ratio measurements [16].

Reproductive parameters in obese men

The relationship between semen quality and BMI has been examined in several observational studies (Table 1 & 2). Studies set in infertility clinics have the advantage of investigating obesity trends within infertile populations whereas populations of unselected healthy men across BMI categories enable evaluation of the association between obesity and fertility without a preconceived bias. Studies designed to measure sperm parameters for obese men tended to report a negative association between semen quality and BMI (Table 1) [17–21]. By contrast, the large INUENDO study of European infertility patients reports no reduction in sperm concentrations or sperm motility in the obese group, whereas moderately overweight men exhibit a small decrease in sperm concentrations [22], which is supported by more recent studies [23,24] that also report no associations between semen parameters and BMI. However, time-to-pregnancy studies support an association between subfecundity and high BMI (Table 1) [25–27]. Sperm concentration/total sperm count seem most likely to be negatively associated with BMI above 25 kg/m² (Table 2) [17–18,20,21,28] with reduced normal sperm morphology and motility less consistent across studies (Table 2).

Many population studies are limited by the small sample size of the obese study population, and tend to report overweight/obese data as a single group, which would be expected to reduce the strength of the associations measured. Possible explanations why these population studies are not more consistent could include lack of sensitivity of BMI as a measurement of adiposity, presence of subfertility, which may manifest by more subtle changes in testicular/sperm physiology not captured by traditional semen assessments, and finally, heterogeneity within the overweight/obese populations.

The association between infertility and obesity has also been examined using reproductive hormone profiles of infertility patients across BMI categories. BMI is negatively correlated with serum testosterone but positively correlated with serum estradiol in these patients [15,22,28,29]; consistent with original reports that visceral obesity in men is associated with decreased free- and total-testosterone levels [30–32] and with increased estrone and estradiol levels [33–35]. Therefore, it seems likely that important reproductive features of morbid obesity in men include hypogonadism, hyperestrogenemia and subfertility (Figure 5) [36].

Mechanisms of obesity-induced male infertility

Hypogonadism

Hypogonadism in males encompasses disrupted testicular functions, including deficient steroidogenesis and/or spermatogenesis. Hypogonadism is classified by the origin of dysfunction, either at the testis or within the HPT axis. Primary hypogonadism results from a testicular deficit (Figure 6A) and may be caused by genetic diseases, including Klinefelter’s syndrome or 5α-reductase deficiency, congenital abnormalities, including cryptorchidism or testicular feminization, or testicular insults, such as trauma, mumps orchitis,

Figure 1. Hypothalamic–pituitary–testicular axis. Systemic regulation of testicular function is provided by the hypothalamic–pituitary–testicular axis. Briefly, the hypothalamus releases GnRH, which acts on the anterior pituitary to release FSH and LH. FSH binds to receptors on testicular Sertoli cells to regulate spermatogenesis. LH binds receptors on testicular Leydig cells. FSH: Follicle-stimulating hormone; GnRH: Gonadotropin-releasing hormone; LH: Luteinizing hormone; T: Testosterone.
radiation or chemotherapy [1], testicular heat stress [37] or varicocele (associated with impaired testicular venous drainage) [38]. Primary testicular deficit is also classified as hypergonadotropic hypogonadism and is clinically characterized by hypogonadism (low free and/or total testosterone and low sperm production) with increased FSH and LH levels, caused by inadequate testosterone levels to provide negative feedback to the HPT axis. Hypogonadism may also be caused by deficits within the hypothalamus or pituitary, a so-called secondary hypogonadism, also termed hypergonadotropic hypogonadism (Figure 6B). This is characterized by low–normal FSH and LH levels and subsequently low testosterone, and is featured in men with Kallman’s syndrome, pituitary disorders and serious illnesses, such as AIDS [39]. We will discuss several hypogonadal mechanisms with either testicular or hypothalamic origins that explain the association between obesity and male hypogonadism.

Adipocyte-derived estrogen & hypogonadism

Adipocyte-derived estrogens in obese men provide feedback inhibition to the HPT axis, modulated by the presence of estrogen receptors (ER-α and -β) localized to the hypothalamus and pituitary, shown in mouse [40] and rat [41,42]. A plausible biological mechanism for obesity-induced hypergonadotropic hypogonadism may result, in part, from increased feedback inhibition of the HPT axis due to high serum levels of estrogens in obese males [43]. This may lead to a hypogonadal–obesity cycle [44], wherein an increased adipose tissue mass represents a significant peripheral source of estrogens, which, in turn, suppress the HPT axis and increase central adiposity (Figure 7). The subsequent reduction in circulating testosterone leads to increased deposits of visceral/abdominal adipose tissue [45–47] and subfertility, whereas the increased production of circulating estrogens supports differentiation of adipocytes [40].

Obesity-induced hypogonadism in males may be treated by weight loss, which should reduce estrogen levels to normal and alleviate the HPT feedback inhibition. Roux-en-Y gastric bypass surgery, one option for the treatment of morbid obesity, has been shown in one study to seemingly reverse abnormal reproductive hormonal profiles, such that total testosterone is increased and serum estradiol is decreased [48]. Other treatment options for hypogonadism include supplementation with alternative or recombinant gonadotropins (e.g., human chorionic gonadotropin [hCG] and recombinant FSH), which stimulate testicular function, including testosterone production [49]. Finally, aromatase inhibitors, letrozole and anastrozole, can be used to prevent enzymatic conversion of androgens to estrogens in adipocytes and other tissues [49,50], thereby reducing serum estradiol and suppression of the HPT axis by interrupting the hypogonadal–obesity cycle [46].

Inter-relationship between hypogonadism & non-insulin-dependent diabetes mellitus

Insulin resistance, together with obesity, are cardinal features of metabolic syndrome [2]. However, it is important to note that not all obese individuals will develop non-insulin-dependent diabetes mellitus (NIDDM) or Type 2 diabetes, a disease that also manifests in normal-weight individuals [51]. Consideration must, therefore, be granted to the differential impacts of adiposity and insulin resistance in the context of fertility in the obese male.

Non-insulin-dependent diabetes mellitus is generally characterized by obesity, insulin resistance, hypogonadism, low sex-hormone binding globulin (SHBG) and reduced free and total testosterone [2,52]. However, it is unclear which of these parameters are causal factors and which are independent. Hypogonadism, for example, has been demonstrated in several studies to predict NIDDM risk (e.g., Multiple Risk Factor Intervention Trial cohort...
Table 1. Male obesity and infertility: observational studies of sperm parameters and BMI.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Study design</th>
<th>Population (n)</th>
<th>Parameters</th>
<th>Support relationship between obesity and male infertility?</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jensen et al. (2004)</td>
<td>Cross-sectional</td>
<td>1558 Danish men, general population</td>
<td>Semen volume, sperm concentration, motility, morphology, total sperm count, testes volume, reproductive hormones</td>
<td>Yes, sperm concentration and total sperm count reduced in underweight men (BMI &lt;20 kg/m²) and in overweight/obese men (BMI &gt; 25 kg/m²) compared with normal-weight men (BMI: 20–25 kg/m²)</td>
<td>[17]</td>
</tr>
<tr>
<td>Hammoud et al. (2008)</td>
<td>Retrospective, cross-sectional</td>
<td>526 male patients attending US fertility center, 390 included</td>
<td>Sperm concentration, progressive motility, morphology, BMI</td>
<td>Yes, results support association between obesity and oligospermia, reduced motile sperm count, increased incidence of abnormal sperm morphology</td>
<td>[18]</td>
</tr>
<tr>
<td>Koloszar et al. (2005)</td>
<td>Cross-sectional</td>
<td>274 Hungarian normozoospermic andrology patients</td>
<td>Sperm concentration, BMI</td>
<td>Yes, results indicate reduced sperm concentration in obese group of men (BMI &gt;30.1 kg/m²) compared with underweight, normal-weight and overweight groups</td>
<td>[20]</td>
</tr>
<tr>
<td>Hofny et al. (2009)</td>
<td>Prospective</td>
<td>122 obese, Egyptian andrology patients</td>
<td>Total sperm count, sperm motility, morphology, BMI, reproductive hormones</td>
<td>Yes, BMI inversely related to sperm concentration, motility and normal morphology</td>
<td>[21]</td>
</tr>
<tr>
<td>Aggerholm et al. (2008)</td>
<td>Retrospective, cross-sectional (INUENDO study)</td>
<td>2139 Danish men, 1989 included</td>
<td>Total sperm count, motility, semen volume, BMI, reproductive hormones</td>
<td>Undear, results support a slight reduction in total sperm count among overweight men (BMI: 25.1–30 kg/m²) compared with normal weight men; no reduction in sperm count in obese men</td>
<td>[22]</td>
</tr>
<tr>
<td>Pauli et al. (2008)</td>
<td>Prospective</td>
<td>87 US men, included fathers and men attending fertility center</td>
<td>Semen volume, sperm concentration, motility, BMI, skin-fold thickness, testicular volume, reproductive hormones</td>
<td>Unclear, no correlation between semen parameters with obesity (BMI/skinfold thickness), men unable to conceive, had higher body fat (BMI/skinfold measurements) compared with men with paternity</td>
<td>[15]</td>
</tr>
<tr>
<td>Duits et al. (2009)</td>
<td>Prospective cohort</td>
<td>1466 male patients attending Dutch fertility center, 1401 included</td>
<td>Sperm concentration, motility, morphology, total sperm count, semen volume, BMI</td>
<td>No, results did not support association between high BMI and any of the sperm parameters measured</td>
<td>[23]</td>
</tr>
<tr>
<td>Nicopoulou et al. (2009)</td>
<td>Retrospective, cross-sectional</td>
<td>349 Greek andrology patients</td>
<td>Total sperm count, BMI</td>
<td>No, results did not support a significant association between BMI and total sperm count</td>
<td>[24]</td>
</tr>
</tbody>
</table>

Fecundity and BMI

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Study design</th>
<th>Population (n)</th>
<th>Parameters</th>
<th>Support relationship between obesity and infertility?</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramlau-Hansen et al. (2007)</td>
<td>Cohort (Danish National Birth Cohort)</td>
<td>64,167 pregnant women, 47,835 couples included</td>
<td>BMI (women’s report), time to pregnancy</td>
<td>Yes, overweight/obese men associated with subfecundity (time to pregnancy &gt;12 months)</td>
<td>[25]</td>
</tr>
<tr>
<td>Nguyen et al. (2007)</td>
<td>Retrospective cohort (Norwegian Mother and Child Cohort)</td>
<td>45,132 Norwegian couples, 26,303 included</td>
<td>BMI (women’s report, self-report), time to pregnancy</td>
<td>Yes, risks of infertility associated with underweight (BMI &lt;20 kg/m²), increased risk of infertility with increase in BMI</td>
<td>[27]</td>
</tr>
<tr>
<td>Study (year)</td>
<td>Obese sample size (total sample size [n])</td>
<td>Population</td>
<td>Association with BMI?</td>
<td></td>
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</table>
| Jensen et al. (2004) | 299 (1558) | Danish men | **Sperm concentration/total sperm count:** yes, BMI >25 kg/m² group exhibited significantly reduced sperm count and reduced sperm concentration; large sample, general population  
**Sperm motility:** no association between sperm motility and BMI; large sample, general population  
**Sperm morphology:** no significant association; large sample, general population, very young (mean age: 19 years) |
| Hammoud et al. (2008) | 296 (390) | Fertility patients | **Sperm concentration/total sperm count:** yes, oligospermia associated with increasing BMI; moderate sample size, large proportion of overweight/obese men  
**Sperm motility:** yes, increasing BMI associated with low sperm motility; moderate sample size, large proportion of overweight/obese men  
**Sperm morphology:** yes, reduced in obese group vs others; moderate sample size, large proportion of overweight/obese men |
| Koloszár et al. (2005) | 149 (274) | Normo-zoospermic infertility patients | **Sperm concentration/total sperm count:** yes, sperm concentration significantly reduced in men with BMI >30 kg/m², not in overweight men (BMI: 25.1–30 kg/m²); simple analysis, determined age effect in obese group, men with abnormalities in reproductive hormones were excluded |
| Hofny et al. (2009) | 122 (122) | Fertile/oligospermic andrology patients | **Sperm concentration/total sperm count:** yes, BMI negatively correlated with sperm count; homogenous sample; BMI >30 kg/m²  
**Sperm motility:** yes, BMI negatively correlated with sperm motility; sperm motility significantly decreased in oligospermic group. Entire sample had BMI >30 kg/m², excluded major illness diabetes, hypertension  
**Sperm morphology:** yes, BMI positively correlated with abnormal sperm morphology, entire sample had BMI >30kg/m², excluded major illness diabetes, hypertension |
| Fejes et al. (2006) | 25 (42) | Oligospermic patients | **Sperm concentration/total sperm count:** yes, reduced sperm concentration vs normal/underweight men; small sample size, subfertile population  
**Sperm motility:** no difference vs normal/underweight men; small sample size, subfertile population  
**Sperm morphology:** no difference vs normal/underweight men; small sample size, subfertile population |
| Duits et al. (2009) | 733 (1401) | Fertility patients | **Sperm concentration/total sperm count:** no association with BMI; thorough analysis, large sample, reduced semen volume in overweight group  
**Sperm motility:** no association with BMI; thorough analysis, found reduced semen volume in overweight group  
**Sperm morphology:** no association with BMI; thorough analysis, found reduced semen volume in overweight group |
| Pauli et al. (2008) | Not listed, mean BMI >25 kg/m² (87) | Fathers/infertility patients | **Sperm concentration/total sperm count:** no association between BMI or skinfold thickness with sperm concentration; proportion of sample with BMI >30 kg/m² not identified, small sample  
**Sperm motility:** No association between BMI or skinfold thickness with sperm motility; proportion of sample with BMI >30 kg/m² not identified, small sample |
| Kort et al. (2006) | Not listed, mean BMI >25 kg/m² (520) | Andrology patients | **Sperm motility:** yes, BMI negatively correlated with total number of normal motile sperm; analysis used normal motile sperm that combines sperm concentration, volume, morphology and motility, no raw data reported |
hyperinsulinemia, somehow directly suppressed Leydig cell testosterone production in a cohort of men with mild-to-moderate obesity [65]. In vitro, Leydig cells express insulin receptors, and insulin has been shown to induce testosterone secretion from Leydig cell cultures [67,68]. The molecular mechanism wherein insulin modulates Leydig cell steroidogenesis is unknown, with the possibility that the testis, as with other organs in individuals with NIDDM, is resistant to insulin signaling [66].

Non-insulin-dependent diabetes mellitus and, therefore, insulin resistance, appears to be associated with ‘mixed hypogonadism’, reflecting the dual actions of insulin resistance at the testis (hypergonadotropic hypogonadism); and the hypothalamus/pituitary (hypogonadotropic hypogonadism). Insulin is predominantly stimulatory, acting at hypothalamic neurons to induce GnRH secretion and gonadotropin secretion from the pituitary gonadotrophs, demonstrated in vitro [69]. In obese men with NIDDM, insulin resistance may blunt the normal, insulin stimulation of the HPT axis [66]. More studies are needed to identify the mechanisms of HPT insulin signaling and the consequences of insulin resistance to the normal endocrine function of these glands.

Although reproductive hormonal profiles have been fairly well established for men with NIDDM, semen quality has been examined in only a few studies. Generally, sperm count is normal or increased, with decreases in sperm motility [70] and semen volume [71], and increases in abnormal sperm morphology [72]. Unfortunately, many published studies of ‘diabetic men’ do not disaggregate the insulin-dependent diabetes mellitus (IDDM) and NIDDM groups; a critical reporting gap in the literature as it appears that NIDDM may be more of a concern with respect to male reproduction. The pathology of IDDM (autoimmune and insulin deficiency) is different from NIDDM (insulin resistance). The stimulatory effects of insulin on the HPT axis in IDDM patients are continued through exogenous insulin, the standard intervention. Insulin resistance in NIDDM patients, however, leads to aberrance in testicular cell signaling and subsequent hypogonadism. With so few studies examining the association between semen quality and insulin resistance, it is impossible to profile ‘typical’ semen parameters of a NIDDM man. More studies using NIDDM men and animal models are urgently needed to verify the causal effect of insulin resistance on hypogonadism, as well as to discern the molecular mechanisms involved.

Leptin’s roles in hypogonadism

Leptin is a 16-kDa protein hormone, encoded by the ob gene [73] and secreted by adipocytes. This adipocytokine plays a major role in energy homeostasis, including neuroendocrine regulation of bodyweight. Leptin is the endogenous agonist for the leptin receptor (Ob-R), a member of the class I cytokine receptor superfamily. Ob-R is produced as alternatively spliced forms and further classified as short (Ob-Ra, Ob-Rc and Ob-Rd) and long (Ob-Rb) and secreted (Ob-Re) receptor types, all with extracellular, transmembrane and variable intracellular domains with the exception of Ob-Re [74–76]. Ob-Rb has the longest intracellular domain and is the functional isof orm in the hypothalamus, whereas Ob-Ra and Ob-Rc are proposed to participate in leptin transport across the blood–brain barrier (BBB) [76,77]. Ob-Re is the putative soluble leptin receptor...
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Lacking the transmembrane and intracellular domains, and proposed to buffer circulating leptin levels, thereby regulating leptin bioavailability [78].

Leptin was initially characterized in ob/ob mice, which, because of a natural mutation, possess no ob gene and are, therefore, leptin deficient [79]. Both male and female ob/ob mice are morbidly obese and infertile [80]. Similar phenotypes are observed in Ob-R-deficient mice [81] and the Zucker fatty (fa/fa) rat [82]. Several human families have also been identified with congenital leptin deficiency owing to recessive mutations in the leptin gene (Delta133G [83], R105W [84,85] and N103K [86]) or the leptin receptor [87]. Clinical features of human congenital leptin deficiency [84–85] include early onset of obesity, hyperphagia (overeating), hypogonadotropic hypogonadism and delayed pubertal onset. Congenital leptin deficiency caused by leptin receptor mutation is associated with a slightly less severe phenotype [87]. Recombinant leptin administration has been used successfully to mitigate some of the features of congenital leptin deficiency, resulting in weight loss and reversal of hypogonadism [83,88,89].

In healthy men, serum leptin positively correlates with BMI and adipose mass [90]. A number of studies in overweight and obese participants report an inverse relationship between serum levels of leptin and testosterone [91–94] but no significant correlation between levels of leptin and the gonadotropins [91,93]. In contrast to the hypogonadism featured in congenital leptin deficiency cases, hypogonadism in obese men is associated with high serum leptin. Altered leptin dynamics may, therefore, contribute to male infertility via at least two mechanisms, both of which may produce hypogonadism. These include leptin resistance or leptin insufficiency at the hypothalamus and leptin modulation of testicular physiology.

Leptin resistance or leptin insufficiency at the hypothalamus

Consider the perplexing presence of the same phenotype (obesity and hypogonadotropic hypogonadism) in individuals with high serum leptin due to obesity, and in humans/animals with leptin deficiencies due to mutations. Leptin resistance has been proposed to explain this apparent paradox in the former group, referring to the inability of leptin to act at the hypothalamus, either due to reduced levels of bioavailable leptin (leptin insufficiency) [95] or impaired leptin signaling [96], thereby mirroring the leptin deficiency present in animal models and congenital genetic conditions [97]. There does not appear to be a model for central leptin excess, in spite of the increased serum leptin levels exhibited by morbidly obese individuals.

Peripheral adipocyte-derived leptin circulating in the bloodstream can cross the BBB via a saturable transport system [95] to act centrally via Ob-Rb in the brain, particularly at the

![Figure 4. Sperm capacitation signaling.](expert-reviews.com)

The process of capacitation includes changes to the sperm membrane and the acquisition of hyperactive motility. Hallmark characteristics of sperm capacitation including intracellular increases in cAMP and HCO₃⁻, cholesterol efflux and tyrosine phosphorylation. Ob-R signal crosstalk is also shown (see text for details). Dotted lines indicate proposed pathways and solid lines indicate established pathways.

cAMP: Cyclic adenosine monophosphate; EGFR: EGF receptor; ERK: Extracellular signal-regulated kinase; JAK: Nonreceptor tyrosine kinase; MEK: Mitogen-activated protein kinase; PDE: Phosphodiesterase; PKA: Protein kinase A; SACY: Soluble adenylyl cyclase; Y-P: Phosphorylated tyrosine residue.

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**Figure 5. Endocrine profile of the obese male.**

- **Obese male**
  - BMI >30 kg/m²
  - High serum estradiol
  - Insulin resistance
  - High serum leptin
  - Hypogonadism
  - Subfertility
was first considered following reports that the ratios of cerebrospinal fluid leptin to plasma leptin are reduced in obese individuals compared with lean counterparts [95]. Thus, in spite of large circulating leptin plasma levels in obese individuals, cerebrospinal fluid leptin levels are not proportionately high [95,100]. As peripheral leptin is ordinarily released in a pulsatile fashion, any number of factors, including circadian rhythm, meal spacing and aging could disrupt this initial leptin pulsatile signal and produce leptin receptor downregulation, thereby attenuating leptin transport at the BBB [101]. Reduced hypothalamic leptin stimulation may produce the morbidly obese phenotype in humans, as these individuals lack leptin-induced suppression of appetite or stimulation of energy expenditure [81], thereby producing a phenotype similar to the ob/ob mouse [102]. The molecular mechanisms of leptin transport saturation at the BBB are not well characterized; however, triglyceride inhibition/reduction of leptin transport at the BBB has been proposed [99]. Alternatively, leptin resistance, characterized by deficits in Ob-R signaling pathways, may impair leptin regulation of eating and energy expenditure. Candidate signaling pathways include SOCS3 and STAT3, both downstream of Ob-Rb [103]. Although impairments in leptin signaling may well accompany central leptin insufficiency, perturbed signaling as a basis for leptin resistance remains to be elucidated [101]. As described previously, ob/ob mice are obese, infertile and exhibit hypogonadism; however, this phenotype can be reversed, including restoration of fertility, by treatment with exogenous leptin [102,104,105]. Gonadotropins and sex-steroid hormones are low in ob/ob mice, consistent with hypogonadotropic hypogonadism and a role for leptin in the regulation of the HPT axis [102]. Leptin acts indirectly to regulate gonadotropin secretion in the hypothalamus by modulating kisspeptins in the arcuate nucleus (Figure 8A) [106]. Kisspeptins are proteins encoded by the Kiss1 gene, transcribed as KiSS1 mRNA and act via a G-protein-coupled receptor (GPR54) [107] to stimulate GnRH release, thereby triggering the gonadotropin cascade [108]. Peripheral leptin, once transported across the BBB to the hypothalamus, binds leptin receptors in the forebrain. Arcuate nucleus neurons in the mouse express both Ob-Rb and KiSS-1 mRNA in approximately 40% of cells, suggesting that so-called KiSS1 neurons (kisspeptins-expressing neurons) are direct targets of leptin. Further, the numbers of KiSS1 neurons are decreased in the hypothalamus of ob/ob mice, indicating that leptin regulates KiSS1 neurons, and indirectly gonadotropin release [108,109]. Modulation of the gonadotropin levels is almost certainly limited to an indirect role of leptin, since leptin receptors are not present on rat GnRH neurons. Supporting these data is the observation of normal fertility in transgenic mice lacking GnRH neuronal leptin receptors [110].

Testicular stress induced by leptin-modulated reductions in circulating gonadotropins, established antiapoptotic agents [111], can induce apoptosis [112]. FSH, a prosurvival factor in rat testis, upregulates expression of antiapoptotic protein Bcl-w in rat Sertoli cells, spermatogonia and spermatocytes, but not spermatids, in vitro [113]. Germ cell death is a normal event during spermatogenesis and may serve to regulate the size of the germ cell population [112,114];
however, in the event of central leptin insufficiency and reduced gonadotropins release, testicular apoptosis may be pathological. This has been demonstrated in leptin-deficient ob/ob mice that exhibit upregulation of nine testicular pro-apoptotic genes involved in both intrinsic and extrinsic apoptosis pathways [115]. Abnormal spermatogenesis and infertility in these mice is likely due to inadequate gonadotropin support of spermatogenesis and accelerated germ cell death by apoptosis [115,116].

Deficiency in hypothalamic leptin signaling resulting in hypogonadotropic hypogonadism is observed in ob/ob mice and humans with leptin deficiency. It would appear that central leptin insufficiency is the causal mechanism of deficient hypothalamic leptin signaling and, thus, the underlying cause of hypogonadotropic hypogonadism (Figure 8B). The stimulation of KiSS1 neurons by leptin provides a link between energy homeostasis and reproduction. Moderate elevations in serum leptin levels due to seasonal weight gain in response to changing environmental conditions may signal reproductive opportunity [80]. While moderate fluctuations in leptin may prove physiologically relevant to seasonal breeders and other animals, high serum leptin levels in the obese human male may be detrimental, as this leads to saturation of the BBB transport system and central leptin insufficiency.

Leptin modulation of testicular physiology

Leptin is found in human and rodent Sertoli cells, Leydig cells, seminiferous tubules and germ cells [117,118] and is able to cross the testis–blood barrier (TBB) [119], suggesting both testicular and peripheral sources of leptin may be involved in reproduction. Unlike the BBB’s saturable transport system for leptin, the TBB system is nonsaturable, enabling leptin to ‘leak’ across the barrier between the blood and the testicular interstitium and traverse the Sertoli cell barrier, dividing the interstitium from the seminiferous tubule fluid. The mechanism at the Sertoli cell barrier has not been elucidated as saturable or leakage [119]. Thus, as serum leptin levels increase, intratesticular leptin levels would be expected to similarly increase with leptin action limited by receptor expression in the testis. Ob-R have been localized to isolated Sertoli and Leydig cells, and testicular germ cells in rodents [119–122], and in seminiferous tubules in humans [117,123,124]. Together, these results strongly suggest that leptin directly modulates testicular functions [80,102].

Leptin is a negative regulator of testicular steroidogenesis; it acts directly on testicular Leydig cells. In response to LH, Leydig cells activate PKA-dependent gene expression, which triggers steroidogenesis (i.e., the production of testosterone) [125]. Leydig cells in the rat [121,126] and human [117], but not mouse [127], express leptin receptors, thus providing a mechanism for direct leptin modulation of Leydig cell functions. In rat Leydig culture, leptin suppresses hCG-stimulated testosterone secretion, supporting a role for leptin in the negative regulation of steroidogenesis [126,128,129]. Leydig cells exhibit differential sensitivity to leptin, according to the developmental expression profiles of Ob-R, such that embryonic and adult but not prepubertal rat Leydig cells demonstrate leptin suppression of hCG-induced testosterone secretion [121]. In an elegant experiment designed by Tena-Sempere and colleagues [122], hCG-stimulated rat testicular samples exposed to increasing doses of human recombinant leptin decreased expression of steroidogenic enzymes mRNAs and, subsequently, reduced testosterone secretion. Leptin is also able to decrease hCG-induced expression levels of steroidogenic factor (SF)-1, steroidogenic acute regulatory protein (StAR) and cytochrome P450 cholesterol side-chain cleavage enzyme (P450scC) in a dose-dependent manner [122].

Drawing from the findings reported by the teams of Caprio and Tena-Sempere, a model of leptin regulation of Leydig cell steroidogenesis is proposed (Figure 9). Leptin signaling via Ob-R is relatively well characterized [76] and is mediated by the JAK–STAT pathway [130–132], with JAK2 [133] and STAT3, primarily described [134]. STAT3 ultimately regulates expression of steroidogenic genes including SF-1, StAR and P450scC [135]. Alternatively, steroidogenic gene expression can be regulated via the PI3K/Akt or MAPK cascades, triggered by leptin binding to Ob-R [76]. Leptin’s repression of steroidogenic gene expression,
particularly StAR, the rate-limiting step in steroidogenesis [136] would, therefore, counteract LH-mediated testosterone production. Leptin’s negative regulation of steroidogenesis provides subtle control over reproductive functions and represents yet another mechanism for leptin in reproduction.

Leptin’s modulation of male infertility also involves direct action of leptin on the sperm itself. Studies in mice indicate that leptin and its receptor are expressed in specific types of male germ cells, implicating leptin in cell proliferation and differentiation. Leptin protein and mRNA expression are present in gonocytes from neonatal mice, spermatogonia from 10-day-old mice and in spermatocytes from adult mice [118]. Neonatal and adult mice express Ob-Ra, Ob-Rb and Ob-Re in the testes [118,120], suggesting leptin normally functions in an autocrine or paracrine manner to regulate spermatogenesis. It remains to be elucidated whether leptin exclusively acts as a positive or negative regulator of spermatogenesis, or whether leptin’s role is more refined, dependent on the receptor isoforms expressed at different stages of spermatogenesis. Leptin signaling via STAT3 suggests a role in the proliferation of undifferentiated germ cells [120]; consistent with reports that stem cell STAT3 phosphorylation prevents differentiation and enables continuous stem cell renewal [137]. Leptin STAT3 signaling may enable undifferentiated germ cells to replicate without loss of potency while triggering later-stage spermatocytes to undergo development and differentiation [118,120]. In obese males, serum leptin levels are elevated, which may lead to downregulation of testicular Ob-R, previously demonstrated in vitro in the rat [138]. Downregulation of leptin receptor expression would disrupt autocrine/paracrine testicular signaling and perhaps spermatogenesis. Taken together, the localization studies demonstrating testis and spermatocyte-specific leptin and leptin receptor expression [117,123,124], along with the studies of testicular leptin signaling [122], suggest that elevated serum leptin levels exhibited by obese males are likely to perturb normal testicular physiology.

Ejaculated sperm are not able to fertilize the egg and must undergo further structural and functional changes during capacitation (Figure 3 & 4). The purpose of seminal plasma leptin is still unclear [17,123,139–143], and is required to modulate sperm capacitation and the acquisition of hyperactive motility. Human seminal plasma leptin levels are positively correlated with serum leptin levels [17,139–141] but inversely correlated with serum testosterone and normal sperm parameters [123,139,142]. Seminal plasma leptin levels tend to be disproportionately lower than serum leptin levels [139] but are still positively correlated [139,142]. Obese men would be expected to have greatly increased seminal plasma levels of leptin, relative to their lean counterparts; however, very few studies have
reporting seminal plasma leptin levels in morbidly obese men, and the capacitation/acrosome reaction status of their sperm has not been investigated.

A role for leptin in sperm capacitation has been proposed by the Aquila research team, drawing upon their studies in humans and boars. Leptin and leptin receptor expression are localized exclusively on the plasma membrane overlaying the acrosome in boar sperm, consistent with their putative regulations of sperm fertilizing ability [135,144]. Leptin has been localized to midpiece/equatorial segment in uncapsulated human sperm, undergoing an overall decrease in expression and more uniform localization in capacitated sperm [141,145], with leptin receptor confirmed at the tail region in ejaculated spermatozoa [145,146]. These expression patterns support a physiological role for leptin, perhaps in the modulation of human sperm motility. Following acute leptin exposure (30–60 min), uncapacitated boar and human sperm undergo leptin-enhanced cholesterol efflux and protein tyrosine phosphorylation [135,141], both hallmark steps in capacitation [144]. Furthermore, boar sperm acrosin activity (acrosomal trypsin-like enzyme) is stimulated by leptin [135]. It is important to note that leptin’s stimulation of these capacitation-related events in both studies [135,141] is not dose dependent, with very low concentrations of leptin (0.63 nM human leptin and 1–10 nM porcine leptin) producing the greatest response. This may be explained by receptor downregulation at higher leptin levels [138] and may hold significance for obese men who would be expected to exhibit seminal plasma leptin levels in the highest ranges.

A model of capacitation signaling crosstalk emerges, wherein leptin signaling via the leptin receptor induces tyrosine phosphorylation downstream of MAPK pathway activation (Figure 4). The process of capacitation includes changes to the sperm membrane and the acquisition of hyperactivated motility. Molecular events include HCO₃⁻-dependent increase in intracellular cAMP, subsequent activation of PKA and, ultimately, tyrosine kinase activation and protein tyrosine phosphorylation, necessary for acquisition of hyperactivated sperm motility [6,7]. Leptin activation of prosurvival pathways may lead to the activation of ERK1/2 signaling [135,140], representing capacitation signaling crosstalk. It is intriguing to speculate that acrosomal leptin receptor expression is associated with cholesterol efflux and acrosome reaction, whereas tail leptin receptor expression in human sperm may reflect leptin’s modulation of hyperactivated sperm motility.

In summary, leptin regulation of normal male reproduction includes central (HPT) and testicular actions. In morbidly obese men, central leptin insufficiency produces hypogonadotropic hypogonadism, reducing circulating gonadotropins [95,98,99], and subsequently inducing testicular apoptosis [111]. It is believed that high serum leptin is able to perturb testicular steroidogenesis and spermatocyte differentiation and development [118,120]. It can also be hypothesized that ejaculated sperm from obese males exhibit reduced capacitation through leptin receptor downregulation in response to high seminal plasma leptin [138]. Many knowledge gaps remain to be elucidated, including a greater understanding of BBB and TBB leptin transport, the differential roles and associated signaling pathways associated with soluble and transmembrane leptin receptor isoforms in the brain, testis and sperm, with further study required to enable the characterization of leptin’s modulation for reproduction in the obese male.
Research studies using animals provide better models and opportunity to investigate the biological effects of thermal stress on testicular functions and structure. Various experimental approaches have been used. One mechanism used to elevate testicular temperatures in rats [353] and mice [354,155] is transient exposure of the animals to temperatures exceeding 40°C. Alternatively, surgically induced cryptorchidism is used in mice to elevate testicular temperature to the body temperature [356]. In another design, mice are housed at 35–38°C over a period of several hours to induce thermal stress [157,158]. Major reproductive outcomes in responses to hyperthermia include decreased testicular weights, germ cell loss and increased rates of apoptosis. Testicular heat-stress responses involve complex signaling pathways with interconnection among hypoxia, apoptosis, gene expression and inhibition of DNA repair, which eventually culminates in altered spermatogenesis.

Elevations in temperature and other environmental stressors, including oxidative stress and chemical exposures, are known to trigger intracellular changes in gene expression, resulting in altered cell survival/apoptosis pathways. Heat stress induces increased expression of a class of 70-kDa heat-shock proteins (Hsp70), a class of chaperone proteins that help regulate protein folding and assembly [159,160]. Hsp70 also play an important role in the prevention of apoptosis [161,162]. At least two testis-specific Hsp70 have been identified. Spermatocyte-specific Hsp70.2 (mice) is continuously expressed by pachytene spermatocytes during meiosis and is not heat inducible [159]. Testis-specific Hsc70t is similarly expressed in round spermatids. Expression of inducible Hsp70 by germ cells is conflicting. Rockett and colleagues demonstrated upregulation of Hsp70-1 and Hsp70-3 in mouse spermatocytes following acute hyperthermia (10 min at 43°C) [163]. Other researchers reported that testicular heat stress did not increase expression of heat shock genes at the mRNA or protein levels in mouse germ cells [161,164]. Transgenic mice designed to express constitutively active heat-shock factor (HSF1) in spermatocytes did not induce Hsp70 gene expression but did activate caspase-dependent apoptosis pathways in the absence of heat shock [161]. HSF1 is a transcription factor activated during stress (hypoxia and hyperthermia); it trimerizes, translocates to the nucleus and binds heat-shock response elements in the promoter regions of HSF1 target genes [165,166]. Hsp70 does not appear to be sufficient to prevent HSF1-induced

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**Heat stress, hypoxia-induced testicular apoptosis**

It is well established that the testis is sensitive to heat, evidenced by the approximately 3°C lower temperature of the scrotum compared with core body temperature, a condition critical for efficient spermatogenesis [417]. Many environmental/lifestyle factors are associated with elevated testicular temperatures including varicocele [148,149], tight fitting undergarments, sedentary work positions, laptop position of portable computers, saunas and occupational heat exposures [37]. Although it is likely that suprapubic and inner thigh adipose tissue in obese males combined with sedentary behaviour increases scrotal temperatures [37], there are no studies that report scrotal temperature measurements in an obese population. As increased scrotal temperature during sedentary activities is associated with impaired semen quality [150–152], it follows that obese men would be at risk of genital heat stress and infertility. Testicular scrotal temperature measurements are difficult to obtain in humans and data regarding duration of sustained sedentary position is not often collected, underscoring the need for more well-designed testicular thermal stress research studies.

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**Figure 10. Model of testicular heat stress.** In response to testicular heat stress, apoptosis pathways may be activated through several mechanisms. Caspase-2 perturbs the ratio of apoptotic proteins Bax:Bcl2 and directly activates apoptosis. p38MAPK (also known as MAPK14) phosphorylates Bcl2, thereby removing the inhibitory block to apoptosis. Caspase-2 and/or caspase-3 degrade ICAD, leading to activation of CAD and DNA fragmentation. Caspase-2 activates HSF1, which downregulates expression of DNA repair genes, ICAD, Hsp70, germ cell antioxidants, upregulates Hif1α, HSF1 and testicular antioxidants, and induces apoptosis. Dotted lines indicate proposed mechanisms and solid lines indicate established mechanisms.

apoptosis as mouse spermatocytes with constitutive expression of both Hsp70 and HSF1 exhibited characteristic DNA strands breaks consistent with apoptosis [161]. The mechanism underlying heat-stress-induced apoptosis appears to be primarily regulated by HSF1 (Figure 10). Intriguingly, the endogenously established temperature ‘set-point’ for HSF1 activation is higher for core somatic tissues compared with the testis, consistent with external location of the male reproductive organ in mammalian species. Thus, testicular tissues are exquisitely sensitive to even mild hyperthermia through regulation by HSF1. Persistent testicular HSF1 activation is observed in cryptorchid mice and is associated with disrupted spermatogenesis [164]. HSF1 may even down-regulate Hsp70 to ensure unopposed activation of apoptosis pathways. In early developmental stages of mouse spermatocytes that express HSF1, Hsp70.2 protein undergoes translocation from the cytoplasm to the nucleus. At later stages of development, germ cells exhibit HSF1-mediated repression of Hsp70.2 following hyperthermia. Widlak and colleagues propose that a reduction in both Hsp70.2 mRNA and protein levels occurs prior to heat-stress induction of apoptosis, with both events mediated by HSF1 [167]. Depending on the conditions of experimentally induced testicular heat stress and the subsequent assays to detect Hsp70, HSF1’s negative regulation of Hsp70 expression and nuclear translocation may explain the inconsistencies regarding spermatocyte expression of Hsp70 in the literature discussed earlier.

Other important changes in testicular cells in response to heat stress include downregulation of DNA repair pathways and activation of p38MAPK signaling (Figure 10). Mouse testis exposed to acute heat stress (43°C for 10 min) downregulate DNA repair genes, including Ogg1 (base excision repair), Xpg (nucleotide excision repair) and Rad54 (double-strand break repair) [163]. Decreased expression of poly (ADP-ribose) polymerase PARP (base excision repair/nucleotide excision repair pathways) also occurs in the rat testis in response to heat stress [168]. With DNA repair pathways disabled, apoptosis is induced via multiple mechanisms, including direct hyperthermic / hypoxic stress. Heat stress induces activation of p38MAPK (also known as MAPK14), which, in turn, phosphorylates Bcl2, thereby removing the inhibitory block to apoptosis [169]. Apoptosis is regulated, in part, by the balance between proapoptotic proteins (e.g., Bax) and antiapoptotic proteins (e.g., Bcl2). Caspase-2 is proposed to be activated upstream of p38MAPK by hyperthermia and/or reactive oxygen species produced during hypoxia. Caspase-2 may also perturb the ratio of Bax:Bcl2 [170] and directly activate caspase-3, thereby promoting apoptosis [170, 171]. Finally, heat-stress-induced cleavage of inhibitor of caspase-activated DNase (ICAD) via caspase-3 activation [154] or directly via caspase-2 activation [171] produces the final fragmentation of nuclear DNA. Another mechanism, yet to be defined, links hyperthermic stress with induced activation of HSF1, which, in turn, promotes apoptosis of pachytene spermatocytes [164]. Testicular heat stress is accompanied by localized hypoxia that induces oxidative stress, apoptosis and reduction in DNA repair gene expression (Figure 10). Increases in testicular cell metabolism during heat stress may be so high that testicular blood flow cannot provide sufficient tissue oxygenation, thereby creating oxidative stress to the tissue [155]. During hypoxia, hypoxia-inducible factor (Hif)-1α translocates from the cytoplasm to the nucleus to form a heterodimer with Hif-1β (also known as aryl hydrocarbon receptor nuclear translocator), now known as HIF1. The formation of the Hif-1α–Hif-1β heterodimer provides genomic protection from oxidative stress [172]. Following mild hyperthermia, the mouse testis undergoes hypoxia and oxidative stress and responds with increased expression of Hif-1α mRNA/protein in the interstitial compartment and increased expression of Hif-1α protein in the nuclei of germ cells [155]. Antioxidants, expressed during hypoxia, are downregulated in mouse germ cells following hyperthermia [163], but not in testicular somatic cells, as whole mouse testis exhibit increased expression of testicular antioxidants (HMOX1, GPX1 and GSTA) following heat stress [155]. Thus, germ cells are particularly vulnerable to hypoxia and hyperthermia, which, in turn, induce apoptosis pathways and ultimately loss of male germ cells.

![Figure 11. Spermatocytes exhibit stage-specific susceptibility to heat stress. Testicular germ cells exhibit stage-specific susceptibility to heat stress. Pre-meiotic germ cells express cell survival factors that protect them against heat stress. By contrast, meiotic germ cells (pachytene spermatocytes and round spermatids) are most susceptible to heat stress; they also have limited capacity for DNA repair.](image-url)
The testis exhibits germ cell stage-specific susceptibility to heat stress (Figure 11). As described previously, premeiotic germ cells express cell survival factors, including HSF1 and Hsp70, that protect against heat stress \[161,164\]. In response to heat stress, as spermatocytes undergo meiosis, they begin to exhibit significant DNA damage that subsequently initiates apoptosis pathways \[154\]. HSF1 seems to be associated with cell survival only in premeiotic and somatic cells, such that meiotic (pachytene spermatocytes and early spermatids) and postmeiotic stages are most susceptible to heat stress \[153,154,173\].

Taken together, it can be speculated that a combination of factors, including sedentary lifestyle and suprapubic and inner thigh adipose tissue deposits, increase testicular temperatures, thereby triggering apoptosis pathways. As discussed, heat-stress-induced apoptosis of actively dividing germ cells would reduce sperm counts, thus contributing to male infertility in morbidly obese men. Animal studies also point to diminished embryo survival following paternal heat stress \[157,158,163,174\], providing another mechanism by which testicular heat stress contributes to reproductive loss. There remain significant technical limitations in measuring scrotal temperatures in humans in a relatively noninvasive manner. These limitations must be overcome to further investigate the relationship between testicular hyperthermia, obesity and male infertility.

**Endocrine disruption**

It has been well established that environmental chemicals (endocrine disrupters) are reproductive toxicants and can be associated with impaired semen quality and reproductive potential in animals and humans \[175–178\]. An endocrine disruptor is defined as \[176\]:

“as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that is responsible for the maintenance of homeostasis, reproduction, development and/or behavior.”

Baillie-Hamilton proposed that the obesity epidemic over the past 40 years may also be related to the increased use of industrial chemicals with demonstrated endocrine-modulating effects.

**Figure 12. Fetal origins of adult-onset diseases.**

![Figure 12. Fetal origins of adult-onset diseases.](image)

**Figure 13. ER-α- and PPAR-γ-dependent gene expression.**

(A) Estrogens. Following ligand (estrogens) binding, ER-α forms a homodimer that together with transcription factors, assemble as a pre-initiation complex at the ERE present in the promoter region of estrogen-responsive genes. (B) PPAR-γ. Once activated by ligand (fatty acids, putative obesogens), PPAR-γ forms a heterodimer with RXR and binds as a complex to PPRE in target gene promoter regions. (C) DES-mediated ER-α and PPAR-γ signal crosstalk. ER-α may also bind the PPRE consensus sequence. DES-binding to ER-α induces adipogenic effects in the rodent penis, possibly through PPAR-γ signal crosstalk. (D) PPAR-γ and ER-α signal crosstalk. Similarly, PPAR-γ may bind the ERE consensus sequence. Although the consequences on male reproduction are unknown, such signal crosstalk may represent yet another mechanism by which putative obesogens including phthalates, organotins and phytoestrogens may disrupt endocrine function.

DES: Diethylstilbestrol; ER: Estrogen receptor; ERE: Estrogen response element; PPRE: PPAR response elements; RXR: Retinoid X receptor.
activity [179–181]. Male infertility and obesity may therefore share an environmental etiology caused by perturbations in normal endocrine pathways.

The Barker hypothesis relates poor fetal nutrition to adult-onset diseases including coronary heart disease, Type 2 diabetes and metabolic dysfunction [182], and has formed the basis for the developmental origins of health and disease paradigm, which similarly posits a correlation between perinatal health and the eventual development of chronic diseases (Figure 12) [183,184]. Toxicologists have also identified neonatal development as a ‘critical window of exposure’, such that chemical exposures (e.g., endocrine disruptors) have been linked to adult-onset reproductive cancers [177,185,186]. Taken together, these models support the extreme sensitivity of the neonatal period to environmental influences, and as proposed by Baillie-Hamilton [179] and Newbold and colleagues [187], the models provide an explanation for fetal origins of adult obesity risk.

The intersection between estrogenic and adipogenic pathways has been best examined in a series of studies defining adipogenesis in penile corpus cavernosa induced by activation of ER-α by potent estrogen disrupter, diethylstilbestrol (DES) (Figure 13A) [188–190]. Neonatal exposure to DES (3–4 mg/kg) generates adult rats that are infertile with gross penile abnormalities, including the appearance of adipocytes in the cavernous spaces of the corpora cavernosa penis. Similarly, histological changes to the penis along with male infertility were observed following postnatal exposures to DES (1 mg/kg) [188,189]. Goyal and colleagues have hypothesized that estrogen-dependent differentiation and proliferation of stromal cells occur following estrogen exposure during a critical development period (1–12 postnatal days) [190]. This transformation replaces endothelial and smooth muscle cells in the corpora cavernosa with adipocytes, resulting in grossly abnormal morphology of the penis and infertility. Transgenic mice lacking ER-α (α-ERKO) are ‘resistant’ to neonatal DES exposure and do not exhibit penile deformities. These studies thereby confirm a role for ER-α in DES-mediated abnormalities of the corpora cavernosa in both rats and mice [191]. Furthermore, this developmental disruption has been recently elucidated as a ‘biological overlap’ between PPAR-γ and ER-α (Figure 13B & C) [192].

The PPAR family includes three isotypes PPAR-α, -β and -γ. PPAR-γ, a nonsteroidal nuclear receptor, regulates proliferation and differentiation of adipocytes through the promotion of genes involved in fatty acid storage and the repression of genes necessary for adipocyte lipolysis. Once activated by ligand (fatty acids, putative obesogens), PPAR-γ forms a heterodimer with retinoid X receptor and binds as a complex to PPAR response elements of adipogenic pathways. Dotted lines indicate proposed mechanisms and solid lines indicate established mechanisms. E2: Estradiol; FSH: Follicle-stimulating hormone; GnRH: Gonadotropin-releasing hormone; LH: Luteinizing hormone; T: Testosterone; t: Temperature.

Figure 14. Mechanisms of obesity-induced male infertility. (A) Normal-weight males. In normal-weight individuals, male reproduction is stimulated by the hypothalamus–pituitary–testis (HPT) axis, with negative feedback provided by testosterone. Increasing evidence points to the stimulatory roles of leptin and insulin in the HPT axis including testis functions. (B) Obese males. Male obesity is characterized by high serum estrogen, leptin and insulin levels. Testicular testosterone production is reduced, but may still provide negative feedback to the HPT axis (not shown), although to a lesser extent than in the normal-weight males. Three main biological mechanisms link obesity to impaired male reproductive function: (1) hypogonadism: hypogonadotropic hypogonadism caused by negative feedback by estrogens or insulin/leptin resistance, and hypergonadotropic hypogonadism caused by direct actions of leptin on the testis, (2) testicular heat-stress-/hypoxia-induced apoptosis, and (3) endocrine disruption by ‘obesogens’: environmental chemicals that may be stored in adipose tissue and have the potential to modulate both estrogenic and adipogenic pathways. Dotted lines indicate proposed mechanisms and solid lines indicate established mechanisms.

Table 1: Summary of obesity-induced male infertility mechanisms.

<table>
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<tr>
<th>Mechanism</th>
<th>Description</th>
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<tr>
<td>(1) Hypogonadism</td>
<td>Hypogonadotropic hypogonadism caused by negative feedback by estrogens or insulin/leptin resistance, and hypergonadotropic hypogonadism caused by direct actions of leptin on the testis.</td>
</tr>
<tr>
<td>(2) Testicular heat-stress-/hypoxia-induced apoptosis</td>
<td>Testicular heat-stress-/hypoxia-induced apoptosis.</td>
</tr>
<tr>
<td>(3) Endocrine disruption by ‘obesogens’</td>
<td>Environmental chemicals that may be stored in adipose tissue and have the potential to modulate both estrogenic and adipogenic pathways.</td>
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Modulation of adipocyte function and differentiation is now recognized as a subset of endocrine disruption. Such environmental chemicals have been termed ‘metabolic disrupters’ [198] or ‘obesogens’ [199]. Obesogenic pathways proposed...
include nuclear receptors involved in lipid metabolism, such as PPAR-γ, liver X receptor and farnesoid X receptor [206]. Several xeno-PPAR-γ ligands have been identified, including phthalate monoesters [201], such as di(2-ethylhexyl) phthalate [202] and its metabolite mono(2-ethylhexyl) phthalate [198], organotin compounds triphenyltin and tributyltin [200,203–206], dioxins [207] and phytoestrogen genistein [208], many of which have established toxicity to the reproductive system [178,209]. Studies performed in laboratory and wildlife animals provide additional evidence for crosstalks between endocrine and adipogenic pathways, further implicating that environmental contaminants, which can be endocrine disrupters, probably also modulate adipocyte differentiation and contribute to reproductive pathologies (Figure 13C & D).

Conclusion
Three main biological mechanisms can be used to explain the relationship between obesity and male infertility: hypogonadism; testicular heat-stress/hypoxia-induced apoptosis and endocrine disruption by obesogens (Figure 14). Obesity-induced hypogonadism appears to be present in most morbidly obese men, driven by peripheral conversion of androgens to estrogens and leptin/insulin resistance. Obese men diagnosed with hypogonadism may have several treatment options, including weight loss and short-term aromatase inhibitor treatment. Similarly, testicular heat stress, although difficult to ascertain medically, could be anticipated following gradation of the pannus (suprapubic/abdominal fat overhang). Testicular heat stress may be alleviated through increased activity, weight loss and use of air-cooling devices during sleep. Future areas for investigation include the roles of adipocytokines and endocrine disrupters in male fertility and obesity. Prenatal exposures to endocrine disrupters may reprogram not only reproductive and endocrine pathways but also molecular modulation of adipocytes. Obesity is, therefore, a causal factor in male infertility, and for some individuals, prenatal exposures may confer additional risk via intersecting endocrine and adipogenic signaling pathways.

Expert commentary
Clinical knowledge in the identification and treatment of obesity-induced male infertility is still lacking. The assessment of male infertility relies on numbers of sperm and morphological abnormalities, with little functional analysis beyond estimates of motility. Subfertility is undoubtedly underestimated, simply because clinics lack tools to provide functional semen analysis, including sperm–egg binding and acrosome/capacitation assays. Screening for insulin resistance and hypogonadism should be considered as part of infertility diagnostics for overweight and obese male patients. As the age of onset for Type 2 diabetes is declining, men under the age of 40 years should be included in this screening. BMI and waist:hip ratio data should be collected for male infertility patients along with standard endocrine profiles to identify who would benefit from weight loss or treatment with hormone therapies. Medications used to treat Type 2 diabetes (sulfonylureas, biguanides, e.g., metformin and thiazolidinediones) have not been well examined for their impacts on male fertility. Information on exposure to endocrine disrupters and other environmental contaminants should be gathered by occupation and lifestyle questionnaires and it may help explain idiopathic forms of male infertility. In general, it is anticipated that the obese man is likely to exhibit hypogonadism and is hyperestrogenic, conditions that would cause the greatest risk to male infertility.

Five-year view
The issue of infertility in the obese male is critical in the context of the obesity epidemic in the Western world. Within the next 5 years, the obesity epidemic will begin to evolve into the Type 2 diabetes epidemic, with an explosion of men and women developing insulin resistance. As we learn more about the molecular biology underlying insulin resistance, the intriguing findings of concomitant suppression of Leydig cell testosterone production may be further elucidated. Certainly, there is a need for more robust studies examining semen quality in insulin-resistant men. Adipocytokines are hormones released from adipocytes, including leptin (reviewed here), resistin and adiponectin. Investigation of the physiological roles of these adipocytokines is in its infancy, although links between adipocytokines and insulin resistance have already been postulated. Kisspeptins and their modulation of the hypothalamic–pituitary–gonadal axis in both men and women is a field that is developing at a rapid pace. Understanding the neuroendocrine modulation of the reproductive system and its potential sensitivity to endocrine disrupters and nutritional stressors will contribute significantly to this field. The contribution of environmental stressors (nutrition, endocrine disrupters and infectious agents) to fetal development, and ultimately adult-onset diseases, including obesity and infertility, will be investigated using molecular approaches. Although this review has focused on male infertility, the potential for environmental stressors to create epigenetic changes to the germ line will have important implications for future generations. Endocrine disrupters, particularly environmental estrogens, are well-established reproductive toxicants and target steroid hormone signaling pathways associated with nuclear receptor superfamilies. The recent characterization of non-genomic steroid hormone receptors has the potential to revolutionize the field of endocrinology. Chemicals with the capacity to perturb adipose physiology have only recently been identified and termed obesogens or metabolic disrupters. The importance of these chemicals to obesity, diabetes and male infertility remains to be elucidated.

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Key issues

- BMI is negatively correlated with subfertility.
- Metabolic syndrome, including obesity and diabetes, intersects with male infertility due to hypogonadism.
- Adipose tissue secretes leptin and estrogen, both of which modulate male reproduction.
- Leptin has central effects at the hypothalamus, which indirectly stimulates release of gonadotropins.
- Leptin has local testicular effects, negatively regulating spermatogenesis and steroidogenesis.
- Testicular temperature elevations in obese, sedentary men may be sufficient to impair spermatogenesis.
- Fetal period represents a critical window of development, wherein environmental stressors (nutrition, chemical exposures) may contribute to adult onset diabetes, obesity or infertility.
- Obesogens, or metabolic disrupters, are chemicals in the environment that modulate adipogenic pathways and may also impair reproduction.

References


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