Human Reproduction Update, Vol.17, No.5 pp. 667–683, 2011
Advanced Access publication on May 4, 2011 doi:10.1093/humupd/dmr017

human reproduction update

Functional relationship between obesity and male reproduction: from humans to animal models

K.J. Teerds*, D.G. de Rooij, and J. Keijer

Human and Animal Physiology, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

*Correspondence address. E-mail: katja.teerds@wur.nl

Submitted on November 11, 2010; resubmitted on January 26, 2011; accepted on April 1, 2011

TABLE OF CONTENTS

BACKGROUND: The increase in the incidence of obesity has a substantial societal health impact. Contrasting reports have been published on whether overweight and obesity affect male fertility. To clarify this, we have reviewed published data on the relation between overweight/obesity, semen parameters, endocrine status and human male fertility. Subsequently, we have used results obtained in animal models of obesity to explain the human data.

METHODS: Pubmed, Scopus, Web of Science and Google Scholar databases were searched between September 2009 and October 2010 for a comprehensive publication record. Available studies on adult human males were examined. The included animal studies examined obesity and fertility, and focused on leptin, leptin receptor signaling, kisspeptins and/or NPY.

RESULTS: Most overweight/obese men do not experience significant fertility problems, despite the presence of reduced testosterone alongside normal gonadotrophin levels. Only a subgroup of subjects suffers from hypogonadotropic hypogonadism. Animal models offer several explanations and show that reduced leptin signaling leads to reduced GnRH neuronal activity. This may be due to decreased hypothalamic *Kiss1* expression, a potent regulator of GnRH/LH/FSH release. As the *Kiss1* neurons express leptin receptors, the *Kiss1* system may participate in transmitting metabolic information to the GnRH neurons, thus providing a bridge between metabolic regulation and fertility.

CONCLUSIONS: Infertility in overweight/obese males may be explained by leptin insensitivity. This implies a possible role for the KISS1 system in human obesity-related male infertility. If substantiated, it will pave the way for methods to restore fertility in these subjects.

Key words: human male fertility / semen parameters / endocrine status / leptin / animal models

Introduction

Overweight and obesity: some facts

Overweight and obesity have become a major societal problem and their incidence is rapidly increasing. Globally, more than 1.6 billion adults are overweight and at least 300 million of these subjects are clinically obese (WHO, 2006). In general, obesity rates have increased more than 3-fold since 1980 in North America, the UK, Eastern Europe, the Middle East, the Pacific Islands, Australasia and China (WHO, 2003). In the USA, the incidence of obesity in adult males has increased from 11.7% in 1991, 17.9% in 1998, to 30.6% in 2004 (Hedley *et al.*, 2004), emphasizing that the incidence of overweight and obesity is reaching epidemic levels. Although there is no generally accepted definition for obesity in children and adolescents, it is clear that the prevalence of overweight is also increasing for children. For the USA, the WHO reported that ~17% of the children (age .6–11) and adolescents (age 12–19) were overweight or obese in the period 2001–2004 (WHO, 2006). Recently, the WHO has emphasized that the prevalence of childhood obesity is growing at an alarming rate (WHO, 2007). Intriguingly, the rise in the incidence of obesity is not restricted to the industrialized societies, but is even more pronounced in developing countries (WHO, 2003).

Overweight and obesity are characterized by an excess of fat mass and are most commonly defined by the ratio between weight (kg) and the square of the height (m), the so-called BMI. The WHO considers persons with a BMI over 25 kg/m^2 as overweight, and as obese when BMI is over 30 kg/m^2. Another way to express the severity of overweight/obesity is by determining waist circumference and waist/hip ratio (Fejes *et al.*, 2005). Although it has been suggested that these may be even better markers for overweight and obesity, the majority of the studies uses BMI as the marker. We therefore use BMI as the indicator of overweight and obesity in the present review.

Overweight and obesity are known risk factors for diseases such as diabetes, coronary heart disease, stroke, hypertension and sleep apnea, and hence have become major contributors to the global burden of chronic disease (NIDDK, 2007). Changes in food composition and diet, such as increased consumption of energy-dense, highly palatable, nutrient-poor foods with high levels of sugar and saturated fats, combined with reduced physical activity are considered primary causes of this problem.

Negative effects of obesity on male fertility have been described as early as the 10th century by Avicenna, a Persian scientist and medical doctor in his encyclopedic medical book *The Canon of Medicine*. However, until recently, the possible relationship between obesity and male reproduction has been largely ignored. Here, we provide an overview of what is known about effects of overweight and obesity on human male fertility. We will try to find answers to questions like 'Does obesity indeed contribute to male infertility? What are the consequences of maternal obesity for the fertility of the male offspring? And, if one considers fat tissue as an endocrine organ, how can hormones released by fat tissue (adipokines) modulate testicular functioning?' In the second part of the review, the effects of these adipokines on male reproduction will be further analyzed using results obtained in animal models for obesity research and related to human fertility in overweight/obese male individuals.

Methods

In the first part of this review, we systematically analyze the effects of overweight and obesity on semen parameters and endocrine hormones in human males of reproductive age. In the second part of the review, selected animal models for obesity are analyzed and related to the human studies, in order to find an explanation for obesity-related fertility problems. The included animal studies involve models for leptin and leptin signaling deficiency and NPY signaling in relation to hypothalamic–pituitary function. Only peer-reviewed studies have been included in the review. Review articles have been used as a source of references and are refered to as such.

Inclusion criteria and outcomes of interest

Included are those studies in which the participants were adult human males of reproductive age, independent of the number of subjects involved. The outcomes included are: (i) any measures of semen volume, sperm concentration, sperm motility, sperm morphology and sperm DNA integrity, or (ii) any basal measurements of the following hormones: steroid hormone-binding globulin (SHBG), total testosterone (T), free-testosterone (free-T), estradiol (E_2), LH, FSH, inhibin-B and leptin.

In general, the outcome of all eligible studies must be related to BMI. We also included a few cases in which waist/hip ratio was used as the marker for overweight and obesity. No exclusion is applied to the statistical methods used in the included papers, although in Tables I and II only true values are included, not correlations.

The included animal model studies were only those that investigated leptin and leptin receptor dysfunction, leptin receptor signaling, kisspeptins or NPY in relation to male fertility.

Exclusion criteria

Studies in which the individuals included had not yet reached adulthood are excluded from the analysis. No upper-age limit is applied. Studies that included primarily men with particular diseases or a history of disorders of the reproductive organs not related to overweight/obesity (e.g. varicocele, cryptorchidism, trauma, vasectomy, testicular torsion) are excluded. Studies that involve primarily males with azoospermia are also excluded, as it is not possible to determine a relationship between semen parameters and BMI in these subjects. Similarly, studies focusing on men who had been exposed to particular environmental toxicants known to compromise male fertility, such as pesticide factory workers, are excluded.

Studies that reported reproductive hormone measurements only after stimulation are excluded, as are studies in which overall fertility was assessed by pregnancy rate. In those cases where duplicate data sets are used in multiple articles, only the article including the largest sample size is included.

Search strategy

The Pubmed, Web of Science, Scopus and Google Scholar databases are comprehensively searched. At least two searches were conducted for each database. Search terms used included combinations of the following terms: BMI, body weight, overweight, obesity, male fertility, semen quality, semen volume, sperm morphology, sperm count, sperm motility, sperm

Table I Summary of studies that have investigated semen parameters in overweight and obese men compared with normal-weight men with a BMI of >18.5 and <24.99 (↑, increased; ↓, decreased; NI, not clearly indicated in reference).

Semen parameters	Overweight BMI >25 <29.99 (kg/m^2)	Number of subjects	Obese BMI >30 (kg/m^2)	Number of subjects
Semen volume (normal)	Jensen *et al.* (2004)	299	Strain *et al.* (1982)	21
	Chavarro *et al.* (2010)	233	Chavarro *et al.* (2010)	127
	Ramlau-Hansen *et al.* (2010)	63	Ramlau-Hansen *et al.* (2010)	21
Sperm concentration↓	Jensen *et al.* (2004)	299	Koloszár *et al.* (2005)	58
	Magnusdottir *et al.* (2005)	25	Hammoud *et al.* (2008)	128
	Fejes *et al.* (2006)	81	Roth *et al.* (2008)	1
	Hanafy *et al.* (2007)	50	Stewart *et al.* (2009)	35
	Qin *et al.* (2007)	241	Chavarro *et al.* (2010)	19
	Hammoud *et al.* (2008)	168	Hofny *et al.* (2010)	80
	Chavarro *et al.* (2010)	35	Wegner *et al.* (2010)	36
Sperm concentration (normal)	Koloszár *et al.* (2005)	91	Strain *et al.* (1982)	21
	Magnusdottir *et al.* (2005)	47	Aggerholm *et al.* (2008)	163
	Hanafy *et al.* (2007)	30	Chavarro *et al.* (2010)	108
	Aggerholm *et al.* (2008)	773	Hofny *et al.* (2010)	42
	Chavarro *et al.* (2010)	198	Ramlau-Hansen *et al.* (2010)	21
	Ramlau-Hansen *et al.* (2010)	63		
Sperm motility↓	Magnusdottir *et al.* (2005)	25	Kort *et al.* (2006)	NI
	Kort *et al.* (2006)	NI	Hammoud *et al.* (2008)	128
	Hammoud *et al.* (2008)	168	Chavarro *et al.* (2010)	55
	Chavarro *et al.* (2010)	105	Hofny *et al.* (2010)	80
			Wegner *et al.* (2010)	35
Sperm motility (normal)	Jensen *et al.* (2004)	299	Strain *et al.* (1982)	21
	Magnusdottir *et al.* (2005)	47	Aggerholm *et al.* (2008)	163
	Aggerholm *et al.* (2008)	773	Chavarro *et al.* (2010)	72
	Chavarro *et al.* (2010)	128	Hofny *et al.* (2010)	42
	Ramlau-Hansen *et al.* (2010)	63	Ramlau-Hansen *et al.* (2010)	21
Abnormal sperm morphology↑	Qin *et al.* (2007)	241	Hammoud *et al.* (2008)	128
	Chavarro *et al.* (2010)	57	Stewart *et al.* (2009)	17
			Chavarro *et al.* (2010)	29
			Hofny *et al.* (2010)	80
			Wegner *et al.* (2010)	19
No abnormal sperm morphology	Jensen *et al.* (2004)	299	Chavarro *et al.* (2010)	98
	Hammoud *et al.* (2008)	168	Hofny *et al.* (2010)	42
	Chavarro *et al.* (2010)	176	Ramlau-Hansen *et al.* (2010)	21
	Ramlau-Hansen *et al.* (2010)	63		
Sperm DNA integrity↓	Kort *et al.* (2006)	NI	Kort *et al.* (2006)	NI
			Chavarro *et al.* (2010)	127

DNA integrity, SHBG, (free)testosterone, estradiol, LH, FSH, inhibin, leptin, NPY, kisspeptin, GnRH, Stat3, hypothalamus, arcuate nucleus and pituitary. Appropriate synonyms, variant spelling and truncations are included for each search term. Only papers published in English are included, while abstracts are excluded.

Results

Overweight and obesity: consequences for male reproduction

Semen parameters

Several reports suggest that an increasing BMI or waist/hip ratio is associated with alterations in testicular volume, semen parameters and fertility. Other investigators were unable to link changes in BMI to reduced fertility. In Table I, an overview of the available literature on the relation between BMI and various semen parameters is presented, revealing contradictory observations by different investigators. While some studies confirm the existence of a relation between BMI and male fertility, a similar number of studies do not. The studies that do show a relation between overweight/obesity and male fertility implicate a wide range of mechanisms. Altogether, obesity seems to enhance a predisposition to fertility problems rather than cause it, except in cases of developmental obesity due to gene mutations. Below, selected studies are discussed to illustrate these points.

Among the studies implicating a relation between overweight/obesity and semen parameters, Fejes *et al.* (2005) found a negative correlation between semen volume and waist/hip ratio but did not observe changes in total sperm count and numbers of motile sperm. The presence of a negative correlation between semen parameters and overweight/obesity was confirmed by Kort *et al.* (2006) reporting the presence of a significantly decreased percentage of normal motile

sperm in both overweight and obese men. Unfortunately, in this study, the percentage of normal motile sperm is based on semen volume × concentration × %motility × %morphology, making it impossible to evaluate effects on separate semen parameters.

In line with these observations, Sallmen et al. (2006) reported a relationship between increasing male BMI and infertility with adjusted odds ratio of 2.13 and 1.83 for the two highest male BMI categories of 32–34 and >35 kg/m^2, respectively. These observations were confirmed by Nguyen et al. (2007) after adjustment for the female partners, BMI, age and smoking habits of both partners. Based on these observations, it may not come as a surprise that overweight and obesity not only in males but also in females coincides with a higher risk of subfecundity, particularly for couples where both partners are overweight or obese. In that case, the odds ratio for subfecundity were 1.41 (95% CI: 1.28–1.56) and 2.74 (95% CI: 2.27–3.30), respectively, compared with couples where both partners had a normal BMI. Thus, weight reduction may very well improve fecundity (Ramlau-Hansen et al., 2007a).

Another important question is whether maternal obesity leads to semen abnormalities in male offspring. Ramlau-Hansen et al. (2007b) hypothesized in a pilot study that maternal overweight/obesity may have a programming effect on testicular development during fetal life. These authors observed that sons of mothers who were overweight/obese during pregnancy had a tendency toward a higher birthweight, a condition that persisted, as these sons had a higher BMI in adulthood compared with sons of normal-weight mothers. Possibly, the reduced SHBG levels and consequently higher free estrogen levels in overweight/obese mothers (reviewed in Pettigrew and Hamilton-Fairley, 1997), and hence in the fetus, may interfere with the hormonal control of testicular development (reviewed in Sharpe, 2003) and, together with an elevated BMI, affect semen quality in adulthood. Although an interesting hypothesis, in a more recent study, the same group reported that no obvious relation existed between high birthweight and prepubertal body fat on semen quality in young adult life. This study did not include an analysis of the possible influence of maternal BMI on male offspring, and suggested an effect of adult BMI rather than an effect of childhood BMI on semen parameters (Ramlau-Hansen et al., 2010). Taken together, these studies implicate that maternal BMI during pregnancy is not an important determinent of male offspring fertility in adulthood.

Fertility parameters do not correlate negatively to BMI in all overweight and obese subjects. On the contrary, in many of these subjects, semen quality has consistently shown to be normal (reviewed in Hammoud et al., 2006). Nevertheless, a higher prevalence of obesity appears to occur among men with poor semen quality compared with those with normal semen quality (Magnusdottir et al., 2005). Thus, one can wonder as to what the decisive factor is: obesity or pre-existing fertility problems? In line with this, Ramlau-Hansen et al. (2007a) have suggested that a high BMI may lead to sterility in persons with additional fecundity problems.

Another explanation for the affected fertility in obese men may be the accumulation of fat tissue in the suprapubic and thigh region, causing elevated scrotal temperatures that may have detrimental effects on spermatogenesis (Shafik and Olfat, 1981a, b). In obese subjects, specific patterns of scrotal lipid tissue accumulation may occur that are not seen in infertile, non-obese subjects. According to the authors, surgical removal of excessive fat pads resulted in a significant improvement of semen quality. Unfortunately, this study did not include a control group of obese subjects with normal fertility, and therefore, it is difficult to draw any firm conclusions.

As adipose tissue is an important site of hormone production and metabolism, accumulation of large amounts of body fat may interfere with the hormonal regulation of testicular function. Several older studies suggest that metabolic parameters associated with obesity, such as high levels of plasma cholesterol and/or triglycerides, have direct adverse effects on testicular function, leading to poor semen quality and infertility (Jones et al., 1979; Padron et al., 1989). These observations are expanded by a more recent investigation that reported a 65% incidence of dyslipidemia as defined by isolated hypercholesterolemia, triglyceridemia or both, in 106 male partners from infertile couples (Ramirez-Torres et al., 2000).

Obesity-related fat accumulation is associated with increased oxidative stress and lipid peroxidation (Vincent et al., 2007). Several studies have shown that reactive oxygen species (ROS), causing lipid peroxidation, are extremely toxic to human spermatozoa (Jones et al., 1979; Selly et al., 1991), implicating a significant role for oxidative stress as cause of male infertility (Tremellen, 2008; Agarwal et al., 2009; Kefer et al., 2009). In line with this, spermatozoa from infertile men show signs of greater oxidative injury compared with normal fertile controls (Kodama et al., 1997). These observations are confirmed by an *in vitro* study in which endogenously generated ROS led to an increase in sperm DNA fragmentation (Aitkin et al., 1998; Twigg et al., 1998). From these data, Kasturi et al. (2008) concluded that oxidative stress may result in lipid peroxidation in the sperm plasma membrane. This could then lead to decreased motility and membrane dysfunction possibly by impairing cell membrane ion exchange essential for normal sperm motility (Rao et al., 1989). Excessive oxidative stress may also result in DNA damage with diminished genetic variability of the affected sperm. Kasturi et al. (2008) suggested that the elevated DNA fragmentation index noted in obese men by Kort et al. (2006) may indeed reflect an abnormal oxidative state in the testicular microenvironment and efferent duct system of these men. In support of these data, it has been suggested that α-tocopherol (vitamin E), a compound with strong antioxidant properties, might help to preserve the functional competence of spermatozoa subjected to an oxidative attack. *In vitro* studies made clear that α-tocopherol indeed protected sperm DNA against oxidative damage (Sierens et al., 2002), and that the percentage of motile sperm in a semen sample is correlated with sperm α-tocopherol content (Thérond et al., 1996). The protective role of antioxidants against sperm DNA damage is confirmed in an animal study in which male rats were fed a high-fat diet containing 1% cholesterol. Due to the diet-induced hypercholesterolemia, fertility was reduced in these rats, a condition that could be improved again by the concomitant administration of α-tocopherol and the hypolipidemic and hypocholesterolemic drug simvastatin (Shalaby et al., 2004). The antioxidant treatment apparently protected the spermatozoa from lipid peroxidation and in this way restored membrane fluidity and thus sperm function and fertilizing capacity. Independent of obesity, the general concept that antioxidant therapy may be beneficial for sperm quality is supported by a recent systematic review (Ross et al., 2010).

Taken together, though Table I may give the impression that obesity affects fertility in ~50% of the studies cited, one has to keep in mind

that in only four of these studies, overweight or obesity was related to an increased incidence in oligospermia due to hypogonadotropic hypogonadism (Magnusdottir *et al*., 2005; Fejes *et al*., 2006; Hanafy *et al*., 2007; Hammoud *et al*., 2008). In most studies, the observed effects on sperm concentration are small, and sperm numbers and quality in overweight and obese men are within the normal range (e.g. Jensen *et al*., 2004; Koloszár *et al*., 2005; Hofny *et al*., 2010; Stewart *et al*., 2009; Chavarro *et al*., 2010; Wegner *et al*., 2010). It has therefore been suggested that a high BMI may lead to reduced fertility only in those males who suffer from additional fecundity problems. In line with the above conclusions, in a recent meta-analysis on the impact of BMI on semen parameters and reproductive hormones in human males, MacDonald *et al.* (2010) using very strict inclusion and exclusion criteria also conclude that there was no evidence for a relationship between BMI and sperm concentration or total sperm count. Our approach expands the analysis by MacDonald *et al*. as we use less strict inclusion and exclusion criteria and present the relevant references and the number of subjects involved in these studies in Table I.

Hormones and testicular function

Although changes in plasma cholesterol and triglyceride levels may be important factors in obesity-related infertility, these factors are in general not taken into account when analyzing endocrine parameters in overweight and obese subjects. On the contrary, most studies focus on changes in gonadotropic hormone levels, steroid hormone-binding globulin (SHBG), testosterone, estradiol and inhibin levels. A few studies also include the levels of the adipokine leptin. As is the case in the relation between overweight/obesity and semen parameters, the reports on correlations between overweight/obesity and endocrine parameters are contradictory. An overview of the relevant literature is presented in Table II.

Steroid hormone-binding globulin

There is a general consensus that obesity is negatively correlated with plasma SHBG levels (Table II), although in some cases, this correlation is only observed in obese men with fertility problems (Jarow *et al*., 1993). The relation between BMI and SHBG is further strengthened by the observation that when massively obese men loose weight, SHBG steroid binding capacity increases again (Vermeulen *et al*., 1996) and plasma SHBG levels return to the normal range when a near-normal body weight is achieved (Pasquali *et al*., 1997). The cause of the reduction in plasma SHBG levels is that obese males are at risk of developing insulin resistance and consequently hyperinsulinemia. Hyperinsulinemia, by affecting liver function, is thought to be the major determinant in the obesity-related decrease in SHBG levels (Isidori *et al*., 1999).

Testosterone and estradiol

The majority of the studies investigating the effect of obesity on total and free-testosterone levels report decreased levels (Table II). This reduction is presumably at least in part caused by the decrease in SHBG levels that will affect the half-life of testosterone (Jarow *et al*., 1993; Giagulli *et al*., 1994; Foresta *et al*., 2009). Whether the reduction in testosterone levels is sufficient to exert a biological effect, influencing spermatogenesis, is a subject of debate. In mild to moderate obese males, the decrease in total testosterone is thought to be biologically ineffective, as SHBG levels are concomitantly decreased (Kley *et al*., 1981). In massively obese males, however, the decrease in SHBG is too small to compensate for the decrease in testosterone levels, resulting in a marked reduction in free-testosterone levels in this subgroup of subjects.

Several explanations have been offered for the decrease in testosterone levels in overweight and obese subjects. Due to the decrease in SHBG levels, more testosterone is available to be converted into estradiol in fat tissue (e.g. Foresta *et al*., 2009). In some cases, this has led to a modest increase in plasma estradiol levels (e.g Schneider *et al*., 1979) but even if estradiol levels are not increased, the testosterone (T)/estradiol (E_2) ratio may be decreased due to the decreased testosterone levels. In humans, a decrease in T/E_2 ratio has been shown to be associated with infertility (Pavlovich *et al*., 2001). Although it is doubtful whether these relatively small changes are sufficient to alter testicular steroid concentrations and influence spermatogenesis, one has to keep in mind that estradiol can exert a physiological effect at a much lower concentration than testosterone (Hammoud *et al*., 2008).

Treatment of morbid obese patients with aromatase inhibitors, such as testolactone, letrozole or anastrozole, to prevent the conversion of testosterone into estradiol, results in normalization of testosterone levels, suppression of estradiol levels and in some cases, to normalization of spermatogenesis and fertility (Pavlovich *et al*., 2001; Raman and Schlegel, 2002; Zumoff *et al*., 2003; De Boer *et al*., 2005; Roth *et al*., 2008). Unfortunately, in most cases, these studies are not case-controlled and include a limited number of subjects. Therefore, it is too early to draw any conclusions about the use of aromatase inhibitors as a possible treatment for obesity-related fertility problems.

Another way to improve the T/E_2 ratio is by suppressing estradiol levels with anti-estrogens. Treatment of normal-weight men suffering from idiopathic oligozoospermia with the anti-estrogen tamoxifen citrate results in improved sperm quantity and quality (Adamopoulos *et al*., 1997). When this treatment is combined with testosterone undecanoate, sperm variables are improved even further, resulting in a higher pregnancy incidence (Adamopoulos *et al*., 1997, 2003; reviewed in Liu and Handelsman, 2003). Despite these positive results, no case-controlled study has been published yet in which this treatment was applied to overweight/obese subjects suffering from reduced fertility.

It has also been suggested that the mildly elevated estradiol levels in obese men influence pituitary gonadotrophin release by affecting LH pulsatility and bioactivity without influencing plasma LH levels (Veldhuis and Dufau, 1987). In line with these observations, Vermeulen (1993) reported a reduction in the frequency of the large amplitude LH pulses in obese subjects.

FSH and LH

Sertoli cells provide developing germ cells with structural and hormonal support. The number of Sertoli cells in the adult testis determines both testis size and daily sperm production, as each Sertoli cell can only support and nourish a fixed number of germ cells (Orth *et al*., 1988; Brendtson and Thompson, 1990). In human males, Sertoli cells undergo two distinct periods of proliferation, in the neonatal and in the (peri)pubertal period. Suppression of FSH levels during these periods reduces the final number of Sertoli cells in adulthood

(reviewed in Sharpe 2003). Both the neonatal and (peri)pubertal increases in Sertoli cell number are paralleled by a rise in circulating inhibin-B levels (Andersson *et al*., 1998; Winters and Plant, 1999; Winters *et al*., 2006). Winters *et al.* (2006) further observed that inhibin-B levels were lower in obese young adult men compared with normal-weight men, whereas inhibin-B levels were unrelated to BMI among prepubertal boys. Winters *et al* hypothesize that this reduction in inhibin-B levels reflects suppressed Sertoli cell proliferation during puberty. Support for this assumption comes from Globerman *et al.* (2005) who report that the low levels of inhibin-B in some morbidly obese men fail to increase after weight loss, despite a rise in testosterone levels. This implicates that the negative impact of obesity on Sertoli cell proliferation during (peri)puberty might compromise male reproductive function in adulthood. Unfortunately, these conclusions are based on inhibin-B levels as a surrogate marker for Sertoli cell numbers, while Sertoli cell proliferation and plasma FSH levels as such have not been determined in obese (peri)-pubertal boys. Therefore, the hypothesis by Winter *et al*. still needs to be confirmed.

As discussed above, estradiol levels are mildly elevated in overweight and obese subjects. Besides influencing LH pulsatility (Veldhuis and Dufau, 1987), it has been hypothesized that these elevated estradiol levels may also influence GnRH, LH and FSH release, resulting in hypogonadotropic hypogonadism and decreased fertility in overweight and obese subjects (reviewed in Strain *et al*., 1982; Jensen *et al*., 2004; Hammoud *et al*., 2006; Fig. 1). Careful screening of the available literature shows that there is not much support for this assumption as the majority of the studies do not report changes in LH and FSH levels in overweight and obese men (Table II). The reason for this may be that the, in general, small elevation in estradiol levels is counteracted by the concomitant small decrease in testosterone levels with as net result no change in gonadotrophin release. Despite this, there is a subset of men in whom obesity is correlated with significantly reduced gonadotrophin levels and subsequent hypogonadotropic hypogonadism (Table II). Possibly, these men suffer from a genetic predisposition that together with the obese condition leads to reduced fertility or even infertility. In the second part of this review, an attempt will be made to offer an explanation for this phenomenon.

Leptin

An alternative explanation for the obesity-related reduction in testosterone levels may be the impairment of Leydig cell function. Testosterone levels are inversely correlated with circulating leptin levels, an adipokine produced by adipocytes and which receptor is expressed by Leydig cells (Caprio *et al*., 1999; Isidori *et al*., 1999; Ishikawa *et al*., 2007). In an *in vitro* study using rodent Leydig cells, it is shown that leptin at concentrations within the range present in obese men directly inhibits hCG-stimulated testosterone production by inhibiting the conversion of 17OH-progesterone to testosterone (Caprio *et al*., 1999; Isidori *et al*., 1999).

Besides influencing testicular function through Leydig cell testosterone production, leptin may also have a direct effect on germ cells. Spermatocytes express a functional leptin receptor in a stage-dependent way (El-Hefnawy *et al*., 2000). Furthermore, the presence of leptin has been demonstrated in spermatocytes in human male testes, while dysfunction of spermatogenesis is associated with increased testicular leptin levels (Ishikawa *et al*., 2007). Regrettably, it is not clear from this study how leptin is able to reach the spermatocytes.

Leptin levels are correlated with adipose tissue mass (Tatti *et al*., 2001). Interestingly, plasma leptin levels correlate positively with semen leptin levels, thus an increase in plasma leptin levels may concomitantly lead to an elevated exposure of germ cells to leptin (Von Sobbe *et al*., 2003). In an *in vitro* study using washed human spermatozoa from normozoospermic donors, it is further demonstrated that leptin stimulates sperm motility (Lampio and du Plessis, 2008). However, the possible consequences of prolonged increased exposure to leptin for sperm cell quality and function are at present not clear.

Summary of endocrine effects

In conclusion, the endocrine profile of overweight and obese men is more clearly affected than semen parameters. Most studies report decreased plasma SHBG and testosterone levels (Table II). Surprisingly, in 88% of the studies, the decrease in testosterone levels does not coincide with reduced LH and FSH levels (Table II). Therefore, in analyzing the effects of overweight and obesity on male fertility, it is important to determine semen parameters and relate these to the endocrine profile of the men. Specific emphasis should be given to the pituitary–testis axis to determine whether the decrease in testosterone levels is caused by hypogonadotropic hypogonadism or Leydig cell dysfunction. This information would provide important clues for possible treatment.

In a recently published meta-analysis, MacDonald *et al*. show that 18 of the 20 included studies measuring testosterone levels and 15 of the 16 studies measuring SHBG levels report a negative relationship between increased BMI and plasma testosterone, free-testosterone and SHBG levels (MacDonald *et al*., 2010). Of the 12 studies that investigate free-testosterone levels, 10 report a negative correlation with BMI, although this relationship is not as strong as that for total testosterone or SHBG (MacDonald *et al*., 2010). The fact that, despite this negative correlation, most studies do not report changes in LH and FSH levels is not further discussed by these authors. The present review offers possible physiological explanations for the decrease in testosterone levels and the absence of changes in gonadotrophin levels.

Genetic obesity syndromes and human male fertility

Overweight and obesity are complex traits, in which interactions between environmental and multiple genetic factors lead to varied clinical presentations (Zondervan and Cardon, 2004). Indeed, a human genome-wide association study has shown that many loci are associated with obesity (Speliotes *et al*., 2010). The first gene to be causally implicated in obesity was leptin, originally identified in rodents (Zhang *et al*., 1994). Analysis of animal models of obesity has led to the identification of many other obesity genes. Most of these genes, as well as additional obesity-predisposing genes identified in association studies, encode molecular components of the physiological systems regulating energy balance (Duarte *et al*., 2007; Chamberland *et al*., 2009; Vimaleswaran and Loos, 2010). The most strong association with obese susceptibility has recently been revealed for gene variants of fat-mass- and obesity-associated (FTO) (Speliotes *et al*., 2010). However, it is beyond the scope of this review to provide an overview on the genetic predisposition to obesity, especially, since

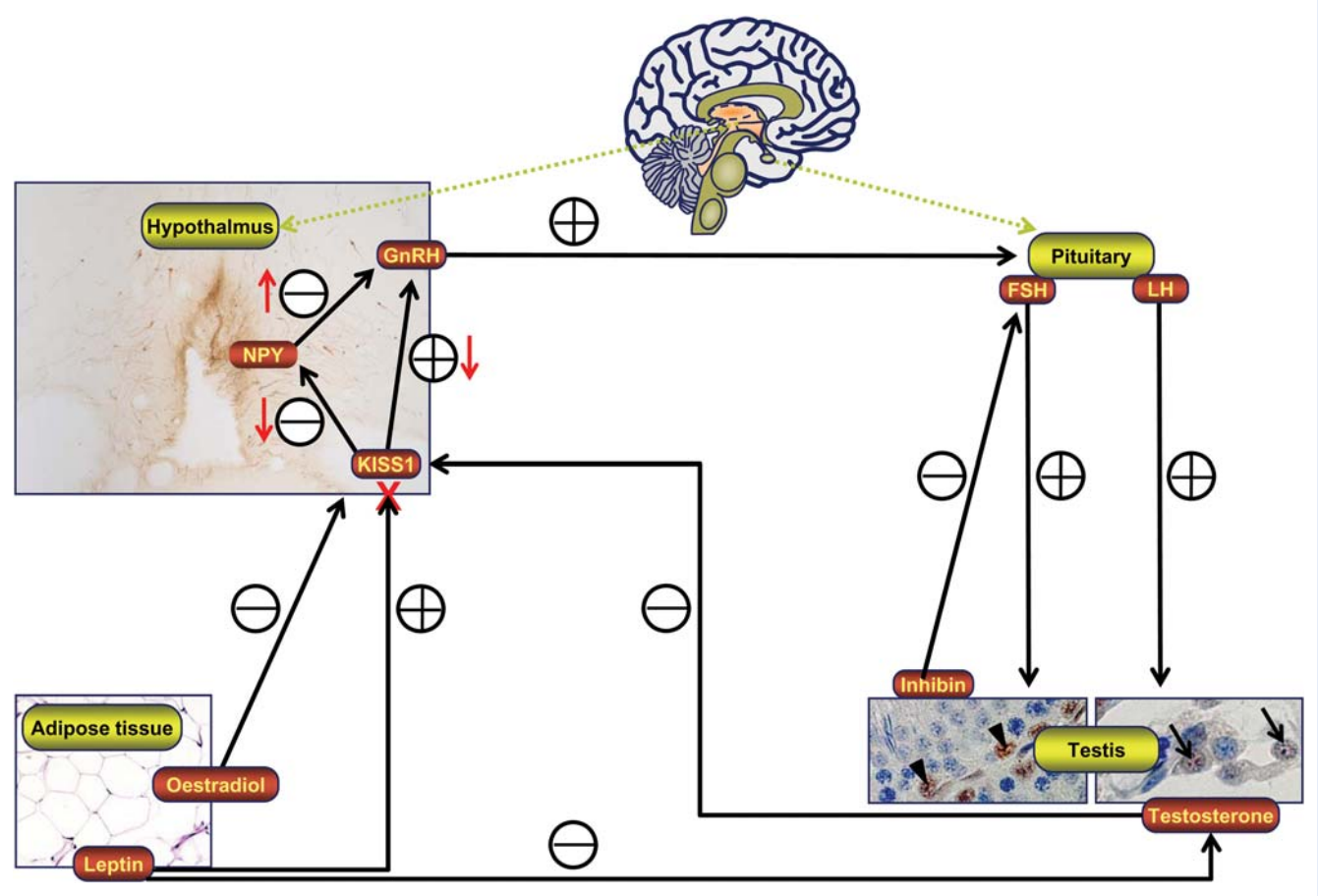

Figure 1 Schematic overview of the hypothalamic–pituitary–testicular axis: KISS1 and leptin resistance. GnRH released by the ARC of the hypothalamus stimulates the synthesis and release of the gonadotrophins FSH and LH by the pituitary. These gonadotrophins are transported to the testis where FSH stimulates, among others, inhibin production by the Sertoli cells (indicated by black arrowheads). Inhibin has a direct and specific negative feedback effect on the pituitary release of FSH. LH stimulates testosterone production by the Leydig cells (indicated by small black arrows). Testosterone has an indirect local effect on spermatogenesis by binding to its receptors in the Sertoli cell nuclei. After entering the circulation, testosterone can either be transported to the hypothalamus or to the adipose tissue, where it is converted to estradiol. Both testosterone and estradiol exert an indirect negative feedback effect on hypothalamic GnRH release through activation of KISS1 neurons. These neurons are found in large numbers in the ARC, possess androgen and estrogen receptors, and have been shown to make contact with the GnRH neurons in the ARC. KISS1 stimulates GnRH release. Besides influencing GnRH release, estradiol and testosterone have a direct negative effect on the release of FSH and LH by the pituitary. In normal-weight men, estradiol concentrations are extremely low and have a negligible effect on hypothalamic–pituitary function. On the contrary, in overweight and obese subjects, with increased adipose tissue volumes, more testosterone will be converted to estradiol. As a consequence, testosterone levels become slightly decreased while estradiol levels are somewhat increased. In general, these changes in steroid hormone levels compensate for each other, resulting in normal (unaffected) FSH and LH levels in most overweight and obese subjects (see also Table II). The major secretory product of the adipose tissue is the adipokine leptin. Leptin exerts several different effects in the body; among these, the inhibition of testosterone production. In normal-weight men, this effect is negligible, but in overweight and obese men, leptin levels increase and can have a direct inhibitory effect on Leydig cell testosterone production. Furthermore, leptin can stimulate, indirectly through activation of its receptors on the KISS1 neurons, GnRH release. Besides projecting to the GnRH neurons, KISS1 neurons also make contact with the NPY neurons in the ARC. In this way, leptin can influence the expression of NPY and prevent the NPY neurons from inhibiting GnRH release. In normal-weight men, NPY expression is kept in abeyance by leptin and therefore its effect on GnRH release is thought to be negligible. In overweight and obese men with normal fertility, leptin levels are normal or elevated (Table II). The leptin receptors (LRb) on the KISS1 neurons function normally, indirectly stimulating GnRH release and suppressing NPY. We hypothesize that the subcategory of overweight/obese men with fertility problems may suffer from leptin resistance; LRb no longer functions properly (indicated by a red cross), possibly due to a genetic predisposition or due to prolonged elevation of leptin levels. As a consequence, KISS1 expression becomes reduced, resulting in less stimulation of the GnRH neurons and reduced suppression of NPY expression (red arrows), leading to increased NPY levels. As a result, GnRH levels become even further suppressed, resulting in reduced LH and FSH release and hypogonadotropic hypogonadism. Although under this condition testosterone levels will also be reduced and therefore KISS1 neurons will be less suppressed by testosterone, this effect is too small to compensate for the effects induced by hypothalamic leptin resistance. Besides influencing GnRH release, NPY also has a direct effect on pituitary LH release, although this is not considered to be an important regulatory pathway.

Table II Overview of reported changes in hormone levels due to overweight and obesity (SHBG, steroid hormone-binding globulin; T, testosterone; E_2, estradiol; LH, luteinizing hormone; FSH, follicle-stimulating hormone; NI, not clearly indicated in reference).

Hormone levels	Overweight BMI >25 <29.99 (kg/m^2)	Number of subjects	Obese BMI >30 (kg/m^2)	Number of subjects
SHBG↓	Jensen et al. (2004)	299	Glass et al. (1977)	10
	Fejes et al. (2005)	81	Kley et al. (1981)	NI
	Aggerholm et al. (2008)	773	Strain et al. (1988)	11
	Wu et al. (2008)	1590	Jarow et al. (1993)	15
	Chavarro et al. (2010)	233	Giagulli et al. (1994)	40
	Ramlau-Hansen et al. (2010)	63	Tchernof et al. (1995)	51
			Vermeulen et al. (1996)	50
			Pasquali et al. (1997)	63
			Isidori et al. (1999)	28
			Allen et al. (2002)	41
			Osuna et al. (2006)	NI
			Vincennati et al. (2006)	22
			Winters et al. (2006)	8
			Aggerholm et al. (2008)	163
			Wu et al. (2008)	786
			Foresta et al. (2009)	31
			Hammoud et al. (2009)	42
			Stewart et al. (2009)	36
			Chavarro et al. (2010)	127
			Ramlau-Hansen et al. (2010)	21
SHBG normal range	Winters et al. (2006)	21	Jarow et al. (1993)	9
	Osuna et al. (2006)	NI	Pasquali et al. (1995)	8
			Roth et al. (2008)	1
			Hofstra et al. (2008)	149
Total T↓	Jensen et al. (2004)	299	Glass et al. (1977)	10
	Fejes et al. (2005)	81	Amatruda et al. (1978)	22
	Aggerholm et al. (2008)	773	Schneider et al. (1979)	NI
	Wu et al. (2008)	1590	Kley et al. (1981)	NI
	Chavarro et al. (2010)	233	Strain et al. (1982)	21
	Ramlau-Hansen et al. (2010)	63	Strain et al. (1988)	11
			Zumoff et al. (1990)	NI
			Jarow et al. (1993)	15
			Giagulli et al. (1994)	40
			Pasquali et al. (1995)	8
			Tchernof et al. (1995)	51
			Vermeulen et al. (1996)	50
			Isidori et al. (1999)	28
			Allen et al. (2002)	41
			Zumoff et al. (2003)	6
			Luboshitzky et al. (2005)	5
			Mohr et al. (2005)	460
			Vincennati et al. (2006)	22
			Osuna et al. (2006)	NI
			Aggerholm et al. (2008)	163
			Pauli et al. (2008)	30
			Roth et al. (2008)	1
			Hofstra et al. (2008)	51
			Wu et al. (2008)	786
			Foresta et al. (2009)	31
			Hammoud et al. (2009)	42
			Stewart et al. (2009)	36
			Chavarro et al. (2010)	127
			Hofny et al. (2010)	80
			Ramlau-Hansen et al. (2010)	21
Total T normal range	Winters et al. (2006)	21	Jarow et al. (1993)	9
	Osuna et al. (2006)	NI	Giagulli et al. (1994)	11
	Hanafy et al. (2007)	80	Leenen et al. (1994)	37
			Winters et al. (2006)	8
			Hofstra et al. (2008)	98
			Hofny et al. (2010)	42

Continued

Table II *Continued*

Hormone levels	Overweight BMI >25 <29.99 (kg/m^2)	Number of subjects	Obese BMI >30 (kg/m^2)	Number of subjects
Free T↓	Wu *et al.* (2008)	1590	Amatruda *et al.* (1978)	10
			Kley *et al.* (1981)	NI
			Strain *et al.* (1982)	21
			Strain *et al.* (1988)	11
			Zumoff *et al.* (1990)	NI
			Giagulli *et al.* (1994)	22
			Pasquali *et al.* (1995)	8
			Vermeulen *et al.* (1996)	50
			Isidori *et al.* (1999)	28
			Allen *et al.* (2002)	41
			Zumoff *et al.* (2003)	6
			Mohr *et al.* (2005)	460
			Hofstra *et al.* (2008)	160
			Roth *et al.* (2008)	1
			Wu *et al.* (2008)	786
			Foresta *et al.* (2009)	31
			Hammoud *et al.* (2009)	42
Free T normal range	Jensen *et al.* (2004)	299	Glass *et al.* (1977)	10
	Winters *et al.* (2006)	21	Schneider *et al.* (1979)	NI
			Giagulli *et al.* (1994)	18
			Winters *et al.* (2006)	8
			Hofstra *et al.* (2008)	160
T / E_2 ratio↓	Tchernof *et al.* (1995)	51	Schneider *et al.* (1979)	NI
	Jensen *et al.* (2004)	299	Jarow *et al.* (1993)	15
	Fejes *et al.* (2005)	81	Isidori *et al.* (1999)	28
	Fejes *et al.* (2006)	NI	Zumoff *et al.* (2003)	6
	Aggerholm *et al.* (2008)	773	Aggerholm *et al.* (2008)	163
	Chavarro *et al.* (2010)	233	Hofstra *et al.* (2008)	149
	Ramlau-Hansen *et al.* (2010)	63	Pauli *et al.* (2008)	30
			Roth *et al.* (2008)	1
			Foresta *et al.* (2009)	31
			Hammoud *et al.* (2009)	42
			Chavarro *et al.* (2010)	80
			Hofny *et al.* (2010)	127
			Ramlau-Hansen *et al.* (2010)	21
T / E_2 ratio normal range	Winters *et al.* (2006)	21	Jarow *et al.* (1993)	9
			Winters *et al.* (2006)	8
LH↑			Hofny *et al.* (2010)	80
LH↓			Kley *et al.* (1981)	NI
			Strain *et al.* (1988)	11
			Giagulli *et al.* (1994)	22
			Allen *et al.* (2002)	41
			Luboshitzky *et al.* (2005)	5
			Roth *et al.* (2008)	1
LH normal range	Jensen *et al.* (2004)	299	Glass *et al.* (1977)	9
	Fejes *et al.* (2006)	NI	Amatruda *et al.* (1978)	22
	Aggerholm *et al.* (2008)	773	Schneider *et al.* (1979)	NI
	Winters *et al.* (2006)	21	Strain *et al.* (1982)	21
	Wu *et al.* (2008)	1590	Jarow *et al.* (1993)	24
	Chavarro *et al.* (2010)	233	Giagulli *et al.* (1994)	18
	Ramlau-Hansen *et al.* (2010)	63	Pasquali *et al.* (1995)	8
			Isidori *et al.* (1999)	28
			Vincennati *et al.* (2006)	22
			Winters *et al.* (2006)	8
			Aggerholm *et al.* (2008)	163
			Hofstra *et al.* (2008)	149
			Pauli *et al.* (2008)	30
			Wu *et al.* (2008)	786
			Foresta *et al.* (2009)	31
			Hammoud *et al.* (2009)	42
			Chavarro *et al.* (2010)	127
			Hofny *et al.* (2010)	42
			Ramlau-Hansen *et al.* (2010)	21

Continued

Table II *Continued*

Hormone levels	Overweight BMI >25 <29.99 (kg/m^2)	Number of subjects	Obese BMI >30 (kg/m^2)	Number of subjects
FSH↑			Hofny *et al.* (2010)	80
FSH↓			Strain *et al.* (1982)	21
			Strain *et al.* (1988)	11
			Zumoff *et al.* (2003)	6
			Qin *et al.* (2007)	17
			Pauli *et al.* (2008)	30
			Roth *et al.* (2008)	1
FSH normal range	Jensen *et al.* (2004)	299	Glass *et al.* (1977)	9
	Fejes *et al.* (2006)	NI	Amatruda *et al.* (1978)	22
	Winters *et al.* (2006)	21	Schneider *et al.* (1979)	NI
	Qin *et al.* (2007)	241	Pasquali *et al.* (1995)	8
	Aggerholm *et al.* (2008)	773	Isidori *et al.* (1999)	28
	Chavarro *et al.* (2010)	233	Vincennati *et al.* (2006)	22
	Ramlau-Hansen *et al.* (2010)	63	Winters *et al.* (2006)	8
			Aggerholm *et al.* (2008)	163
			Hofstra *et al.* (2008)	149
			Roth *et al.* (2008)	1
			Foresta *et al.* (2009)	31
			Hammoud *et al.* (2009)	42
			Stewart *et al.* (2009)	36
			Chavarro *et al.* (2010)	127
			Hofny *et al.* (2010)	42
			Ramlau-Hansen *et al.* (2010)	21
Inhibin-B↓	Jensen *et al.* (2004)	299	Winters *et al.* (2006)	8
	Chavarro *et al.* (2010)	233	Aggerholm *et al.* (2008)	163
	Ramlau-Hansen *et al.* (2010)	63	Pauli *et al.* (2008)	30
			Stewart *et al.* (2009)	36
			Chavarro *et al.* (2010)	127
			Ramlau-Hansen *et al.* (2010)	21
Inhibin-B normal range	Winters *et al.* (2006)	21		
	Aggerholm *et al.* (2008)	773		
Leptin↑	Hanafy *et al.* (2007)	50	Isidori *et al.* (1999)	28
			Hofny *et al.* (2010)	80
Leptin↓			Strobel *et al.* (1998)	1
Leptin normal range	Hanafy *et al.* (2007)	30	Hofny *et al.* (2010)	42
	Zorn *et al.* (2007)	210		

to our knowledge neither in the genome-wide association studies, nor in studies focusing on specific obesity-associated polymorphisms, the relation with male fertility has been investigated.

The most convincing evidence that the leptin gene and leptin receptor gene are involved in the development of obesity comes from subjects who suffer from complete loss of gene function. Characteristic features of the rare condition of classical congenital leptin dysfunction include hyperphagia and morbid obesity. Although polymorphisms in the leptin gene have not been related to reproductive disorders in males, subjects in which the leptin gene is completely ablated are unable to pass through puberty, resulting in infertility in adulthood when not treated. Thus far, 12 subjects have been identified who suffer from this genetic defect; in most cases, the defect in leptin synthesis is identified during childhood (Montague *et al.*, 1997; Farooqi *et al.*, 2002; Gibson *et al.*, 2004; Farooqi and O'Rahilly, 2009). hCG treatment of an adult male suffering from this defect restored total testosterone and free-testosterone levels to the control range within 3 days, while a GnRH test in this patient revealed a normal FSH and LH response (Strobel *et al.*, 1998). In another study, males with this mutation received recombinant leptin over a period of 18 months, which improved their physical and metabolic condition enormously, and resulted in the initiation of puberty, growth of a penis and testes and normal ejaculation (Licinio *et al.*, 2004).

Complete loss of function mutations in the leptin receptor gene are even more rare than in the leptin gene. So far five nonsense and four missense mutations have been identified in eight subjects. In general, these patients are of prepubertal age at the time of diagnosis, except for a 15-year-old male subject who demonstrated clear clinical signs of hypogonadotropic hypogonadism. His sex steroid hormone, LH and FSH levels were low, suggesting that pubertal development was prevented (Farooqi *et al.*, 2007). Surprisingly, the phenotypic features of subjects with a mutation in the leptin receptor are less severe than in cases of leptin deficiency (Farooqi *et al.*, 2007). Possibly, the leptin receptor can make use of more than one signaling pathway. Nevertheless, for normal reproductive development and fertility in humans, leptin and a functional canonical leptin receptor seem to be essential.

Animal models for overweight and obesity: effects on reproduction

Obese model animals frequently display reproductive problems. In the following section, an overview is presented of those animal models that may help us to understand the sometimes contradictory observations made in human studies concerning the relation between obesity and male infertility.

Leptin, leptin signaling and Kiss1

Mice with a loss of function mutation in the obesity (ob) gene are in general infertile with low sex steroid and gonadotrophin levels, as is the case in humans with the same defect. Testes of *ob/ob* mice are characterized by multinucleated spermatids, few spermatozoa and hypothrophic interstitial tissue with Leydig cells that have an abnormal fibroblast-like morphology. However, within the *ob/ob* testis, there are also regions in which tubular morphology and spermatogenesis are apparently normal (Mounzih *et al*., 1997; Bhat *et al*., 2006), possibly offering an explanation for the few occasions that *ob/ob* males have been reported to reproduce successfully (Lane and Dickie, 1954; Mounzih *et al*., 1997).

The underlying nature of the sterility in the *ob/ob* mice is thought to be of a central origin, presumably due to a hypothalamic defect, as evidenced by low levels of FSH, LH and sex steroids (Runner, 1954). Low levels of gonadal steroids should stimulate gonadotrophin secretion in the presence of a functioning hypothalamic–pituitary–gonadal feedback mechanism (Fig. 1). However, in the *ob/ob* mouse, this is obviously not occurring. Indeed, more recent results from Swerdloff *et al.* (1976) indicate that the hypothalamic–pituitary sensitivity in *ob/ob* mice is altered. Further evidence for a hypothalamic defect comes from a study showing that exogenous GnRH administration to *ob/ob* mice leads to increased LH levels (Swerdloff *et al*., 1978). Leptin treatment of *ob/ob* mice results in a significant decrease in body weight as well as an increase in testis and seminal vesicle weights, plasma FSH levels, and normal spermatogenesis and Leydig cell morphology (Barash *et al*., 1996; Chehad *et al*., 1996; Cioffi *et al*., 1996; Mounzih *et al*., 1997). The observation that male *ob/ob* mice on a feed-restricted diet losing about the same amount of weight, are unable to impregnate normal lean female mice, indicates that, with leptin treatment, it is leptin itself that is responsible for the restoration in fertility and not the decrease in body weight (Mounzih *et al*., 1997).

The genetic background on which the mutation is maintained may influence the severity of the infertility-associated state of *ob/ob* mice. The sterility of *ob/ob* mice on a C57BL/6J background was at least partially corrected in the absence of leptin by cross-breeding with BALB/cJ mice. The transfer of the *ob* mutation onto a BALB/cJ-background results in a clear improvement of the obesity-associated fertility problem. Not surprisingly, the testicular histology of F_2 *ob/ob* males on a BALB/cJ mixed background is significantly better than on a homogenous C57BL/6J background. This suggests that the stimulatory effect of leptin on the reproductive system can, at least in part, be substituted genetically by modifier genes or allelic variants brought onto the obese-sterile phenotype by a mouse strain different from the one on which the *ob* mutation is maintained. Interestingly, the leptin-independent genetic rescue of sterility in the F_2 BALB/cJ *ob/ob* mice was applicable only to obese males and not to obese females, implicating that leptin is essential for the initiation of reproduction in females and cannot be substituted at least partially, as in males, by the effect of modifier genes (Ewart-Toland *et al*., 1999).

The *db/db* mouse has a loss of function mutation in the leptin receptor. As in humans, this mutation, among others, results in severe early-onset obesity, insulin resistance, hyperphagia and infertility. There are multiple alternatively spliced isoforms of the leptin receptor (LRa to LRf), of which only the long form (isoform b, LRb) is able to mediate leptin signaling (Lee *et al*., 1996; Tartaglia, 1997). Mutations of the leptin receptor have also been described in the rat. The Zucker rat has a recessive mutation in the leptin receptor and shows many similarities with the *db/db* mouse. In young male rats, fertility can be improved by food restriction or by treating the males with extremely high doses of testosterone. Although adult male Zucker rats have a normal LH and FSH response upon GnRH stimulation, testicular morphology is different from lean control rats. The Leydig cells show a hypertrophied morphology and contain many lipid droplets indicative of a disturbed function. Infertility in these rats is thought to be the consequence of reduced testosterone levels (Young *et al*., 1982).

The nonsense mutation in the leptin receptor of the obese Koletsky rat causes a phenotype very similar to that in the Zucker rat, as obese male Koletsky rat are also infertile and have reduced GnRH levels in the hypothalamus. What the consequences of the leptin receptor mutation are for the testis of the Koletsky rat is not known (Rhinehart *et al*., 2004).

Animal model studies have made clear that following binding to its receptor, leptin can activate several signaling pathways. In order to investigate which pathway is involved in the effects of leptin on male fertility, several mutant leptin receptor mouse models have been generated. Binding of leptin to LRb activates Jak 2, leading to phosphorylation and dimerization of signal transducer and activator of transcription 3 (Stat3) which then acts as a transcription factor to regulate transcription of leptin target genes (Münzberg *et al*., 2005; Piper *et al*., 2008). Furthermore, leptin has been suggested to signal via the phosphatidylinositol 3-kinase and ERK pathways. This assumption is supported by the fact that the *db/db* phenotype is less severe than the *ob/ob* phenotype (Niswender *et al*., 2001; Münzberg *et al*., 2005; Xu *et al*., 2005). Additional support for an alternative signaling pathway comes from Farooqi *et al.* (2007) who reported that, as in mice, in general the phenotypic features seen in human subjects with leptin-receptor deficiency are not as severe as those in subjects with leptin deficiency.

To study whether leptin influences fertility through the Stat3 pathway or one of the other signaling pathways, mouse models have been established in which leptin receptor-mediated Stat3 signaling was partially or completely prevented. Bates *et al.* (2003) generated a $lepr^{S1138}$ mutant in which the encoded protein fails to induce Stat3 tyrosine phosphorylation and nuclear translocation, while LRb is expressed on the cell surface and normally mediates other leptin signals. $Lepr^{S1138}$ homozygotes are hyperphagic and obese, but in contrast to *db/db* mice, these mutant mice are fertile, suggesting that the hypothalamic control of reproduction by leptin is predominantly regulated by signaling pathways other than Stat3 (Bates *et al*., 2003). These observations were confirmed by Piper *et al.* (2008) who used a genetic strategy to disrupt Stat3 function, especially in neurons expressing LRb, such that Stat3 signaling was abolished only in relevant cell

types and only after the onset of LRb expression. In contrast to these studies, Gao et al. (2004), using a different approach, generated a neural-specific deletion of Stat3 by using gene targeting to create a conditional Stat3 allele. These $Stat3^{N-/-}$ mutants were like *db/db* mice hyperleptinemic and obese, suggesting that leptin did not lower body weight in these animals, thus rendering a phenotype closely resembling that of leptin-deficient mice. In contrast to the $lepr^{S1138}$ and neural $Stat3^{-/-}$ mutants (Bates et al., 2003; Piper et al., 2008), $Stat3^{N-/-}$ mutants were infertile (Gao et al., 2004). The inconsistency between the Stat3 mutants is probably due to the nature of the mutations, with $Stat3^{N-/-}$ mice ablating Stat3 directly and $lepr^{S1138}$ mice having only diminished Stat3 activation by leptin (Bates et al., 2003; Gao et al., 2004). This does not, however, explain the conflicting results between the studies of the groups of Gao et al. (2004) and Piper et al. (2008). Possibly, the genetic background on which the mutants were generated provides an explanation for this apparent discrepancy. The $Stat3^{N-/-}$ mice were generated on a background different from the mixed genetic background of the mutants generated by Piper et al. (2008), which is less severely affected by leptin deficiency as the C57BL/6J background (Ewart-Toland et al., 1999).

There are other indications that obesity in model animals does not always coincides with fertility problems. The effects of leptin in the hypothalamus are thought at least in part to be mediated by pro-opiodmelanocortin (*POMC*) and its derivatives. Mutations in the *POMC* gene lead to severe obesity and reduced adrenal function both in humans and in mice (Krude et al., 1998; Yaswen et al., 1999). One of the products of the *POMC* gene is α-melanocyte-stimulating hormone (α-MSH), which binds with high affinity to the melanocortin 4 (MC4) receptor to inhibit apetite (Yeo et al., 1998). Mutation of the MC4 receptor in humans and mice leads to obesity (Yeo et al., 1998; reviewed in Carroll et al., 2004). The obesity observed in humans with a POMC null mutation or in *pomc* knockout mice is thought to be the result of the absence of MC4 stimulation (Krude et al., 1998; Yeo et al., 1998; Yaswen et al., 1999). Despite their obesity, MC4 receptor knockout mice are fertile (reviewed in Schiöth and Watanobe, 2002), while in *pomc* knockout mice, the fertility status is not known. As the focus of this part of the review is on animal models in which obesity coincides with infertility, these animal models are not further discussed.

Although leptin contributes to the regulation of the hypothalamic–pituitary–gonadal axis, GnRH neurons do not express leptin receptors, suggesting that leptin must act indirectly to regulate GnRH neuronal activity (Quennell et al., 2009). Downstream targets for leptin action include neuropeptide Y (NPY), agouti gene-related peptide, POMC, kisspeptin and nitric oxide synthase, all of which modulate GnRH neuronal activity and/or gonadotrophin levels (reviewed in Ojeda et al., 2006). In support of a role for kisspeptins (*Kiss1*, nomenclature as suggested by Gottsch et al., 2009) as intermediate in the communication between leptin and GnRH neurons, *Kiss1* mRNA levels in the arcuate nucleus (ARC) of the hypothalamus, a nucleus that contains large numbers of GnRH neurons, were significantly reduced in *ob/ob* mice in the absence of leptin (Smith et al., 2006). This effect could be reversed by treatment with leptin, implicating a causative relationship between the absence of leptin and suppressed *Kiss1* mRNA levels. These authors also showed that almost half of the *Kiss1* mRNA expressing neurons in the ARC expressed LRb, suggesting that *Kiss1* neurons in the ARC are direct targets for regulation by leptin, providing further evidence for the presence of a neuro-endocrine leptin–kisspeptin–GnRH pathway (summarized in Fig. 1).

Several studies further support the importance of kisspeptins in the regulation of GnRH neuronal function. Irwig et al. (2004) were the first to show that centrally administered *Kiss1* stimulated the release of LH and FSH by the pituitary, and that this stimulation was dependent upon activation of the GnRH neurons. *In situ* hybridization and immunohistochemical studies demonstrated the presence of kisspeptin receptors (*kiss1r*) on GnRH neurons (Irwig et al., 2004; Herbison et al., 2010). Direct evidence for *Kiss1* modulation of GnRH neuronal activity came from *in vivo* studies in rats and monkeys treated with a potent kisspeptin antagonist. Intracerebroventricular injections of kisspeptin antagonist reduced serum LH levels in castrated male rats, and inhibited pulsatile GnRH release in female monkeys (Roseweir et al., 2009). Moreover, *Kiss1* mRNA expression in the ARC was increased following castration in male rats (Irwig et al., 2004), an effect that could be reversed by sex steroid replacement (Smith et al., 2005a, b). Analogous observations in other species, such as sheep, monkey and human (reviewed in Oakley et al., 2009; Pineda et al., 2010), suggested that *Kiss1* gene expression was under the negative feedback control of sex steroids. Indeed, most *Kiss1* neurons in the ARC co-expressed either the estrogen receptor α or the androgen receptor (Smith et al., 2005b).

These observations have led to the concept that the kisspeptins belong to the most potent activators of the hypothalamic GnRH system (Rao et al., 2008; reviewed in Oakley et al., 2009; Fig. 1). Moreover, as kisspeptin neurons also express functional leptin receptors, it is tempting to propose the hypothesis that the hypothalamic KISS1 system participates in the transmission of metabolic information to the hypothalamic–pituitary–gonadal axis (reviewed in Castellano et al., 2009).

NPY and leptin signaling

The hypothalamic neuropeptide NPY exerts rather complex effects on the hypothalamic–pituitary axis. In ovariectomized steroid-primed rats, NPY stimulates the gonadotropic axis (Kalra and Crowley, 1984a, b), while in castrated male rats and mice, central NPY administration inhibits the release of LH (Kerkerian et al., 1985; Raposinho et al., 1999). In line with the latter observations, in intact male rats chronic NPY infusion or repeated injections into the lateral ventricle result into severely reduced plasma LH and testosterone levels, reduced testicular and seminal vesicle weights, impaired sexual motivation, and hence a profound inhibition of the hypothalamic–pituitary–gonadal axis (Clark et al., 1985; Pierroz et al., 1996; Raposinho et al., 2000, 2001). Clearly, NPY is a major central regulator of reproductive functions.

The expression of NPY is negatively influenced by leptin as in *ob/ob* mice, which lack leptin, hypothalamic NPY levels are elevated (Stephens et al., 1995; Schwartz et al., 1996; Widdowson and Wilding, 2000). Treatment of *ob/ob* mice with leptin reduces NPY mRNA expression and induces fertility by improving the function of the hypothalamic–pituitary–gonadal axis (Stephens et al., 1995; Chehad et al., 1996; Mounzih et al., 1997). Food restriction in *ob/ob* mice results in a similar weight loss as leptin treatment but does not

reduce central NPY expression nor does it restore fertility (Mounzih *et al*., 1997).

In massively obese *db/db* mice, hypothalamic NPY levels are dramatically increased. Generation of *db/db* transgenic mice carrying a neuron-specific leptin receptor results in a complete correction of obesity and related phenotypes, normalizes expression of NPY and restores fertility (De Luca *et al*., 2005). Together, these data indicate that leptin is an important regulator of NPY expression.

The Y receptor family mediates the various functions of NPY and consists of several members, including Y4 (Blomqvist and Herzog, 1997). Significant amounts of Y4 mRNA have been found in the hypothalamus in areas involved in the regulation of reproductive functions, such as the paraventricular nucleus (Parker and Herzog, 1999; Larsen and Kristensen, 2000). Crossing the Y4 receptor knockout to an *ob/ob* background restores fertility in *ob/ob* mice. Plasma testosterone levels in the double knockouts are significantly higher than in *ob/ob* mice, as are testis and seminal vesicle weights, while testicular morphology is normal (Sainsbury *et al*., 2002). Although these observations suggest an important role for the Y4 receptor in regulation of reproductive functions, it cannot be excluded that other NPY receptor subtypes are also involved. For example, the Y5 receptor subtype appears to be involved in the regulation of GnRH/LH release (Raposinho *et al*., 1999, 2001), while the Y1 receptor subtype has been implicated in the inhibition of hypothalamic GnRH release (Sahab *et al*., 2003).

Further support for a role of NPY in infertility of *ob/ob* mice comes from a study by Erickson *et al.* (1996) who crossed the *ob/ob* strain onto NPY-deficient mice. Fertility in these double knockout mice is significantly improved compared with the *ob/ob* single knockout mice.

NPY neurons in the ARC express LRb, suggesting that leptin can directly influence NPY activity. Recent evidence suggests, however, that binding of leptin to its receptor on these NPY neurons leads to activation of the STAT3 signalling transduction pathway (Draper *et al*., 2010), a pathway most likely not involved in leptins effects on fertility (Bates *et al*., 2003; Piper *et al*., 2008). The fertility-related effects of leptin on NPY expression most likely take place indirectly through the kisspeptin system, as Fu and Pol (2010) have recently shown that *Kiss1* inhibits NPY neuronal activity (Fig. 1).

Discussion

How do animal models help to understand the relation between obesity and fertility in humans?

The most surprising conclusion that can be drawn from the present analysis of the literature is that most overweight and obese men do not experience significant fertility problems, despite the presence of reduced testosterone levels and normal gonadotrophin levels, a conclusion recently also drawn by MacDonald *et al*. (2010). Only a subgroup of overweight and obese men suffers from hypogonadotropic hypogonadism. The animal models described in the second part of this review offer several possible explanations for this phenomenon. First of all, adipose tissue can be considered as an important endocrine organ (Wang *et al*., 2010). It synthesizes and releases hormones and cytokines that can exert their effects elsewhere in the body. Under conditions of overweight or obesity, the levels of these adipokines may increase, resulting in a disturbance of homeostasis. For example, elevated levels of leptin may negatively influence Leydig cell testosterone synthesis by inhibiting the conversion of 17OH-progesterone into testosterone.

Despite its direct effects on testosterone production at the testicular level, the peripheral effects of leptin on the activity of the GnRH neurons in the hypothalamus seem to be far more important in the regulation of testosterone production. Leptin does not directly affect the activity of the GnRH neurons in the hypothalamic ARC, but exert its effect through activation of the LRb expressing kisspeptin neurons in the ARC. The kisspeptin neurons in the ARC belong to the most potent activators of the hypothalamic (GnRH)/pituitary (LH/FSH)/gonadal axis, as they not only mediate the effects of leptin but also of sex steroids, on GnRH neuronal activation.

As discussed above, several animal models for obesity have made clear that NPY also plays an important role in obesity-related infertility (Fig. 1). NPY expression is under normal conditions negatively influenced by leptin. In the infertile obesity model animals, such as the *ob/ob* and *db/db* mice, NPY expression is significantly increased. Administration of leptin to *ob/ob* mice leads to a decrease in hypothalamic NPY expression and activation of reproductive function. ICV infusion of NPY to lean control animals leads to a reduction in GnRH and LH release and a disturbed reproductive function, suggesting that NPY directly influences fertility by inhibiting hypothalamic and pituitary function.

Obesity can lead to leptin resistance by either a defect in leptin transport over the blood–brain barrier, defects within the LRb signaling cascade (cellular leptin resistance) or defective neuronal wiring and functioning (reviewed in Münzberg, 2010). This may prevent a suppressive effect of leptin on NPY (Fig. 1).

Turning back to humans, chronically elevated leptin levels in males resulting from overweight and obesity may lead to leptin resistence. This can have two consequences: first of all, this will result in elevated NPY expression; secondly, the stimulatory effect of leptin on KiSS1 gene expression will be reduced, alleviating the negative effect of KiSS1 on NPY. Together this will affect hypothalamic GnRH and pituitary LH levels. Since the NPY phenotype does not explain leptin resistence in full, it seems likely that kisspeptins also affect GnRH release along other pathways. Ultimately, this may lead to a reduced activity of the hypothalamic–pituitary–gonadal axis and subsequent hypogonadotropic hypogonadism. It would be of interest to determine whether infertile overweight and obese males suffer from the same degree of leptin insensitivity as fertile overweight and obese subjects. This could, indirectly, give information about the possible role of kisspeptins and NPY in human obesity-related male infertility, thus offering a physiological basis for observations made in earlier reviews such as by Hammoud *et al*. (2006) and MacDonald *et al*. (2010). Based on the outcome of these investigations, animal model studies could then be used to develop methods to influence hypothalamic KiSS1 and NPY levels and restore human male fertility in overweight and obese subjects who suffer from this type of hypogonadotropic hypogonadism.

Funding

K.J.T. was funded by Wageningen University. J.K. was funded by FP7-KBBE-244995 and Wageningen University.

References

Adamopoulos DA, Nicopoulou S, Kapolla N, Karamertzanis M, Andreou E. The combination of testosterone undecanoate with tamoxifen citrate enhances the effects of each agent given independently on seminal parameters in men with iodiopathic oligozoospermia. *Fertil Steril* 1997;**67**:756–762.

Adamopoulos DA, Pappa A, Billa E, Nicopoulou S, Koukkou E, Michopoulos J. Effectiveness of combined tamoxifen citrate and testosterone undecanoate treatment in men with idiopathic oligozoospermia. *Fertil Steril* 2003;**80**: 914–920.

Agarwal A, Sharma RK, Desai NR, Prabakaran S, Tavares A, Sebenegh E. Role of oxidative stress in pathogenesis of varicocele and infertility. *Urology* 2009; **73**:461–469.

Aggerholm AS, Thulstrup AM, Gunnar T, Ramlau-Hansen CH, Bonde JP. Is overweight a risk for reduced semen quality and altered serum sex hormone profile? *Fertil Steril* 2008;**90**:619–626.

Aitkin RJ, Gordon E, Harkiss D, Twigg JP, Jennings Z, Irvine DS. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. *Biol Reprod* 1998;**59**:1037–1046.

Allen NE, Appleby PN, Davey GK, Key TJ. Lifestyle and nutritional determinants of bioavailable androgens and related hormones in British men. *Cancer Causes Control* 2002;**13**:353–363.

Amatruda JM, Harman SM, Pourmatabbed G, Lockwood DH. Depressed plasma testosterone and fractional binding of testosterone in obese males. *J Clin Endocrinol Metab* 1978;**47**:268–271.

Andersson AM, Muller J, Skakkebaek NE. Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibin B levels. *J Clin Endocrinol Metab* 1998;**83**:4451–4458.

Barash IA, Cheung CC, Weigle DS, Ren H, Kabingting EB, Kuijper JL, Clifton DK, Steiner RA. Leptin is a metabolic signal to the reproductive system. *Endocrinology* 1996;**137**:3144–3147.

Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AWK, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E *et al.* STAST3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* 2003;**421**:856–859.

Bhat GK, Sea TL, Olatinwo MO, Simorangkir D, Ford GD, Ford BD, Mann DR. Influence of a leptin deficiency on testicular morphology, germ cell apoptosis, and expression levels of apoptosis-related genes in the mouse. *J Androl* 2006; **27**:302–310.

Blomqvist AG, Herzog H. Y-receptor subtypes. How many more? *Trends Neurosci* 1997;**20**:294–298.

Brendtson WE, Thompson TL. Changing relationships between testis size, Sertoli cell number and spermatogenesis in Sprague–Dawley rats. *J Androl* 1990; **11**:429–435.

Caprio M, Isidori AM, Carta AR, Moretti C, Dufau ML, Fabbri A. Expression of functional leptin receptors in rodent Leydig cells. *Endocrinology* 1999; **140**:4939–4947.

Carroll L, Voisey J, Van Daal A. Mouse models of obesity. *Clin Dermatol* 2004; **22**:345–349.

Castellano JM, Roa J, Luque RM, Dieguez C, Aquilar E, Pinella L, Tena-Sempere M. Ks and the metabolic control of reproduction: physiologic roles and putative physiological implications. *Peptides* 2009;**30**:139–145.

Chamberland A, Tremblay N, Gilbert B, Pérusse L, Vohl M-C, Laprise C. Association study between candidate genes and obesity-related phenotypes using a sample of lumberjacks. *Public Health Genomics* 2009;**12**:253–258.

Chavarro JE, Toth TL, Wright DL, Meeker JD, Hauser R. Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertil Steril* 2010;**93**:2222–2231.

Chehad FF, Lim ME, Ronghua L. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nat Genet* 1996;**12**:318–320.

Cioffi J, Shafer A, Zupancic T, Smith-Gbur J, Mikhail A, Platika D, Snodgrass H. Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. *Nat Med* 1996;**2**:585–588.

Clark JT, Kalra PS, Kalra SP. Neuropeptide Y stimulates feeding but inhibits sexual behavior in rats. *Endocrinology* 1985;**117**:2435–2442.

De Boer H, Verschoor L, Ruinemans-Koerts J, Jansen M. Letrozole normalizes serum testosterone in severely obese men with hypogonadotropic hypogonadism. *Diabetes Obes Metab* 2005;**7**:211–215.

De Luca C, Kowalsky TJ, Zhang Y, Elmquist JK, Lee C, Kilimann MW, Ludwig T, Liu SM, Chua SC. Complete rescue of obesity, diabetes, and infertility in *db/db* mice by neuron-specific LEPR-B transgenes. *J Clin Invest* 2005; **11**:3484–3493.

Draper S, Kirigiti M, Galvas M, Grayson B, Chong CAN, Jiang B, Smith MS, Zeltser LM, Grove KL. Differential gene expression between neuropeptide Y expressing neurons of the dorsomedial nucleus of the hypothalamus and the arcuate nucleus: microarray analysis study. *Brain Res* 2010;**1350**:139–150.

Duarte SFP, Francischetti EA, Genelhu VA, Cabello PH, Pimentel MMG. *LEPR* p.Q223R, *β3-AR* p.W64R and *LEP* c.-2548G > A gene variants in obese Brazilian subjects. *Genet Mol Res* 2007;**6**:1035–1043.

El-Hefnawy T, Ioffe S, Dym M. Expression of the leptin receptor during germ cell development in the mouse testis. *Endocrinology* 2000;**141**:2624–2630.

Erickson JC, Hollopeter G, Palmiter RD. Attenuation of the obesity syndrome of *ob/ob* mice by the loss of neuropeptide Y. *Science* 1996;**27**:1704–1707.

Ewart-Toland A, Mounzih K, Qui J, Chehab FF. Effect of the genetic background on the reproduction of leptin-deficient obese mice. *Endocrinology* 1999; **140**:732–738.

Farooqi IS, O'Rahilly S. Leptin: a pivotal regulator of human energy homeostasis. *Am J Clin Nutr* 2009;**89**:980S–984S.

Farooqi IS, Materese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S *et al.* Beneficial effects of leptin on obesity, T cell hyporesponsiveness and neuroendocrine.metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest* 2002;**110**:1093–1103.

Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM, Lank E, Bottomley B, Lopez-Fernandez J, Ferraz-Amaro I *et al.* Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med* 2007;**35**:237–247.

Fejes I, Koloszár S, Szöllősi J, Závaczki Z, Pál A. Is semen quality affected by male body fat distribution? *Andrologia* 2005;**37**:155–159.

Fejes I, Koloszár S, Závaczki Z, Daru J, Szöllősi J, Pál A. Effect of body weight on testosterone/estradiol ratio in oligozoospermic patients. *Arch Androl* 2006; **52**:97–102.

Foresta C, Di Mambro A, Pagan C, Garolla A, Vettor R, Ferlin A. Insulin-like factor 3 as a marker of testicular function in obese men. *Clin Endocrinol* 2009;**7**:422–426.

Fu LY, Pol AN. Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism. *J Neurosci* 2010;**30**:10205–10219.

Gao Q, Wolfgang MJ, Neschen S, Morino K, Horvath TL, Shulman GI, Fu X-Y. Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and themal dysregulation. *Proc Natl Acad Sci USA* 2004;**101**:4661–4666.

Giagulli VA, Kaufman JM, Vermeulen A. Pathogenesis of the decreased androgen levels in obese men. *J Clin Endocrinol Metab* 1994;**79**:997–1000.

Gibson WT, Farooqi IS, Moreau M, DePaoli AM, Lawrence E, O'Rahilly S, Trussell RA. Congenital leptin deficiency due to homozygosity for the delta 133G mutation: report of another case and evaluation of response to four years of leptin therapy. *J Clin Endocrinol Metab* 2004;**8**:4821–4826.

Glass AR, Swerdloff RS, Bray GA, Dahms WT, Atkinson RL. Low serum testosterone and sex-hormone-binding-globulin in massively obese men. *J Clin Endocrinol Metab* 1977;**4**:1211–1219.

Globerman H, Shen-Orr Z, Karnieli E, Aloni Y, Charuzi I. Inhibin B in men with severe obesity and after weight reduction following gastroplasty. *Endocr Res* 2005;**3**:17–26.

Gottsch ML, Clifton DK, Steiner RA. From KISS1 to kisspeptins: an historical perspective and suggested nomenclature. *Peptides* 2009;**30**:4–9.

Hammoud AO, Gibson M, Peterson CM, Hamilton BD, Carrell DT. Obesity and male reproductive potential. *J Androl* 2006;**2**:619–626.

Hammoud AO, Wilde N, Gibson M, Parks A, Carrell DT, Meikle W. Male obesity and alteration in sperm parameters. *Fertil Steril* 2008;**9**:2222–2225.

Hammoud A, Gibson M, Hunt SC, Adams DT, Carrell DT, Kolotkin RL, Meikle AW. Effect of Roux-en-Y gastric bypass surgery on the sex steroids and quality of life in obese men. *J Clin Endocrinol Metab* 2009;**9**:1329–1332.

Hanafy S, Halawa FA, Mostafa T, Mikhael NW, Khalil KT. Serum leptin correlates in infertile oligozoospermic males. *Andrologia* 2007;**39**:177–180.

Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. *JAMA* 2004;**291**:2847–2850.

Herbison AE, dÁglemont de Tassigny X, Doran J, Colledge WH. Distribution of postnatal development of Gpr54 gene expression in mouse brain and gonadotropin-releasing hormone neurons. *Endocrinology* 2010;**151**:312–321.

Hofny ERM, Ali ME, Abdel-Hafez HZ, El-Dien Kamal E, Mohamed EE, Abd El-Azeem HG, Mostafa T. Semen parameters and hormonal profile in obese fertile and infertile males. *Fertil Steril* 2010;**94**:581–584.

Hofstra J, Loves S, van Wageningen B, Ruinemans-Koerts J, Janssen I, de Boer H. High prevalence of hypogonadotropic hypogonadism in men referred for obesity treatment. *Neth J Med* 2008;**66**:103–109.

Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ, Gottsch ML, Clifton DK, Steiner RA. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. *Neuroendocrinology* 2004; **80**:264–272.

Ishikawa T, Fujioka H, Ishimura T, Takenaka A, Fujisawa M. Expression of leptin and leptin receptor in the testis of fertile and infertile patients. *Andrologia* 2007; **39**:22–27.

Isidori AM, Caprio M, Strollo F, Moretti C, Frajese G, Isisori A, Fabbri A. Leptin and androgen in male obesity: evidence for leptin contribution to reduced androgen levels. *J Clin Endocrinol Metab* 1999;**84**:3673–3680.

Jarow JP, Kirkland J, Koritnik DR, Cefalu WT. Effect of obesity and fertility status on sex steroid levels in men. *Urology* 1993;**42**:171–174.

Jensen TK, Andersson AM, Jørgensen N, Andersen AG, Carlsen E, Petersen JH, Skakkebæk NE. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil Steril* 2004;**82**:863–870.

Jones R, Mann T, Sherins R. Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides and protective action of seminal plasma. *Fertil Steril* 1979;**31**:531–537.

Kalra SP, Crowley WR. Differential effects of pancreatic polypeptide on luteinizing hormone release in female rats. *Neuroendocrinology* 1984a;**3**:511–513.

Kalra SP, Crowley WR. Norepinephrin-like effects of neuropeptide Y in the rat. *Life Sci* 1984b;**35**:1173–1176.

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

Kefer JC, Agarwal A, Sabanegh E. Role of antioxidants in the treatment of male infertility. *Int J Urol* 2009;**16**:449–457.

Kerkerian L, Guy J, Lefèvre G, Pelletier G. Effects of NPY on the release of anterior pituitary hormones in the rat. *Peptides* 1985;**6**:1201–1204.

Kley HK, Deselaers T, Peerenboom H. Evidence for hypogonadism in massively obese males due to decreased free testosterone. *Horm Metab Res* 1981; **13**:639–641.

Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertil Steril* 1997;**68**:519–524.

Koloszár S, Fejes I, Závaczki Z, Daru J, Szöllosi J, Pál A. Effect of bodyweight on sperm concentration in normozoospermic males. *Arch Androl* 2005; **51**:299–304.

Kort HI, Massey JB, Elsner CW, Mitchell-Leef D, Shapiro DB, Witt MA, Roueboush WE. Impact of body mass index values on sperm quantity and quality. *J Androl* 2006;**27**:450–452.

Krude H, Biebermann H, Luck W, Brabant G, Grüters A. Severely early-onset obesity, adrenal insufficiency and red hair pigmentation caused by *POMC* mutations in humans. *Nat Genet* 1998;**19**:155–157.

Lampio F, du Plessis SS. Insulin and leptin enhance human sperm motility, acrosome reaction and nitric oxide production. *Asian Androl* 2008;**10**:799–807.

Lane PW, Dickie MM. Fertile obese male mice. Relative sterility in obese males corrected by dietary restriction. *J Hered* 1954;**45**:56–58.

Larsen PJ, Kristensen P. Central Y4 receptor distribution. Radioactive nucleotide probe in sity hybridization with in vitro receptor autoradiography. *Meth Mol Biol* 2000;**153**:185–198.

Lee G, Proenca R, Montez JM, Carroll KM, Darvishzadeh H, Lee JI, Friedman JM. Abnormal splicing of leptin receptor in diabetic mice. *Nature* 1996;**379**:632–635.

Leenen R, van der Kooy K, Seidell JC, Deurenberg P, Koppeschaar HPF. Visceral fat accumulation in relation to sex hormones in obese men and women undergoing weight loss therapy. *J Clin Endocrinol Metab* 1994;**78**:1515–1520.

Licinio J, Caglayan S, Ozata M, Yildiz BO, de Miranda P, O'Kirwan F, Whitby R, Liang L, Cohen P, Bhasin S *et al*. Phenotypic effects of leptin replacement on morbid obesity, diabetis mellitus, hypogonadism, and behavior in leptin-deficient adults. *Proc Natl Acad Sci USA* 2004;**101**:4531–4536.

Liu PY, Handelsman DJ. The present and future state of hormonal treatment for male infertility. *Hum Reprod Update* 2003;**9**:9–23.

Luboshitzky R, Lavie L, Shen-Orr Z, Herer P. Altered luteinizing hormone and testosterone secretion in middle-aged obese men with obstructive sleep apnea. *Obes Res* 2005;**13**:780–786.

MacDonald AA, Herbison GP, Showell M, Farquhat CM. The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. *Hum Reprod Update* 2010; **16**:293–311.

Magnusdottir EV, Thorsteinsson T, Thorsteinsdottir S, Heimisdottir M, Olafsdottir K. Presistent organochlorines, sedentary occupation, obesity and human male subfertility. *Hum Reprod* 2005;**20**:208–215.

Mohr BA, Guay AT, O'Donnell AB, McKinlay JB. Normal, boud and nonbound testosterone levels in normally ageing men: results from the Massachusetts male ageing study. *Clin Endocrinol* 2005;**62**:64–73.

Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA *et al*. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 1997;**387**:903–908.

Mounzih K, Lu R, Chehab FF. Leptin treatment rescuss the sterility of genetically obese *ob/ob* males. *Endocrinology* 1997;**138**:1190–1193.

Münzberg H. Leptin-signalling pathways and leptin resistance. *Forum Nutr* 2010; **63**:123–132.

Münzberg H, Bjornholm M, Bates SH, Myers MG Jr. Leptin receptor action and mechanisms of leptin resistance. *Cell Mol Life Sci* 2005;**62**:642–652.

Nguyen RHN, Wilcox AF, Skjærven R, Baird DD. Men's body mass index and infertility. *Hum Reprod* 2007;**22**:2488–2493.

NIDDK. Statistics related to overweight and obesity. *Weight-Control Information Network (WIN)*. 2007.

Niswender KD, Morton GJ, Steams WH, Rhodes CJ, Myers MG Jr, Schwartz MW. Intracellular signaling. Key enzyme in leptin-induced anorexia. *Nature* 2001; **413**:794–795.

Oakley AE, Clifton DK, Steiner RA. Kisspeptin signaling in the brain. *Endocr Rev* 2009;**30**:713–743.

Ojeda SR, Lomniczi A, Mastronardi C, Heger S, Roth C, Parent AS, Matagne V, Mungenast AE. Minireview: the neuroendocrine regulation of puberty: is the time ripe for systems biology approach? *Endocrinology* 2006;**147**: 1166–1174.

Orth JM, Gunsalus GM, Lamperti AA. Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on the numbers of Sertoli cells produced during perinatal development. *Endocrinology* 1988; **122**:787–794.

Osuna JA, Gómez-Pérez R, Arata-Bellabarba G, Villaroel V. Relationship between BMI, total testosterone, sex hormone-binding-globulin, leptin, insulin and insulin resistance in obese men. *Arch Androl* 2006;**52**:355–361.

Padron RS, Mas J, Zamora R, Riverol L, Liccea M, Mallea L, Rodriguez J. Lipids and testicular function. *Int Urol Nephrol* 1989;**21**:515–519.

Parker RM, Herzog H. Regional distribution of Y-receptor subtype mRNAs in rat brain. *Eur J Neurosci* 1999;**11**:1431–1448.

Pasquali R, Casimirri F, De Lasio R, Mesini P, Boschi S, Chierici R, Flamia R, Biscotti M, Vicennati V. Insulin regulates testosterone and sex hormone-binding globulin concentrations in adult normal weight and obese men. *J Clin Endocrinol Metab* 1995;**80**:654–658.

Pasquali R, Vicennati V, Scopinaro N, Marinari G, Simonelli A, Flamia R, Casimirri F, Gagliardi L. Achievement of near-normal body weight as the prerequisite to normalize sex hormone-binding globulin concentration in massively obese men. *Int J Obes Relat Metab Disord* 1997;**21**:1–5.

Pauli EM, Legro RS, Demers LM, Kunselman AR, Dodson WC, Lee PA. Diminished paternity and gonadal function with increasing obesity in men. *Fertil Steril* 2008; **90**:345–351.

Pavlovich CP, King P, Goldstein M, Schlegel PN. Evidence of a treatable endocrinopathy in infertile men. *J Urol* 2001;**165**:837–841.

Pettigrew R, Hamilton-Fairley D. Obesity and female reproductive function. *Br Med Bull* 1997;**53**:341–358.

Pierroz DD, Catzeflis C, Aebi AC, Rivier JE, Aubert ML. Chronic administration of neuropeptide Y into the lateral ventricles inhibits both the pituitary–testicular axis and growth hormone and insulin-like growth factor I secretion in intact adult male rats. *Endocrinology* 1996;**137**:3–12.

Pineda R, Aquilar E, Pinilla L, Tena-Sempere M. Ples of the kisspeptin/GPR54 system in the neuroendocrine control of reproduction. *Res Prog Brain Res* 2010; **181**:55–77.

Piper ML, Unger EK, Myers MG Jr, Xu AW. Specific physiological roles for signal transducer and activator of transcription 3 in leptin receptor-expressing neurons. *Mol Endocrinol* 2008;**22**:751–759.

Qin DD, Yuan W, Zhou WJ, Cui YQ, Wu JQ, Gao ES. Do reproductive hormones explain the association between body mass index and semen quality? *Asian J Androl* 2007;**9**:827–834.

Quennell JH, Mulligan AC, Tups A, Liu X, Phipps SJ, Kemp CJ, Herbison AE, Grattan DR, Anderson GM. Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. *Endocrinology* 2009;**15**:2805–2812.

Raman JD, Schlegel PN. Aromatase inhibitors for male infertility. *J Urol* 2002; **167**:624–629.

Ramirez-Torres MA, Carrera A, Zambrana M. High incidence of hyperestrogenemia and dyslipidemia in a group of infertile men. *Ginecol Obstet Mex* 2000; **68**:224–229.

Ramlau-Hansen CH, Thulstrup AM, Nohr EA, Bonde JP, Sørensen TIA, Olsen J. Subfecundity in overweight and obese couples. *Hum Reprod* 2007a; **22**:1634–1637.

Ramlau-Hansen CH, Nohr EA, Thulstrup AM, Bonde JP, Storgaard L, Olsen J. Is maternal obesity related to semen quality in the male offspring? A pilot study. *Hum Reprod* 2007b;**22**:2758–2762.

Ramlau-Hansen CH, Hansen M, Jensen CK, Olsen J, Bonde JP, Thulstrup AM. Semen quality and reproductive hormones according to birth weight and body mass index in childhood and adult life. Two decades of follow-up. *Fertil Steril* 2010;**94**:610–618.

Rao B, Soufir JC, Martin M, David G. Lipid peroxidation in human spermatozoa as related to midpiece abnormalities and motility. *Gamete Res* 1989;**24**:127–134.

Rao J, Aquilar E, Dieguez C, Pinella L, Tena-Sempere M. Nin kisspeptin/GPR54 physiology as fundamental gatekeepers of reproductive function. *Front Neuroendocrinol* 2008;**29**:48–69.

Raposinho PD, Broqua P, Pierroz DD, Hayward A, Dumont Y, Quirion R, Junien JL, Aubert ML. Evidence that the inhibition of LH secretion exerted by central administration of neuropeptide Y (NPY) in the rat is predominantly mediated by NPY-YS receptor subtype. *Endocrinology* 1999;**140**:4046–4055.

Raposinho PD, Castillo E, d'Allèves V, Broqua P, Pralong FP, Aubert ML. Chronic blockade of the melanocortin 4 receptor subtype leads to obesity independently of neuropeptide Y action, with no adverse effects on the gonadotropic and somatotropic axes. *Endocrinology* 2000;**141**:4419–4427.

Raposinho PD, Pierroz DD, Broqua P, White RB, Pedrazzini T, Aubert ML. Chronic administration of neuropeptide Y into the lateral ventricle of C57BL/6J male mice produces obesity syndrome including hyperphagia, hyperleptinemia, insulin resistance, and hypogonadism. *Mol Cell Endocrinol* 2001;**185**:195–204.

Rhinehart EK, Kalra SP, Kalra PS. Neuropeptidergic characterization of the leptin receptor mutated obese Koletsky rat. *Reg Pep* 2004;**119**:3–10.

Roseweir AK, Kauffman AS, Smith JT, Guerriero KA, Morgan K, Pielecka-Fortuna J, Pineda R, Gottsch ML, Tena-Sempere M, Moenter SM *et al.* Dicovery of poptent kisspeptin antagonists delineate physiological mechanisms of gonadotropin regulation. *J Neurosci* 2009;**29**:3920–3929.

Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A, El-Toukhy T. A systematic review of the effect of oral antioxidants on male infertility. *Reprod Biomed Online* 2010, doi:10.1016/j.rbmo.2010.03.008.

Roth MY, Amory JK, Page ST. Treatment of male infertility secondary to morbid obesity. *Nat Clin Pract Endocrinol Metab* 2008;**4**:415–419.

Runner MN. Inherited hypofunction of the female pituitary in the sterile-obese syndrome in the mouse. *Genetics* 1954;**39**:990–991.

Sahab M, Balasubramaniam A, Sahu A, Plant TM. Central nervous system receptors involved in mediating inhibitory action of neuropeptide Y on luteinizing hormone secretion in the male rhesus monkey (*Macaca mulatta*). *J Neuroendocrinol* 2003; **15**:965–970.

Sainsbury A, Schwarzer C, Couzens M, Jenkins A, Oakes SR, Ormandy CJ, Herzog H. Y4 receptor knockout rescues fertility in *ob/ob* mice. *Gen Dev* 2002; **16**:1077–1088.

Sallmen M, Sandler DP, Hoppin JA, Blair A, Baird DD. Reduced fertility among overweight and obese men. *Epidemiology* 2006;**17**:520–523.

Schiöth HB, Watanobe H. Melanocortins and reproduction. *Brain Res Rev* 2002; **38**:340–350.

Schneider G, Kirschner MA, Berkowitz R, Ertel NH. Increased estrogen production in obese men. *J Clin Endocrinol Metab* 1979;**48**:633–638.

Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Punkard DE, Porte DJ, Woods SC, Seeley RJ *et al.* Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y expression in *ob/ob* mice. *Diabetes* 1996;**45**:531–535.

Selly ML, Lacey MJ, Bartlett MR, Copeland CM, Ardlie NG. Content of significant amounts of a cytotoxic endproduct of lipid peroxidation in human semen. *J Reprod Fertil* 1991;**92**:291–298.

Shafik A, Olfat S. Scrotal lipomatosis. *Br J Urol* 1981a;**83**:50–54.

Shafik A, Olfat S. Lipectomy in the treatment of scrotal lipomatosis. *Br J Urol* 1981b; **53**:55–61.

Shalaby MA, El Zorba HY, Kamel GM. Effect of α-tocopherol and simvastatin on male fertility in hypercholesterolemic rats. *Pharmacol Res* 2004;**50**: 137–142.

Sharpe RM. The 'oestrogen hypothesis'—where do we stand now? *Int J Androl* 2003; **26**:2–15.

Sierens J, Hartley JA, Campbell MJ, Leathem AJC, Woodside JV. In vitro isoflavone supplementation reduces hydrogen peroxide-induced DNA damage in sperm. *Teratol Carcinog Mutag* 2002;**22**:227–234.

Smith JT, Cunningham MJ, Rissman EF, Clifton DK, Steiner RA. Regulation of Kiss1 gene expression in the brain of the female mouse. *Endocrinology* 2005a; **146**:3686–3692.

Smith JT, Dungan HM, Stoll EA, Gottsch ML, Braun RE, Eacker SM, Clifton DK, Steiner RA. Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. *Endocrinology* 2005b;**146**:2976–2984.

Smith JT, Acohido BV, Clifton DK, Steiner RA. KiSS-1 neurones are direct targets for leptin in the *ob/ob* mouse. *J Neurosci* 2006;**18**:298–303.

Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, Allen HL, Lindgren CM, Luan J, Magi R. Association analysis of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* 2010;**42**:937–948.

Stephens TW, Bassinski M, Bristow PK, Buc-Valleskey JM, Burgett SC, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A *et al.* The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 1995;**377**:530–532.

Stewart TM, Liu DY, Garrett C, Jørgensen N, Brown EH, Baker HWG. Associations between andrological measures, hormones and semen quality in fertile Australian men: inverse relationship between obesity and sperm output. *Hum Reprod* 2009; **24**:1561–1568.

Strain GW, Zumoff B, Kream J, Strain JJ, Deucher R, Rosenfeld RS, Levin J, Fukushima DK. Mild hypogonadotropic hypogonadism in obese men. *Metabolism* 1982;**31**:871–875.

Strain GW, Zumoff B, Miller LK, Rosner W, Levit C, Kalin M, Hershcopf RJ, Rosenfeld RS. Effect of massive weight loss on hypothalamic–pituitary–gonadal function in obese men. *J Clin Endocrinol Metab* 1988;**66**:1019–1023.

Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat Genet* 1998; **18**:213–215.

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

Swerdloff RS, Peterson M, Vera A, Batt RA, Heber D, Bray GA. The hypothalamic–pituitary axis in genetically obese (*ob/ob*) mice: response to luteinizing hormone-releasing hormone. *Endocrinology* 1978;**103**:542–547.

Tartaglia LA. The leptin receptor. *J Biol Chem* 1997;**272**:6093–6096.

Tatti P, Masselli L, Buonanno A, Di Mauro P, Strollo F. Leptin levels in diabetic and nondiabetic subjects. *Endocrine* 2001;**15**:305–308.

Tchernof A, Desrés JP, Bélanger A, Dupont A, Prud'homme D, Moorjani S, Lupien PJ, Labrie F. Reduced testosterone and adrenal C_{19} steroid levels in obese men. *Metabolism* 1995;**44**:513–519.

Thérond P, Auger J, Legrand A, Jouannet P. α-Tocopherol in human spermatozoa and seminal plasma: relationships with motility, antioxidant enzymes and leukocytes. *Mol Hum Reprod* 1996;**2**:739–744.

Tremellen K. Oxidative stress and male infertility—a clinical prespective. *Hum Reprod Update* 2008;**14**:243–258.

Twigg J, Fulton N, Gomez E, Irvine DS, Aitkin RJ. Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. *Hum Reprod* 1998;**13**:1429–1436.

Veldhuis JD, Dufau ML. Estradiol modulates the pulsatile secretion of biologically active luteinizing hormone in man. *J Clin Invest* 1987;**80**:631–638.

Vermeulen A. Environment, human reproduction, menopause, and andropause. *Environ Health Pers Suppl* 1993;**101**:91–100.

Vermeulen A, Kaufman JM, Giagulli VA. Influence of some biological indexes on sex hormone-binding globulin and androgen levels in aging or obese males. *J Clin Endocrinol Metab* 1996;**81**:1821–1826.

Vimaleswaran KS, Loos RJ. Progress in the genetics of common obesity and type 2 diabetes. *Expert Rev Mol Med* 2010;**12**:e7.

Vincennati V, Ceroni L, Genghini S, Patton L, Pagotto U, Pasquali R. Sex differences in the relationship between the hypothalamic-pituitary-adrenal axis and sex hormones in obesity. *Obesity* 2006;**14**:235–243.

Vincent HK, Innes KE, Vincent KR. Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. *Diabetes Obes Metab* 2007; **9**:813–8–39.

Von Sobbe HU, Koebrick C, Jenne L, Kiesewetter F. Leptin concentrations in semen are correlated with serum leptin and elevated in hypogonadotrohic hypogonadism. *Andrologia* 2003;**35**:233–237.

Wang H, Wu M, Zhu W, Shen J, Shi X, Yanf J, Zhao Q, Ni C, Xu Y, Shen H *et al.* Evaluation of the association between the AC3 genetic polymorphisms and obesity in a Chines Han population. *PLoS ONE* 2010;**5**:e13851 (1–5).

Wegner CC, Clifford AL, Jilbert PM, Henry MA, Gentry WL. Abnormal high body mass index and tobacco use are associated with poor sperm quality as revealed by reduced sperm binding to hyaluronan-coated slides. *Fertil Steril* 2010; **93**:332–334.

Widdowson PS, Wilding JP. Hypothalamic neuropeptide Y and its neuroendocrine regulation by leptin. *Front Horm Res* 2000;**26**:71–86.

Winters SJ, Plant TM. Partial characterization of circulating inhibin-B and pro-alpha C during development in the male rhesus monkey. *Endocrinology* 1999;**140**:5497–5504.

Winters SJ, Wang C, Abdelrahaman E, Hadeed V, Dyky MA, Brufsky A. Inhibin-B levels in healthy young adult men and prepubertal boys: is obesity the cause for the contemporary decline in sperm count because of fewer Sertoli cells? *J Androl* 2006;**27**:560–564.

World Health Organization. Obesity and Overweight. *Global Strategy on Diet, Physical Activity and Health,* 2003.

World Health Organization. Obesity and overweight. *Fact Sheet* 2006; N°311.

World Health Organization. *The Challenge of Obesity in the WHO European Region and the Strategies for Response*. In: Branca F, Nikogosian H, Lobstein T (eds). Copenhagen, 2007.

Wu FCW, Tajar A, Pye SR, Silman AJ, Finn JD, O'Neill TW, Bartfai G, Casanueva F, Forti G, Giwercman A *et al.*, European Male Aging Study Group. Hypothalamic–pituitary–testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European male aging study. *J Clin Endocrinol Metab* 2008;**93**:2737–2745.

Xu AW, Kaelin CB, Takeda K, Akira S, Schwartz MW, Barsh GS. PI3K integrates the action of insulin and leptin on hypothalamic neurons. *J Clin Invest* 2005; **115**:951–958.

Yaswen L, Diehl N, Brennan MB, Hochgeschwender U. Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. *Nat Med* 1999;**5**:1066–1070.

Yeo GSH, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O'Rahilly S. A frameshift mutation in *MC4R* associated with dominantly inherited human obesity. *Nat Genet* 1998;**20**:111–112.

Young RA, Frink R, Longcope C. Serum testosterone and gonadotropins in the genetically obese male Zucker rat. *Endocrinology* 1982;**111**:977–981.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**:425–432.

Zondervan KT, Cardon LR. The complex interplay among factors that influence allelic association. *Nat Rev Genet* 2004;**5**:89–100.

Zorn B, Osredkar J, Meder-Vrtovec H, Majdic G. Leptin levels in male patients are correlated with inhibin B, testosterone and SHBG but not with sperm characteristics. *Int J Androl* 2007;**30**:439–444.

Zumoff B, Strain GW, Miller LK, Rosner W, Senie R, Seres DS, Rosenfeld RS. Plasma free and non-sex-hormone-binding-globulin-bound testosterone are decreased in obese men in proportion to their degree of obesity. *J Clin Endocrinol Metab* 1990; **71**:929–931.

Zumoff B, Miller LK, Strain GW. Reversal of the hypogonadotropic hypogonadism of obese men by administration of the aromatase inhibitor testolactone. *Metabolism* 2003;**52**:1126–1128.