Human Reproduction Update, Vol.21, No.2 pp. 249–261, 2015
Advanced Access publication on November 20, 2014 doi:10.1093/humupd/dmu060

human reproduction update

Obesity and the reproductive system disorders: epigenetics as a potential bridge

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Submitted on April 29, 2014; resubmitted on October 23, 2014; accepted on October 31, 2014

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BACKGROUND: Obesity and overweight are significantly involved in several reproductive pathologies contributing to infertility in men and women. In addition, several cancers of the reproductive system, such as endometrial, ovarian, breast, testicular and prostate cancers, are strongly influenced by obesity. However, the molecular mechanisms involved in the association between obesity and reproductive disorders remain unclear. Our proposal is to review the current scientific evidence regarding the effect of obesity-related factors as the core of the collective mechanisms directly and indirectly involved in the relationship between obesity and reproductive disorders, with a special and original focus on the effect of the obesity state microenvironment on the epigenetic profile as a reversible mechanistic link between obesity and the reproductive disorders.

METHODS: A PubMed search was performed using keywords related to obesity and adipose-related factors and epigenetics and associated with keywords related to reproduction. Full-text articles and abstracts in the English language published prior to 31 December 2013 were reviewed.

RESULTS: The obesity state notably contributes to a reproductive dysfunction in both men and women, ranging from infertility to oncological outcomes. Several epidemiological and experimental studies demonstrate that factors secreted by the adipose tissue and gut in an obesity state can directly induce reproductive disturbances. Relevantly, these same factors are able to alter the epigenetic regulation of genes, a dynamic and reversible mechanism by which the organism responds to environmental pressures critical to the reproductive function.

CONCLUSION: This review outlines the evidence showing that the association between the reproductive pathologies and obesity is not inevitable but is potentially preventable and reversible. The epigenetic marks related to obesity could constitute a therapeutic target for the reproductive disorders associated with obesity.

Key words: adipose tissue / DNA methylation / cancer / infertility / obesity

Introduction

Currently, obesity is a major health problem for all countries due to its increasing prevalence and its substantial health implications related to chronic disease and mortality. The worldwide prevalence of obesity has nearly doubled in the last three decades. According to data from the National Health and Nutrition Examination Survey (NHANES), 35.7% of American adults are obese, and 17% of children and adolescents aged 2–19 years are obese. In Europe, over 50% of the population is overweight, and ~23% is obese. In Spain in 2006/07, 15.5% of men and 15.2% of women were obese. Despite being a country of Mediterranean diet, this prevalence in Spain rose to 22.9% in 2010 (Gutierrez-Fisac *et al.*, 2011).

Obesity is a multifactorial chronic disease whose etiology is an imbalance between the energy ingested in food and the energy expended. This imbalance is promoted by complex interactions between inadequate dietary habits, diminished physical exercise and genetic background (Marti *et al.*, 2008). Excess energy is stored in fat cells that enlarge and/or increase in number. This process results in an adipose tissue dysfunction and the pathological consequences of obesity that include the development of other diseases, such as diabetes mellitus, heart disease, neurological disease and some forms of cancer (Bray, 2004). Obesity is instrumental in the development of type 2 diabetes mellitus (T2D), and abdominal obesity is strongly associated with cardiovascular disease and diabetes, even among patients with low BMI as demonstrated by data that was generated during the International Day for the Evaluation of Abdominal obesity (IDEA) study in Spain (Casanueva *et al.*, 2010). In addition to the widely known relationships of obesity with cardiovascular disease and diabetes mellitus, obesity itself is thought to be a relevant risk factor for reproductive disorders.

Reproductive function is controlled by the hypothalamic–pituitary–gonadal axis, which is regulated by numerous endogenous and environmental factors (Sanchez-Garrido and Tena-Sempere, 2013). Several years ago, it was proposed that the metabolic conditions and nutritional status influence reproductive capacity. Thus, reproductive function is perturbed both by energy insufficiencies and energy excesses. Accordingly, several epidemiological and experimental studies have provided evidence that obesity and overweight can play relevant roles in the early onset of reproductive features which lead to decreased rates of conception and increases in infertility, such as puberty, menopause and andropause, ovulatory disorders and hypogonadism (Table I). Moreover, overweight and obesity have been demonstrated to be important risk factors for several types of cancers including those that involve the reproduction-related tissues. However, the molecular mechanisms that are involved in the association between obesity and reproductive disorders remain unclear.

Here we review the current scientific evidence regarding the effects of obesity-related factors that form the core of the collective mechanisms that are directly and indirectly involved in the relationship between obesity and reproductive disorders, with a special and original focus on the effects of obesity-state microenvironment on epigenetic profile as a reversible mechanistic link between obesity and the reproductive disorders.

Table I Summary of the consequences of overweight and obesity for male and female reproduction.

Reproductive landmarks	Men	Women
Increased time to conception	+	+
Early cessation of reproductive ability	+	?
Hypogonadism	+	+
Spermatogenesis and ovulatory disorders	+	+
Gonadal tumorigenesis	+	+

Methods

The articles selected for this review were English-language, full-text articles and abstracts that were identified by a series of PubMed searches using keywords either alone or in combination and published before the 31 of December 2013. The data were extracted from the identified papers, and secondary data sources were identified within these papers. The keywords used included obesity, reproductive system, reproduction, reproductive pathologies, puberty, menopause, adipose tissue, adipokines, leptin, adiponectin, insulin, ghrelin, inflammation, oxidative stress, PCOS, estrogens, testosterone, ovary, testis, endometrium, breast, prostate, epigenetics and DNA methylation.

Effects of obesity on male and female reproductive function

The increased global prevalence of obesity has occurred concomitantly with an apparent decline in fertility. Even though there is still insufficient evidence to claim that human fecundity was generally declining due to obesity, experimental studies of animal models and humans have shown direct relationships between obesity and infertility in both males and females.

Evidence for an association between high BMI and the periconceptual periods of both women and men exists. Obese women seeking to become pregnant experience longer times to conception, even if they are young and exhibit stable menstrual cycle patterns (Jensen *et al.*, 1999; van der Steeg *et al.*, 2008). In support of this observational evidence, it has been demonstrated that female mice with diet-induced obesity ovulate oocytes that exhibit developmental delays to the blastocyst stage and gave rise to embryos with altered cell type proportions (Minge *et al.*, 2008). Moreover, the aging and function of the ovary in women appears to be influenced by obesity as observed in the general population (Gracia *et al.*, 2005; Freeman *et al.*, 2007) and, very recently, corroborated in a cohort of female survivors of childhood cancer (van Dorp *et al.*, 2013). Thus, the levels of anti-Mullerian hormone (Freeman *et al.*, 2007; van Dorp *et al.*, 2013) and inhibin B (Gracia *et al.*, 2005), which are markers of ovarian aging and ovarian function, respectively, are significantly lower in healthy obese women than in non-obese women in the menopausal transition period.

The aforementioned findings regarding the fertility of women are similar to those for males. High BMIs (>25 kg/m^2) are associated with depletions in sperm concentrations and total sperm counts compared with these measures in subjects with BMIs between 20 and 25 kg/m^2 (Jensen *et al.*, 2004). These alterations in spermatogenesis have also been evidenced in animal models of genetic obesity; for example, obese Zucker rats exhibit decreased sperm production during puberty and increased sperm DNA fragmentation compared with their lean

littermates (Vendramini *et al.*, 2014). Additionally, a recent meta-analysis that included 21 relevant studies that were published before June 2012 (the total sample was 13 077 men) reinforced these conclusions and revealed that overweight and obesity are associated with increased prevalence of azoospermia and oligozoospermia (Sermondade *et al.*, 2013).

With increasing age, men and women reach a period of cessation of reproductive ability, namely menopause in women and andropause or male midlife transition in men. This age-dependent cessation in reproductive function can be influenced by lifestyle factors such as obesity.

Menopause is a natural transition in the life cycle of women that is caused by the depletion of ovarian function followed by the cessation of menstruation. This female transition occurs most frequently at the age of 50–51 years, but there is considerable variability across countries, and the age of menopause can be influenced by several factors. Regarding the effects of obesity, few studies have explored the associations between BMI and age at menopause, and those that have are controversial. Whereas some studies have observed an association between higher BMI and later age at menopause (Henderson *et al.*, 2008), other studies, such as the large-cohort Fragmingham heart study, have shown that age at natural menopause is not associated with measures of body composition, although post-menopausal women have increased BMI, waist circumference, visceral adipose tissue and subcutaneous adipose tissue compared with premenopausal women (Trikudanathan *et al.*, 2013).

Regarding the male midlife transition, longitudinal studies of the US population within the Massachusetts Male Aging Study (MMAS) survey revealed that a 4-5 kg/m^2 increase in BMI has a negative effect on testosterone that is comparable to that of 10 years of ageing (Derby *et al.*, 2006; Mohr *et al.*, 2006; Travison *et al.*, 2007; Lapauw *et al.*, 2008). Consistently, the European Male Aging Study (EMAS), which provided one of the largest data sets in existence for the investigation of hormonal variations in aging men, confirmed the age-related cross-sectional trends in circulating testosterone among community-dwelling men (Wu *et al.*, 2008). This study found that obesity is associated with progressively lower total and free testosterone levels. Indeed, the effect of increasing BMI on circulating testosterone is more substantial than that of age in that a transition in BMI from non-obese to obese is equivalent to the fall in testosterone that would occur over 15 years (Wu *et al.*, 2008). Moreover, a prospective evaluation of the EMAS survey revealed that weight gain is associated with suppressions of testosterone, free testosterone and sex hormone-binding globulin (SHBG). This potential effect of obesity on the hypothalamic–pituitary–testicular axis can be dissociated from those of age; whereas the reductions in testosterone that are observed with obesity are concomitant with unchanged or even reduced LH in obese older men, age is associated with a compensatory increase in the LH, which suggests that obesity impairs hypothalamic/pituitary function and that age affects testicular function (Wu *et al.*, 2008).

In light of these findings, obesity appears to be a relevant determinant of the age-related decline in reproductive function and could accelerate the age-related infertility outcome. Therefore, even though declining fertility in ageing is a prevalent feature across the animal kingdom (Jones *et al.*, 2014), and for men and women it will remain an inevitable fact of life, a significant part of the age-related reproductive dysfunction could potentially be slowed down by maintaining a healthy body weight.

In agreement with this hypothesis, interventional weight loss regimes for obese diabetic patients result in increased testosterone (Grossmann, 2011). Moreover, a prospective evaluation of community-dwelling men in Europe (EMAS) observed that unsupervised weight loss over 4 years of follow-up was associated with increases in testosterone, free testosterone, SHBG and LH (Camacho *et al.*, 2013).

The association between excess body weight and polycystic ovary syndrome (PCOS) is particularly relevant. PCOS is the most common female endocrine disturbance, affects ~10% of the adult reproductive-aged population and is characterized by ovarian hyperandrogenism and chronic oligo-anovulation (Hart *et al.*, 2004; Ehrmann, 2005). A pathogenic role of obesity in the subsequent development of this syndrome and the exacerbation of its phenotype has been suggested because excess body weight frequently precedes the onset of oligomenorrhea and hyperandrogenism and because 50% of PCOS women are overweight or obese (Gambineri *et al.*, 2002; Lim *et al.*, 2013).

Obesity and oncologic outcomes of the reproductive system

Excess body weight has been demonstrated to be a relevant preventable risk factor for several types of cancers (Calle *et al.*, 2003; Renehan *et al.*, 2008; Boeing, 2013). For example, the oncologic outcomes of the gonads can be influenced by increases in BMI. In women, obesity is a risk factor for endometrial, ovarian, uterine cervical and breast cancer malignancies (Reeves *et al.*, 2007). In men, high body weight is associated with a greater incidence of testicular tumor and more aggressive prostate cancers (Fig. 1).

A recent meta-analysis that included 25 157 women with ovarian cancer from 47 epidemiological studies (i.e. the majority of the available epidemiological information worldwide) indicated that highly significant increases in the risk of ovarian cancer accompany increases in weight and BMI (Beral, 2012). These increases in ovarian cancer risk do not vary with other factors that are relevant to the disease, and these effects do not differ across the common histological subtypes of ovarian cancer with the exception of serous tumors with borderline malignancy; for these tumors, the increase in the risk with increasing BMI is considerably greater than that for other tumor subtypes. However, the use of hormone therapy for menopause has a substantial effect on this association; the relative risk of ovarian cancer is increased among obese women who have never used menopausal hormone therapy (relative risk per every 5-unit increase in BMI of 1.10 [95% confidence interval (CI), 1.07–1.13]) but not among those who have used hormone therapy at some point (relative risk per every 5-unit increase in BMI of 0.95 [95% CI, 0.92–0.99]) (Beral, 2012).

The development of cervical cancer (the third most frequent cancer among women worldwide) is also influenced by obesity status. Obese and overweight women appear to be at a 2-fold (odds ratio (OR) 2.1 [95% CI, 1.1–3.8]) higher risk for cervical adenocarcinoma compared with women who are not overweight or obese (Lacey *et al.*, 2003), and recent observations from the Metabolic Syndrome and Cancer Project (MeCan) cohort indicate that a 12% (hazard ratio 1.12; 95% CI, 1.01–1.25) increase in the risk of cervical cancer risk accompanies a 1 SD increase in BMI without differences among the histological types of cervical cancer (Ulmer *et al.*, 2012).

Strikingly, the strongest influences of obesity on the development of gynecologic cancers have been observed for the risks of endometrial and breast cancers (Renehan *et al.*, 2008). Obesity is a major risk

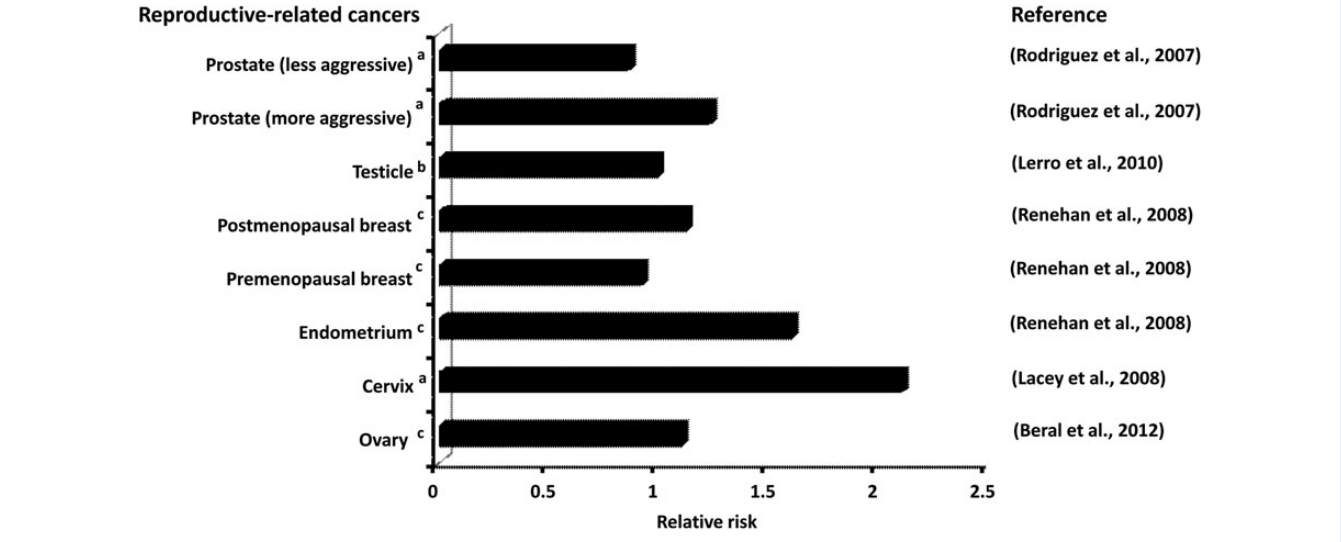

Figure 1 Reproductive-related cancers associated with obesity. The data denote the relative risks of patients with BMI > 30 kg/m^2 (a), relative risk per every unit increase in BMI (b) and relative risk per every 5-units increase in BMI (c).

factor for endometrial cancer; the risk increases by 50–60% for every 5-unit increase in BMI, and it has been estimated that 30–34% of all endometrial cancers can be attributed to overweight and obesity (Webb, 2013). This association is strongest for low-grade endometrial cancers but is present, albeit modest, with more aggressive endometrial cancers (Yang *et al.*, 2012).

Obesity is a known risk factor for breast cancer (Lorincz and Sukumar, 2006). For every 5-unit increase in BMI, the risk of post-menopausal breast cancer increases by 12% (relative risk (RR) 1.12 [95% CI, 1.08–1.16]), and elevated BMIs are associated with a protective effect against the development of breast cancer in premenopausal women (RR 0.92 [95% CI, 0.88–0.97]) (Renehan *et al.*, 2008). This association has recently been corroborated in a study of homogeneous population from Spain that provided evidence that the prevalence of being overweight/obese is significantly higher among post-menopausal breast cancer patients (Crujeiras *et al.*, 2012). However, although it is generally accepted that obese women are at an increased risk for post-menopausal breast cancer and decreased risk for premenopausal breast cancer, a recent study provided new and consistent data that suggests that there are 50 and 70% increases in the risks for premenopausal breast cancer among overweight and obese women, respectively (Cecchini *et al.*, 2012). Indeed, post-menopausal obesity is more frequently associated with positive estrogen receptor (ER) breast cancers, and ages below 50 years and obesity are associated with a higher risk for ER-negative breast cancer (Anderson and Neuhouser, 2012). Therefore, these lines of evidence suggest that reducing obesity should be added to the cancer prevention toolbox for both pre- and the post-menopausal breast cancer.

Regarding the effect of excess body weight on oncologic outcomes in men, the literature has reported heterogeneous findings. Testicular cancer is a relatively rare malignancy in the general population, but it is the most commonly occurring cancer among young men in many countries (Chia *et al.*, 2010). One hypothesis suggests that a high-calorie diet and the resultant increase in body size are among the risk factors for testicular cancer (Dieckmann *et al.*, 2009). However, several epidemiological studies have found positive associations between the risk of testicular cancer and height, and inverse or null associations of testicular cancer with BMI and body weight (OR per every unit increase in BMI 0.99 [95% CI, 0.97–1.00]). A recent systematic review and meta-analysis of body size in relation to testicular cancer corroborated these conclusions (Lerro *et al.*, 2010).

Inconsistent results regarding the association between obesity and prostate cancer have also been published. While some studies of adult BMI and the incidences of all prostate cancers have yielded null results, other studies have reported that adult BMI is associated with a decrease in prostate cancer risk among men who are diagnosed before 60 years of age and those with family histories of prostate cancer (Rodriguez *et al.*, 2007). However, when the association between obesity and prostate cancer is evaluated while accounting for the stage or grade at diagnosis, adult BMI has been positively associated with the risk of more aggressive tumors (RR BMI > 30 kg/m^2 1.23 [95% CI, 1.00–1.55]) and inversely associated with the risk of less-aggressive tumors (RR BMI > 30 kg/m^2 0.86 [95% CI, 0.77–1.06]) (Rodriguez *et al.*, 2007).

The epigenetic impact of obesity on reproductive function

The biological regulatory system through which the organism responds to environmental pressures is mediated by epigenetic modifications to the genome (Herceg and Vaissiere, 2011). These modifications refer to mitotically and/or meiotically heritable changes in gene expression that occur without altering DNA sequences (Holliday, 1987; Berger *et al.*, 2009). Such mechanisms play important roles in many biological processes that occur over a person's lifetime, including embryogenesis and the phenotypic variations of genetically identical individuals (Herceg and Vaissiere, 2011). The epigenetic machinery involves several levels of regulation including DNA methylation, post-translational

histone modifications, nucleosome positioning and non-coding RNAs (Portela and Esteller, 2010; Taby and Issa, 2010; Rodriguez-Paredes and Esteller, 2011). Among these mechanisms, the best-known epigenetic marker is DNA methylation, which occurs in certain areas of the genome with high concentrations of CpG dinucleotides ('CpG islands') and leads to the silencing of both coding and non-coding genes. In addition, the DNA methylation also occurs in areas of low CpG density (<10 CpG/100 bp) named 'CpG deserts'. Although the previous dogma is that epigenetic modifications in CpG islands or shores (regions up to 2 kb away from CpG islands) with highest CpG density are critical, these 'CpG deserts' may be especially important for gene regulation as it is reflected by the maintenance of small CpG clusters in these deserts, despite the high mutation rates observed in CpG sites (Guerrero-Bosagna *et al.*, 2013; Skinner and Guerrero-Bosagna, 2014).

All levels of epigenetic regulation appear to have wide-ranging effects on development and health and can be reversible. Aberrant epigenetic regulation has been described in many human diseases including cancer (Heyn and Esteller, 2012), obesity (Milagro *et al.*, 2013), T2D (Andersen *et al.*, 2014), atherosclerosis (Zaina *et al.*, 2014) and cardiovascular disease (Ordovas and Smith, 2010), neurodegenerative and neurological diseases (Urdinguio *et al.*, 2009; Sanchez-Mut *et al.*, 2013) as well as several inflammatory processes such as inflammatory bowel disease, autoimmune diseases and rheumatoid arthritis (Glossop *et al.*, 2014). Therefore, epigenetic marks might explain the link between lifestyle and the risk for disease and have been proposed to be sensitive biomarkers of disease and potential therapeutic targets for disease management (Hamilton, 2011).

Epigenetics and reproductive function

Reproductive function is highly sensitive to environmental conditions including season, diet and exposure to chemical contaminants. In this regards, recent reports have described epigenetic differences that seem to be critical to reproductive function (Kurian and Terasawa, 2013). The development of the GnRH neuronal circuit is essential for reproductive function and has been described to be influenced by epigenetic modifications. During development and across the onset of puberty, increases in GnRH gene expression occur, and these increases appear to result from DNA demethylation across a CpG island of the GnRH gene (Kurian *et al.*, 2010; Kurian and Terasawa, 2013). Moreover, the epigenetic regulation of the expression of kisspeptin, which is a major stimulant of GnRH release, is associated with development and pubertal maturation (Semaan and Kauffman, 2013). A recent study demonstrated that the timing of puberty is under the regulatory control of an epigenetic mechanism of transcriptional repression (Lomniczi *et al.*, 2013). This study provided evidence for increases in the methylation of two polycomb genes that are related to the transcriptional repression of several genes (e.g. EED; embryonic ectoderm development; and CBX7; chromobox homolog 7) during the onset of puberty. Pharmacological inhibition of the increases in the methylation of the DNA for these two genes is associated with delayed puberty (Lomniczi *et al.*, 2013). Further analysis demonstrated that EED associates with the Kiss1 promoter to reduce its expression and consequently induces a delay of puberty and disruptions of estrous cyclicity and fecundity (Lomniczi *et al.*, 2013).

Epigenetic DNA modifications are also essential for spermatogenesis and male fertility and can be affected by environmental factors. The methylation of testes and epididymis-specific promoters is important for spermatogenesis and sperm maturation, and aberrant changes in the methylation of these promoters are correlated with male infertility, carcinogenesis and development (Wu *et al.*, 2013). Epimutations (frequently hypermethylations) of several genes have been reported to be associated with poor semen parameters or male infertility (Rajender *et al.*, 2010). Moreover, important genes that are expressed in the reproductive tract and are relevant for spermatogenesis and male fertility, such as the X-linked gene family of reproductive homeobox genes on the X chromosome (RHOX) that encodes a set of homeobox genes, are epigenetically regulated. Hypermethylation of the X-linked RHOX cluster may have a causal role in male infertility and may serve as a marker for male idiopathic infertility (Richardson *et al.*, 2014).

On the other hand, during aging, the epigenetic pattern is modified (Fraga and Esteller, 2007; Heyn *et al.*, 2012), and the new pattern is associated with the onset of age-related diseases (Bell *et al.*, 2012). Therefore, although this issue requires further studies, the transitions to menopause and andropause could be conditioned by age-related epigenetic regulation and orchestrated by environmental and lifestyle factors such as increased body weight and obesity-related energetic status.

Recent studies have concluded that inappropriate epigenetic reprogramming is also an important contributing factor to PCOS (Li and Huang, 2008). For example, a genome-wide analysis of DNA methylation via immunoprecipitation found 40 genes in the blood cells of PCOS women that are differentially methylated compared with those of healthy women (Shen *et al.*, 2013). This involvement of epigenetic regulation in PCOS is also supported by evidence from a previous genome-wide analysis of the visceral adipose tissue of female rhesus monkeys that had been exposed to gestational androgen excesses (Xu *et al.*, 2011). More specifically, hyperandrogenism has been found to be associated with significant alterations in the methylation status of genes that are essential for normal reproduction and ovarian function, such as the peroxisome proliferator-activated receptor gamma 1 (PPARG1) and nuclear corepressor 1 (NCOR1) genes, and with alterations in the histone deacetylase 3 (HDAC3)-related acetylation status of blood cells in PCOS women (Qu *et al.*, 2012). Results consistent with those from humans have been observed in high androgen-induced PCOS animal models (Qu *et al.*, 2012).

Relevantly, several types of cancer have been associated with an aberrant pattern of DNA methylation (Weichenhan and Plass, 2013). This DNA methylation pattern is associated with increased risk of disease, poor prognoses and a decreased likelihood of relapse-free survival. For example, DNA hypermethylation of the tumor suppressor genes (BRCA1, p16INK4a and RASSF1) and the methylation of several individual genes were recently found to be associated with clinical features of breast cancer such as predisposition, poor prognosis and relapse-free survival (Hill *et al.*, 2010, 2011). Moreover, a consistent pattern of DNA methylation across several genes has been observed in endometrial and ovarian cancer (Huang *et al.*, 2010; Catasus *et al.*, 2013; Dehan *et al.*, 2013; Sanchez-Vega *et al.*, 2013).

Frequent epigenetic aberrations, such as DNA hypo- and hypermethylation and altered histone acetylation and methylation, have also been observed in prostate cancer, and these aberrations affect the expression and function of a large array of genes and lead to tumorigenesis, tumor progression and metastasis (Dobosy *et al.*, 2007; Li, 2007).

However, despite the extensive evidence regarding the relationship between epigenetic aberrations and reproductive dysfunction, the

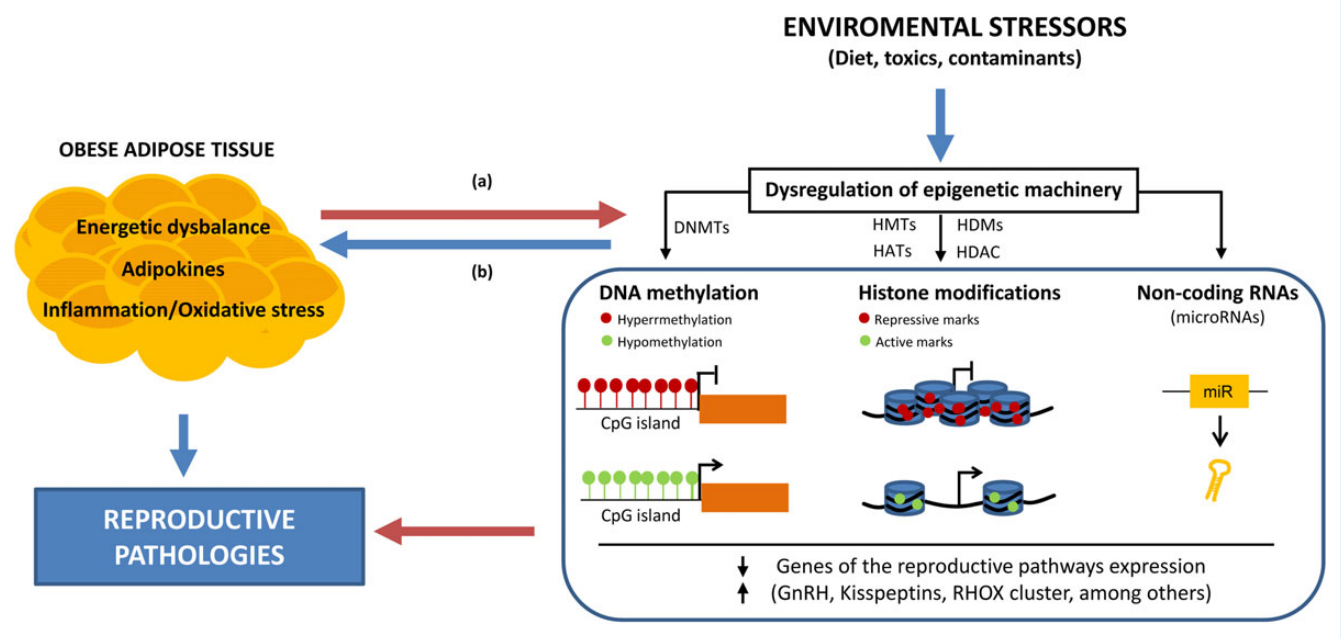

Figure 2 Effects of obesity-related factors on reproductive pathologies. (a) Obesity-related factors may contribute to the process of reproductive dysfunction via the dysregulation of the epigenetic machinery (discontinuous arrows). The obesity-induced microenvironment may influence gene expression levels and the activities of the different enzymes that are implicated in epigenetic control to induce changes in DNA methylation (hyper- or hypomethylation), post-translational histone modifications (active or repressive marks) and non-coding RNAs expression (microRNAs). These epigenetic dysregulations can modify the activities of several genes that are involved in the reproductive pathways. (b) Environmental stressors, such as diet or toxicants, can lead to epigenetic changes that promote obesity, and the obese state can contribute to dys-regulation of adipokines that then causes reproductive disorders (continuous arrows). DNMTs, DNA methyltransferases; HMTs, histone methyltransferases; HATs, histone acetyltransferases; HDMs, histone demethylases; HDAC, histone deacetylases; miR, microRNA; RHOX, reproductive homeobox genes on the X chromosome.

concrete mechanisms responsible for the dysregulation of the epigenetic machinery remain to be elucidated.

All of the aforementioned epigenetic states of different reproductive disorders can be induced by combinations of environmental and nutritional factors because epigenetic regulation may potentially be altered by diet, age, BMI, inflammation and oxidative stress (Milagro *et al.*, 2013). Therefore, we hypothesize that the disturbances in reproductive function that result from obesity may be mediated, at least in part, by the effect of obesity-related factors on epigenetic regulation of reproductive function, or an environmental-related epigenetic regulation can induce an obesity state that leads to dysregulation of adipokines that then causes reproductive disorders (Fig. 2).

Obesity-related factors, epigenetic alterations and reproductive dysfunction

Here we review the evidence for the hypothesis that obesity induces epigenetic alterations, which in turn may increase susceptibility to reproductive dysfunction.

Together with a dysbalance in the energy homeostasis, obesity is characterized by a state of chronic low-grade inflammation that promotes oxidative stress due to the dysfunction of adipose tissue and the alterations of adipocyte-derived hormone secretion and cytokine synthesis (Zou and Shao, 2008).

In addition to its primary role as a fuel reservoir, adipose tissue is a highly active metabolic and endocrine organ that secretes factors that include leptin, adiponectin, and other cytokines (Catalan *et al.*, 2009) and other new signals that have been identified with the proteomics approach (Roca-Rivada *et al.*, 2011). Moreover, adipose tissue is a major site of the metabolism of sex steroids and glucocorticoids (Kershaw and Flier, 2004). Obesity is strongly associated with changes in the physiological function of adipose tissue that lead to adipose tissue dysfunction, which in turn results in increased systemic levels of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin 6 (IL-6), C-reactive protein and matrix metalloprotienases (Dizdar and Alyamac, 2004). The chronic inflammation that is induced by adipocyte dysfunction induces increases in the release and accumulation of reactive oxygen species (ROS). Additionally, obesity alone induces an excessive generation of ROS due to inefficient energy metabolism (Crujeiras *et al.*, 2008). Such obesity-related inflammatory and oxidative stress has been hypothesized to be a link between obesity and its comorbidities (Vincent and Taylor, 2006).

Several enzymes involved in the epigenetic modifications utilize cofactors or substrates that are crucial metabolites in core pathways of intermediary metabolism such as acetyl-CoA, glucose, α-ketoglutarate (α-KG), nicotinamide adenine dinucleotide (NAD+), flavin adenine dinucleotide (FAD), ATP or S-adenosylmethionine (SAM) (Gut and Verdin, 2013). Histone acetylation primarily depends on glucose-derived, cytosolic pools of acetyl-CoA. This chromatin modification allows a feed-forward control mechanism for the selective expression of genes that regulates cellular function (Gut and Verdin, 2013). Therefore, disturbances in energy metabolism such as occur in obesity, might

also lead to stable epigenetic changes that are maintained through the germline or they can occur in adult tissues and may affect the health of the organism, as was recently reviewed (Gut and Verdin, 2013).

Oxidative stress induces DNA damage (e.g. base modifications, deletions, strand breakages and chromosomal rearrangements) that reduces the ability of DNA to be methylated by DNA methyltransferases (DNMTs) and results in global hypomethylation (Wachsman, 1997; Franco *et al.*, 2008). Additionally, ROS can induce the hypermethylation of certain tumor suppressor genes and thus promote carcinogenesis (Govindarajan *et al.*, 2002; Lim *et al.*, 2008). Moreover, oxidative damage has also been implicated in the regulation of histone modifications and microRNA expression (Simone *et al.*, 2009; Mateescu *et al.*, 2011; Rajendran *et al.*, 2011). Inflammation also induces epigenetic alterations in tissues that are associated with disease manifestations, as revealed by recent therapeutic interventions utilizing histone deacetylase and DNMT inhibitors, the effects of certain anti-inflammatory dietary elements on DNA methylation and chromatin remodeling, and the actions of several inflammatory-related transcription factors such as nuclear factor kappa B (NFkB) (Milagro *et al.*, 2013).

This epigenetic impact of obesity on reproductive function may be broadly divided into two areas; somatic effects and germline effects. The obesity-related factors can induce epigenetic alterations in adult target tissues and cells but these factors can induce an epigenetic phenotype in germline cells as well, that not only impede gamete function and contribute to infertility but also that are transmittable to the next generation.

Even though the notion that epigenetic marks are transmitted across generations is still controversial because it is uncertain whether and to what extent such epigenetic inheritance exists (Heard and Martienssen, 2014), over the past few years, evidence has been accumulating that epigenetic transgenerational inheritance occurs in mammals (Skinner *et al.*, 2010; Guerrero-Bosagna *et al.*, 2013; Pembrey *et al.*, 2014; Skinner and Guerrero-Bosagna, 2014). In this regard, obesity status can exert a germline epigenetic impact since a parental obesity effect on the reproductive health of subsequent generations was demonstrated (Fullston *et al.*, 2012). Relatedly, a recent study observed that diet-induced paternal obesity modulates sperm microRNA content and germ cell methylation status (which are potential signals that program the health of the offspring) and impairs the metabolic health of future generations (Fullston *et al.*, 2013). Similarly, maternal obesity adversely affects oocyte quality, embryo development and the health of the offspring. The DNA methylation status of several imprinted genes and metabolism-related genes appears to be the underlying mechanism responsible for the adverse effects of maternal obesity on oocyte quality and the embryo development of the offspring. These findings have recently been corroborated in oocytes from an obesity mouse model and in oocytes and liver from their offspring (Ge *et al.*, 2014). The results of this study revealed that the DNA methylation patterns of several metabolism-related genes are not only altered in the oocytes of the obese mice but also in the oocytes and liver of their offspring (Ge *et al.*, 2014).

Epigenetic alterations, obesity-related factors and reproductive dysfunction

Here we review the evidence for the hypothesis that epigenetic changes can induce obesity, which in turn leads to reproductive dysfunction.

Specific patterns of epigenetic factors, including DNA methylation, have also been found to be associated with obesity itself in a genome-wide DNA methylation analysis in leucocytes and adipose tissue (Carless *et al.*, 2013; Xu *et al.*, 2013; Dick *et al.*, 2014) and in analyses of specific genes, such as the circadian clock genes (e.g. CLOCK; clock circadian regulator, BMAL1; aryl hydrocarbon receptor nuclear translocator-like and PER2; period circadian 2), whose methylation status in human leucocytes is associated with obesity (Milagro *et al.*, 2012). This specific epigenetic profile associated with obesity can be induced by environmental factors on somatic cells but also a number of studies demonstrate that ancestral environmental exposure can promote the epigenetic transgenerational inheritance of obesity through germline epimutations, i.e. the germline (sperm or egg) transgenerational transmission of epigenetic marks that influence physiological parameters and disease, in the absence of direct environmental exposures (Skinner *et al.*, 2010). For example, a recent study demonstrated the presence of differentially methylated regions in the sperm of the F3 generation of male rodents with ancestral exposure to the insecticide dichlorodiphenyltrichloroethane (DDT) and a number of previously identified obesity-associated genes correlated with the epimutations identified (Skinner *et al.*, 2013). This germline transmission of epimutations associated with obesity in rodents was also observed after the exposure to plastics-derived endocrine disruptors (bisfenol-A, Diethylhexyl phthalate and dibutyl phthalate) (Manikkam *et al.*, 2013) or jet fuel hydrocarbons(Tracey *et al.*, 2013).

Strikingly, therapeutic strategies for counteracting excess body weight are able to remodel DNA methylation profiles concomitant with the reduction of body weight. The DNA methylation and expression levels of several genes, which are related to metabolic processes and mitochondrial functions (e.g. peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; PGC-1α and pyruvate dehydrogenase kinase, isozyme 4; PDK4), are altered in the skeletal muscle of obese people and after Roux-en-Y gastric bypass (RYGB), a type of weight-loss surgery, and were normalized to levels observed in normal-weight, healthy controls (Barres *et al.*, 2013). A 6-month intervention of exercise can induce changes in the genome-wide DNA methylation patterns of human adipose tissue that potentially affect adipocyte metabolism (Ronn *et al.*, 2013). Similar results have been observed in the muscle of patients with T2D; in these patients, exercise altered the DNA methylations of genes involved in retinol metabolism and calcium signaling pathways that have known functions in the muscle and T2D (Nitert *et al.*, 2012). The DNA methylation status of specific genes in human leukocytes (Milagro *et al.*, 2011) and adipose tissue can be altered by caloric restriction interventions (Bouchard *et al.*, 2010). Additionally, it has been demonstrated that responses to weight loss treatments can be influenced and predicted by DNA methylation status prior to beginning treatment; i.e. differences in the DNA methylation patterns of specific genes have been found between low and high responders to weight loss therapy (Bouchard *et al.*, 2010; Milagro *et al.*, 2011) and between patients who are prone to regain lost weight and those who are able to maintain weight loss during a free-living period after dieting (Crujeiras *et al.*, 2013).

Once obesity is established, factors secreted by the dysfunctional obese adipose tissue can directly influence reproductive function (Budak *et al.*, 2006; Comninos *et al.*, 2014) and have carcinogenic properties in several tissues (Doyle *et al.*, 2012) (Fig. 3).

Leptin, which is mainly secreted by adipose tissue and is related to body fat, has been demonstrated to play a central role in the metabolic control of the reproductive system (Carro *et al.*, 1997;

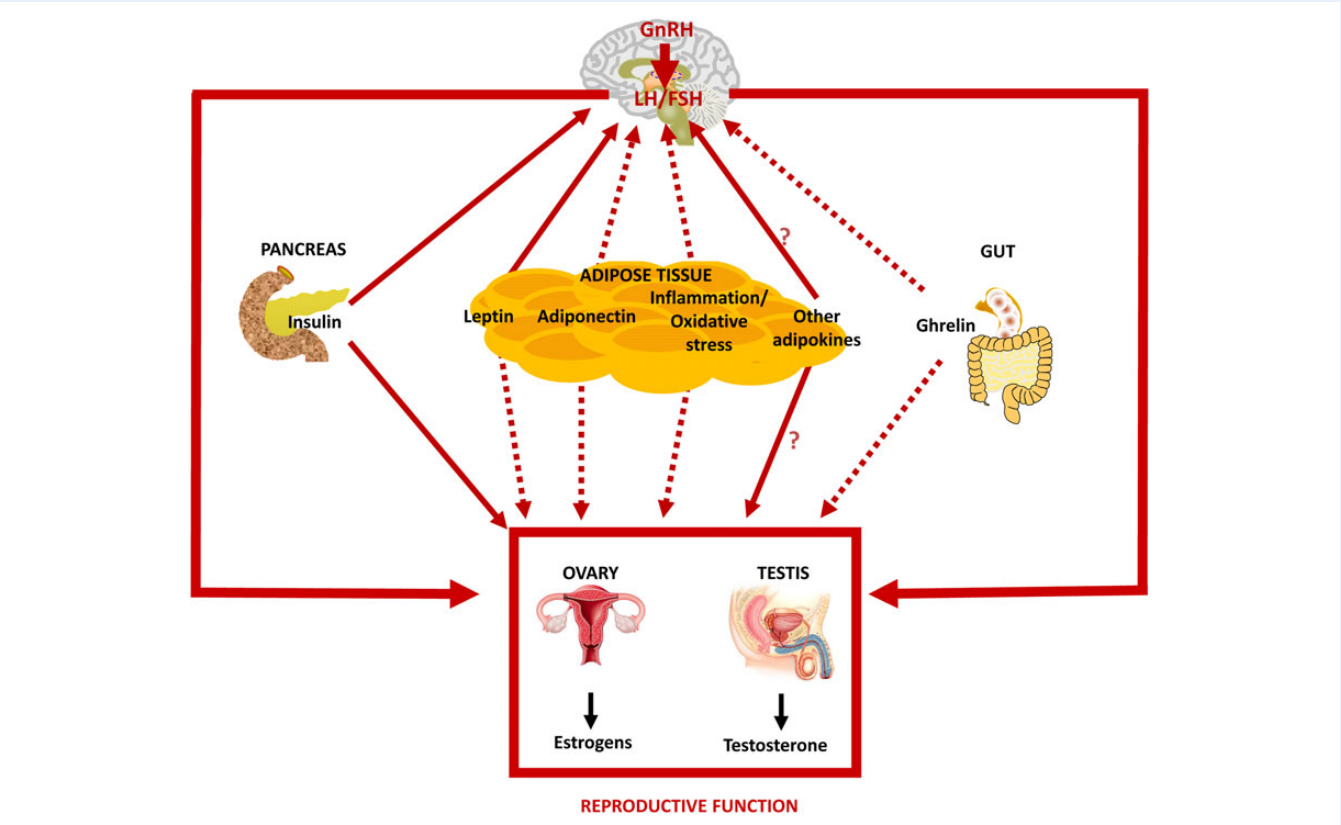

Figure 3 Links between obesity-related factors and reproductive function. The continuous arrows denote stimulatory effects, and the discontinuous arrows indicate inhibitory effects.

Casanueva and Dieguez, 1999; Pinilla *et al.*, 1999; Tena-Sempere *et al.*, 1999). At the central level, endogenous leptin is required for the activation of hypothalamic GnRH secretion and physiological GnRH pulse generation, which are mediated by the intermediate signaling factors of kisspeptin or glutamate (Comninos *et al.*, 2014). At high concentrations, such as those that occur in states of obesity, leptin can directly inhibit some gonadal functions, including sex steroid secretion; thus these inhibitory actions of leptin may explain part of the hypogonadal states frequently observed in obese patients (Sanchez-Garrido and Tena-Sempere, 2013).

Adiponectin is another protein that is abundantly secreted by white adipose tissue and whose serum concentrations are inversely correlated with body fat mass. In addition to its well-known antidiabetic, antiatherogenic and anti-inflammatory properties (Campos *et al.*, 2008), adiponectin has been suggested to play a beneficial role in reproductive tissues. Adiponectin has been suggested to play roles in oocyte maturation, granulosa cell proliferation and death, and as a modulator of estradiol and progesterone secretion (Lagaly *et al.*, 2008; Maillard *et al.*, 2010). The deletion of functional adiponectin in mice results in infertility in female but not male mice (Combs *et al.*, 2004). Moreover, knockdown of the genes for the adiponectin receptors Adipo R1 and Adipo R2 is associated with increased androstenedione secretion by bovine theca cells, which suggest that the hyperandrogenism observed in the obese and overweight can be explained by reductions in adiponectin in obesity (Comim *et al.*, 2013).

The roles of other adipose-related proteins, such as resistin and omentin, remain to be established (Comninos *et al.*, 2014).

Gut hormones, such as ghrelin, may also operate as modifiers of male and female reproduction. Ghrelin is primarily secreted by the oxyntic cells of the gastric mucosa, although it is also produced in the intestine, pancreas, hypothalamus and pituitary (Gualillo *et al.*, 2003). Additionally, further evidence has demonstrated that ghrelin and its functional receptor are expressed in rat and human gonads, suggesting paracrine/autocrine effects of this peptide in different tissues (Barreiro and Tena-Sempere, 2004). Thus, in addition to its roles in the regulation of feeding behavior and energy metabolism as an orexigenic signal that increases food intake and adiposity, ghrelin has been demonstrated to play a relevant role in the regulation of reproductive function via its inhibitory effect, which has previously been reviewed elsewhere (Barreiro and Tena-Sempere, 2004; Comninos *et al.*, 2014). Ghrelin inhibits the proliferative activity of immature Leydig cells (Barreiro *et al.*, 2004), testosterone secretion in the testes (Tena-Sempere *et al.*, 2002), and prevents spermatogenesis (Kheradmand *et al.*, 2012). In the female reproductive tract, ghrelin exerts inhibitory effects on luteal function (Tropea *et al.*, 2007) and the biosynthesis of estradiol and progesterone in granulosa-lutein cells (Viani *et al.*, 2008).

Energy metabolism and reproductive function are also connected by insulin, which is a hormone that is produced by pancreatic β-cells. In addition to its function in the regulation of glucose homeostasis, this hormone can stimulate reproductive function at the hypothalamic and

gonadal levels (Comninos *et al.*, 2014). Hyperinsulinemia is associated with increased LH secretion due to increased expression and secretion of hypothalamic GnRH, particularly in females (Burcelin *et al.*, 2003; Kim *et al.*, 2005). Moreover, insulin can stimulate ovarian (Nestler and Jakubowicz, 1996; Tosi *et al.*, 2012) and testicular (Ahn *et al.*, 2013) steroidogenesis.

As previously mentioned, accumulated body fat is also associated with increased inflammation and oxidative stress (Vincent *et al.*, 2007). The complex interplay between cytokines and oxidative stress plays a role in the etiologies of female and male reproductive disorders that has been highlighted in a recent review (Agarwal *et al.*, 2012). The oxidant status of the cell modulates angiogenesis, which is critical for follicular growth, corpus luteum formation, endometrial differentiation and embryonic growth as highlighted in the aforementioned review. Chronic inflammation has been proposed to promote delays in oocyte development and thus the poor reproductive outcomes that are typically observed in obese women (Robker *et al.*, 2009). Oxidative stress also appears to be a powerful mechanism that can lead to sperm damage, deformity and eventually, male infertility, as oxidative stress is toxic to human spermatozoa (Agarwal *et al.*, 2009; Makker *et al.*, 2009). The inflammatory factors secreted by adipose tissue also appear to play an inhibitory role at the central level of the hypothalamic–pituitary–gonadal axis. For example, cytokines, such as TNF-alpha and IL-6, have been observed to dose-dependently suppress GnRH-stimulated LH release in *in vitro* pituitary glands isolated from female and male rats (Russell *et al.*, 2001). Moreover, in rabbits with high-fat diet (HFD)-induced metabolic syndrome (MetS), it has been observed that hypogonadism-associated MetS is related to an inflammatory state that is concomitant with a decrease in gonadotrophin levels; this finding suggests that HFD-induced metabolic derangements negatively affect GnRH neuron function via inflammatory injuries at the hypothalamic level (Morelli *et al.*, 2014).

The above mentioned obesity-related factors are also associated with carcinogenesis in the reproductive system (Calle and Kaaks, 2004; Catalan *et al.*, 2009; Khandekar *et al.*, 2011; Howe *et al.*, 2013). For example, leptin induces the growth, migration and antiapoptotic functions of several types of cancer cells (Li *et al.*, 2012; Weichenhan and Plass, 2013), and elevated circulating leptin levels in patients are correlated with the risk for and progression of several cancers, such as endometrial and ovarian cancers (Luhn *et al.*, 2013), but no changes have been observed in patients with breast cancer (Aliustaoglu *et al.*, 2010). Insulin has been demonstrated to be a potent mitogen that stimulates DNA synthesis and appears to have a role in tumor initiation and progression (Renehan *et al.*, 2006). *In vitro* studies have demonstrated that insulin increases the neoplastic proliferation of cell lines at both physiological and pharmacological doses (Osborne *et al.*, 1976; Pollak, 2008). Moreover, insulin increases the bioactivity of insulin-like growth factor I (IGF-I) by enhancing hepatic IGF-I synthesis and by reducing the hepatic production of IGF binding protein 1 (IGFBP-1) and 2 (IGFBP-2). IGF-I has mitotic properties, and low levels of the expression of IGFBPs are associated with malignant transformation (Clemmons, 2007). Therefore, although insulin can directly induce tumor growth, many of its mitogenic and anti-apoptotic effects operate through the IGF-I system, through increasing the bioavailabilities of hormones, through increasing the levels of proinflammatory cytokines, and through oxidative stress (Arcidiacono *et al.*, 2012). In contrast, adiponectin has been found to exhibit anticarcinogenic effects *in vitro* (Dalamaga *et al.*, 2012), and epidemiologic studies have shown that low levels of adiponectin are inversely associated with the risks of developing of multiple cancers and the advanced progressions of these diseases (Barb *et al.*, 2007). Moreover, adiponectin inhibits leptin-induced oncogenic signaling *in vitro* (Beales *et al.*, 2014).

Therefore, the systemic alterations induced by obesity, such as hyperleptinemia and hyperinsulinemia, that are concomitant with low levels of adiponectin, chronic inflammation and oxidative stress determine the specific microenvironments in the reproductive system that are favorable to poor reproductive outcomes and determine the onset of the reproductive-related cancers that are typically observed in the obese. These obesity-related factors not only can act directly to dys-regulate reproduction, but also may affect the epigenome, and these epigenetic effects further lead to reproductive disorders (Fig. 2).

Conclusions

Several epidemiological and observational studies provide evidence for the relationships between obesity and reproductive pathologies, ranging from early in the onset of puberty to menopause/andropause, and several types of tumors in the reproductive tissues. Attempts to reduce obesity-related factors could improve reproductive function and reduce the incidence and poor prognoses of cancers in reproductive tissues. Although the molecular mechanisms involved in these associations require further elucidation, the molecular etiology of reproductive disease associated with obesity could be the epigenetic changes that could be either the result of, or cause, obesity. Obesity could contribute to infertility and malignancies of the reproductive system by an altered microenvironment that favors epigenetic changes that increase the susceptibility for inducing and cementing a disease state. In addition, epigenetic changes induced by environmental stressors can increase the susceptibility to obesity with the consequent dys-regulation of the obesity-related factors that obstruct the reproductive function. Therefore, metabolic environment, chronic inflammation, oxidative stress and deregulation of adipokine secretion that are induced by the obesity state may play direct roles in the regulation of the hypothalamic–pituitary–gonadal axis or indirect roles in inducing epigenetic modifications of specific genes in the reproductive pathways.

Taken together, this review highlights the need for further scientific research to elucidate the role of epigenetic regulation as a potential mechanistic link between obesity and disturbances in reproductive function. Because epigenetic modifications are dynamic and reversible and change in response to dietary patterns, physical activity and weight loss, the epigenetic markers related to obesity may constitute therapeutic targets for the prevention of obesity-related disorders including reproductive pathologies. Future efforts will therefore be needed to carry out genome-wide analysis that provides information on reproductive dysfunction-specific epigenetic marks and longitudinal studies will be required to evaluate the effect of dietary- or surgery-induced weight loss on reprogramming these disease-specific epigenetic modifications. Understanding the influence of the obesity-related microenvironment on epigenetic regulation of the reproductive function machinery will provide new tools to improve the management as well as the prevention of reproductive disorders.

Authors' roles

A.B.C. designed the review, performed the literature search, extracted the data, wrote the manuscript and provided final approval of the

manuscript. F.F.C. designed the review, supervised the writing, critically reviewed the complete manuscript and provided final approval of the manuscript.

Funding

The authors' laboratory work is supported by CIBERobn and the INTRASALUD proyect, Instituto de Salud Carlos III (ISCIII) initiatives as well as the Xunta de Galicia and Fundación Lilly. A.B.C. is funded by the ISCIII through a research contract 'Sara Borrell' (C09/00365).

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

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

Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. *Reprod Biol Endocrinol* 2012;**10**:49.

Ahn SW, Gang GT, Kim YD, Ahn RS, Harris RA, Lee CH, Choi HS. Insulin directly regulates steroidogenesis via induction of the orphan nuclear receptor DAX-1 in testicular Leydig cells. *J Biol Chem* 2013;**288**:15937–15946.

Aliustaoglu M, Bilici A, Gumus M, Colak AT, Baloglu G, Irmak R, Seker M, Ustaalioglu BB, Salman T, Sonmez B *et al.* Preoperative serum leptin levels in patients with breast cancer. *Med Oncol* 2010;**27**:388–391.

Anderson GL, Neuhouser ML. Obesity and the risk for premenopausal and postmenopausal breast cancer. *Cancer Prev Res (Phila)* 2012;**5**:515–521.

Andersen GS, Thybo T, Cederberg H, Oresic M, Esteller M, Zorzano A, Carr B, Walker M, Cobb J, Clissmann C *et al.* The DEXLIFE study methods: identifying novel candidate biomarkers that predict progression to type 2 diabetes in high risk individuals. *Diabetes Res Clin Pract* 2014;**5**:515–521.

Arcidiacono B, Iiritano S, Nocera A, Possidente K, Nevolo MT, Ventura V, Foti D, Chiefari E, Brunetti A. Insulin resistance and cancer risk: an overview of the pathogenetic mechanisms. *Exp Diabetes Res* 2012;**2012**:789174.

Barb D, Williams CJ, Neuwirth AK, Mantzoros CS. Adiponectin in relation to malignancies: a review of existing basic research and clinical evidence. *Am J Clin Nutr* 2007;**86**:s858–s866.

Barreiro ML, Tena-Sempere M. Ghrelin and reproduction: a novel signal linking energy status and fertility? *Mol Cell Endocrinol* 2004;**226**:1–9.

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

Barres R, Kirchner H, Rasmussen M, Yan J, Kantor FR, Krook A, Naslund E, Zierath JR. Weight loss after gastric bypass surgery in human obesity remodels promoter methylation. *Cell Rep* 2013;**3**:1020–1027.

Beales IL, Garcia-Morales C, Ogunwobi OO, Mutungi G. Adiponectin inhibits leptin-induced oncogenic signalling in oesophageal cancer cells by activation of PTP1B. *Mol Cell Endocrinol* 2014;**382**:150–158.

Bell JT, Tsai PC, Yang TP, Pidsley R, Nisbet J, Glass D, Mangino M, Zhai G, Zhang F, Valdes A *et al.* Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. *PLoS Genet* 2012; **8**:e1002629.

Beral VoboCgoesooc. Ovarian cancer and body size: individual participant meta-analysis including 25,157 women with ovarian cancer from 47 epidemiological studies. *PLoS Med* 2012;**9**:e1001200.

Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. *Genes Dev* 2009;**23**:781–783.

Boeing H. Obesity and cancer—the update 2013. *Best Pract Res Clin Endocrinol Metab* 2013;**27**:219–227.

Bouchard L, Rabasa-Lhoret R, Faraj M, Lavoie ME, Mill J, Perusse L, Vohl MC. Differential epigenomic and transcriptomic responses in subcutaneous adipose tissue between low and high responders to caloric restriction. *Am J Clin Nutr* 2010;**91**:309–320.

Bray GA. Medical consequences of obesity. *J Clin Endocrinol Metab* 2004; **89**:2583–2589.

Budak E, Fernandez Sanchez M, Bellver J, Cervero A, Simon C, Pellicer A. Interactions of the hormones leptin, ghrelin, adiponectin, resistin, and PYY3-36 with the reproductive system. *Fertil Steril* 2006;**85**:1563–1581.

Burcelin R, Thorens B, Glauser M, Gaillard RC, Pralong FP. Gonadotropin-releasing hormone secretion from hypothalamic neurons: stimulation by insulin and potentiation by leptin. *Endocrinology* 2003;**144**:4484–4491.

Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* 2004;**4**:579–591.

Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003;**348**:1625–1638.

Camacho EM, Huhtaniemi IT, O'Neill TW, Finn JD, Pye SR, Lee DM, Tajar A, Bartfai G, Boonen S, Casanueva FF *et al.* Age-associated changes in hypothalamic-pituitary-testicular function in middle-aged and older men are modified by weight change and lifestyle factors: longitudinal results from the European Male Ageing Study. *Eur J Endocrinol* 2013;**168**:445–455.

Campos DB, Palin MF, Bordignon V, Murphy BD. The 'beneficial' adipokines in reproduction and fertility. *Int J Obes (Lond)* 2008;**32**:223–231.

Carless MA, Kulkarni H, Kos MZ, Charlesworth J, Peralta JM, Goring HH, Curran JE, Almasy L, Dyer TD, Comuzzie AG *et al.* Genetic effects on DNA methylation and its potential relevance for obesity in Mexican Americans. *PLoS One* 2013;**8**:e73950.

Carro E, Pinilla L, Seoane LM, Considine RV, Aguilar E, Casanueva FF, Dieguez C. Influence of endogenous leptin tone on the estrous cycle and luteinizing hormone pulsatility in female rats. *Neuroendocrinology* 1997;**66**:375–377.

Casanueva FF, Dieguez C. Neuroendocrine regulation and actions of leptin. *Front Neuroendocrinol* 1999;**20**:317–363.

Casanueva FF, Moreno B, Rodriguez-Azeredo R, Massien C, Conthe P, Formiguera X, Barrios V, Balkau B. Relationship of abdominal obesity with cardiovascular disease, diabetes and hyperlipidaemia in Spain. *Clin Endocrinol (Oxf)* 2010;**73**:35–40.

Catalan V, Gomez-Ambrosi J, Rodriguez A, Salvador J, Fruhbeck G. Adipokines in the treatment of diabetes mellitus and obesity. *Expert Opin Pharmacother* 2009; **10**:239–254.

Catasus L, Pons C, Munoz J, Espinosa I, Prat J. Promoter hypermethylation contributes to TIMP3 down-regulation in high stage endometrioid endometrial carcinomas. *Histopathology* 2013;**62**:632–641.

Cecchini RS, Costantino JP, Cauley JA, Cronin WM, Wickerham DL, Land SR, Weissfeld JL, Wolmark N. Body mass index and the risk for developing invasive breast cancer among high-risk women in NSABP P-1 and STAR breast cancer prevention trials. *Cancer Prev Res (Phila)* 2012;**5**:583–592.

Chia VM, Quraishi SM, Devesa SS, Purdue MP, Cook MB, McGlynn KA. International trends in the incidence of testicular cancer, 1973–2002. *Cancer Epidemiol Biomarkers Prev* 2010;**19**:1151–1159.

Clemmons DR. Modifying IGF1 activity: an approach to treat endocrine disorders, atherosclerosis and cancer. *Nat Rev Drug Discov* 2007;**6**:821–833.

Combs TP, Pajvani UB, Berg AH, Lin Y, Jelicks LA, Laplante M, Nawrocki AR, Rajala MW, Parlow AF, Cheeseboro L *et al.* A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. *Endocrinology* 2004;**145**:367–383.

Comim FV, Hardy K, Franks S. Adiponectin and its receptors in the ovary: further evidence for a link between obesity and hyperandrogenism in polycystic ovary syndrome. *PLoS One* 2013;**8**:e80416.

Comninos AN, Jayasena CN, Dhillo WS. The relationship between gut and adipose hormones, and reproduction. *Hum Reprod Update* 2014;**20**:153–174.

Crujeiras AB, Parra D, Goyenechea E, Abete I, Gonzalez-Muniesa P, Martinez JA. Energy restriction in obese subjects impact differently two mitochondrial function markers. *J Physiol Biochem* 2008;**64**:211–219.

Crujeiras AB, Cueva J, Vieito M, Curiel T, Lopez-Lopez R, Pollan M, Casanueva FF. Association of breast cancer and obesity in a homogeneous population from Spain. *J Endocrinol Invest* 2012;**35**:681–685.

Crujeiras AB, Campion J, Diaz-Lagares A, Milagro FI, Goyenechea E, Abete I, Casanueva FF, Martinez JA. Association of weight regain with specific methylation levels in the NPY and POMC promoters in leukocytes of obese men: a translational study. *Regul Pept* 2013;**186**:1–6.

Dalamaga M, Diakopoulos KN, Mantzoros CS. The role of adiponectin in cancer: a review of current evidence. *Endocr Rev* 2012;**33**:547–594.

Dehan P, Canon C, Trooskens G, Rehli M, Munaut C, Van Criekinge W, Delvenne P. Expression of type 2 orexin receptor in human endometrium and its epigenetic silencing in endometrial cancer. *J Clin Endocrinol Metab* 2013;**98**:1549–1557.

Derby CA, Zilber S, Brambilla D, Morales KH, McKinlay JB. Body mass index, waist circumference and waist to hip ratio and change in sex steroid hormones: the Massachusetts Male Ageing Study. *Clin Endocrinol (Oxf)* 2006;**65**:125–131.

Dick KJ, Nelson CP, Tsaprouni L, Sandling JK, Aissi D, Wahl S, Meduri E, Morange PE, Gagnon F, Grallert H *et al.* DNA methylation and body-mass index: a genome-wide analysis. *Lancet* 2014;**383**:1990–1998.

Dieckmann KP, Hartmann JT, Classen J, Diederichs M, Pichlmeier U. Is increased body mass index associated with the incidence of testicular germ cell cancer? *J Cancer Res Clin Oncol* 2009;**135**:731–738.

Dizdar O, Alyamac E. Obesity: an endocrine tumor? *Med Hypotheses* 2004; **63**:790–792.

Dobosy JR, Roberts JL, Fu VX, Jarrard DF. The expanding role of epigenetics in the development, diagnosis and treatment of prostate cancer and benign prostatic hyperplasia. *J Urol* 2007;**177**:822–831.

Doyle SL, Donohoe CL, Lysaght J, Reynolds JV. Visceral obesity, metabolic syndrome, insulin resistance and cancer. *Proc Nutr Soc* 2012;**71**:181–189.

Ehrmann DA. Polycystic ovary syndrome. *N Engl J Med* 2005;**352**:1223–1236.

Fraga MF, Esteller M. Epigenetics and aging: the targets and the marks. *Trends Genet* 2007;**23**:413–418.

Franco R, Schoneveld O, Georgakilas AG, Panayiotidis MI. Oxidative stress, DNA methylation and carcinogenesis. *Cancer Lett* 2008;**266**:6–11.

Freeman EW, Gracia CR, Sammel MD, Lin H, Lim LC, Strauss JF III. Association of anti-mullerian hormone levels with obesity in late reproductive-age women. *Fertil Steril* 2007;**87**:101–106.

Fullston T, Palmer NO, Owens JA, Mitchell M, Bakos HW, Lane M. Diet-induced paternal obesity in the absence of diabetes diminishes the reproductive health of two subsequent generations of mice. *Hum Reprod* 2012;**27**:1391–1400.

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

Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R. Obesity and the polycystic ovary syndrome. *Int J Obes Relat Metab Disord* 2002;**26**:883–896.

Ge ZJ, Luo SM, Lin F, Liang QX, Huang L, Wei YC, Hou Y, Han ZM, Schatten H, Sun QY. DNA methylation in oocytes and liver of female mice and their offspring: effects of high-fat-diet-induced obesity. *Environ Health Perspect* 2014;**122**:154–164.

Glossop JR, Emes RD, Nixon NB, Haworth KE, Packham JC, Dawes PT, Fryer AA, Mattey DL, Farrell WE. Genome-wide DNA methylation profiling in rheumatoid arthritis identifies disease-associated methylation changes that are distinct to individual T- and B-lymphocyte populations. *Epigenetics* 2014;**9**:1228–1237.

Govindarajan B, Klafter R, Miller MS, Mansur C, Mizesko M, Bai X, LaMontagne K Jr, Arbiser JL. Reactive oxygen-induced carcinogenesis causes hypermethylation of p16(Ink4a) and activation of MAP kinase. *Mol Med* 2002;**8**:1–8.

Gracia CR, Freeman EW, Sammel MD, Lin H, Nelson DB. The relationship between obesity and race on inhibin B during the menopause transition. *Menopause* 2005; **12**:559–566.

Grossmann M. Low testosterone in men with type 2 diabetes: significance and treatment. *J Clin Endocrinol Metab* 2011;**96**:2341–2353.

Gualillo O, Lago F, Gomez-Reino J, Casanueva FF, Dieguez C. Ghrelin, a widespread hormone: insights into molecular and cellular regulation of its expression and mechanism of action. *FEBS Lett* 2003;**552**:105–109.

Guerrero-Bosagna C, Savenkova M, Haque MM, Nilsson E, Skinner MK. Environmentally induced epigenetic transgenerational inheritance of altered Sertoli cell transcriptome and epigenome: molecular etiology of male infertility. *PLoS One* 2013;**8**:e59922.

Gut P, Verdin E. The nexus of chromatin regulation and intermediary metabolism. *Nature* 2013;**502**:489–498.

Gutierrez-Fisac JL, Guallar-Castillon P, Leon-Munoz LM, Graciani A, Banegas JR, Rodriguez-Artalejo F. Prevalence of general and abdominal obesity in the adult population of Spain, 2008–2010: the ENRICA study. *Obes Rev* 2011;**13**:388–392.

Hamilton JP. Epigenetics: principles and practice. *Dig Dis* 2011;**29**:130–135.

Hart R, Hickey M, Franks S. Definitions, prevalence and symptoms of polycystic ovaries and polycystic ovary syndrome. *Best Pract Res Clin Obstet Gynaecol* 2004; **18**:671–683.

Heard E, Martienssen RA. Transgenerational epigenetic inheritance: myths and mechanisms. *Cell* 2014;**157**:95–109.

Henderson KD, Bernstein L, Henderson B, Kolonel L, Pike MC. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. *Am J Epidemiol* 2008; **167**:1287–1294.

Herceg Z, Vaissiere T. Epigenetic mechanisms and cancer: an interface between the environment and the genome. *Epigenetics* 2011;**6**:804–819.

Heyn H, Esteller M. DNA methylation profiling in the clinic: applications and challenges. *Nat Rev Genet* 2012;**13**:679–692.

Heyn H, Li N, Ferreira HJ, Moran S, Pisano DG, Gomez A, Diez J, Sanchez-Mut JV, Setien F, Carmona FJ *et al.* Distinct DNA methylomes of newborns and centenarians. *Proc Natl Acad Sci USA* 2012;**109**:10522–7.

Hill VK, Hesson LB, Dansranjavin T, Dallol A, Bieche I, Vacher S, Tommasi S, Dobbins T, Gentle D, Euhus D *et al.* Identification of 5 novel genes methylated in breast and other epithelial cancers. *Mol Cancer* 2010;**9**:51.

Hill VK, Ricketts C, Bieche I, Vacher S, Gentle D, Lewis C, Maher ER, Latif F. Genome-wide DNA methylation profiling of CpG islands in breast cancer identifies novel genes associated with tumorigenicity. *Cancer Res* 2011; **71**:2988–2999.

Holliday R. The inheritance of epigenetic defects. *Science* 1987;**238**:163–170.

Howe LR, Subbaramaiah K, Hudis CA, Dannenberg AJ. Molecular pathways: adipose inflammation as a mediator of obesity-associated cancer. *Clin Cancer Res* 2013; **19**:6074–6083.

Huang YW, Luo J, Weng YI, Mutch DG, Goodfellow PJ, Miller DS, Huang TH. Promoter hypermethylation of CIDEA, HAAO and RXFP3 associated with microsatellite instability in endometrial carcinomas. *Gynecol Oncol* 2010;**117**:239–247.

Jensen TK, Scheike T, Keiding N, Schaumburg I, Grandjean P. Fecundability in relation to body mass and menstrual cycle patterns. *Epidemiology* 1999;**10**:422–428.

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

Jones OR, Scheuerlein A, Salguero-Gomez R, Camarda CG, Schaible R, Casper BB, Dahlgren JP, Ehrlen J, Garcia MB, Menges ES *et al.* Diversity of ageing across the tree of life. *Nature* 2014;**505**:169–173.

Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004; **89**:2548–2556.

Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. *Nat Rev Cancer* 2011;**11**:886–895.

Kheradmand A, Dezfoulian O, Alirezaei M, Rasoulian B. Ghrelin modulates testicular germ cells apoptosis and proliferation in adult normal rats. *Biochem Biophys Res Commun* 2012;**419**:299–304.

Kim HH, DiVall SA, Deneau RM, Wolfe A. Insulin regulation of GnRH gene expression through MAP kinase signaling pathways. *Mol Cell Endocrinol* 2005;**242**:42–49.

Kurian JR, Terasawa E. Epigenetic control of gonadotropin releasing hormone neurons. *Front Endocrinol (Lausanne)* 2013;**4**:61.

Kurian JR, Keen KL, Terasawa E. Epigenetic changes coincide with *in vitro* primate GnRH neuronal maturation. *Endocrinology* 2010;**151**:5359–5368.

Lacey JV Jr, Swanson CA, Brinton LA, Altekruse SF, Barnes WA, Gravitt PE, Greenberg MD, Hadjimichael OC, McGowan L, Mortel R *et al.* Obesity as a potential risk factor for adenocarcinomas and squamous cell carcinomas of the uterine cervix. *Cancer* 2003;**98**:814–821.

Lagaly DV, Aad PY, Grado-Ahuir JA, Hulsey LB, Spicer LJ. Role of adiponectin in regulating ovarian theca and granulosa cell function. *Mol Cell Endocrinol* 2008; **284**:38–45.

Lapauw B, Goemaere S, Zmierczak H, Van Pottelbergh I, Mahmoud A, Taes Y, De Bacquer D, Vansteelandt S, Kaufman JM. The decline of serum testosterone levels in community-dwelling men over 70 years of age: descriptive data and predictors of longitudinal changes. *Eur J Endocrinol* 2008;**159**:459–468.

Lerro CC, McGlynn KA, Cook MB. A systematic review and meta-analysis of the relationship between body size and testicular cancer. *Br J Cancer* 2010; **103**:1467–1474.

Li LC. Epigenetics of prostate cancer. *Front Biosci* 2007;**12**:3377–3397.

Li Z, Huang H. Epigenetic abnormality: a possible mechanism underlying the fetal origin of polycystic ovary syndrome. *Med Hypotheses* 2008;**70**:638–642.

Li L, Wu J, Pu D, Zhao Y, Wan C, Sun L, Shen CE, Sun W, Yuan Z, Shen Q *et al*. Factors associated with the age of natural menopause and menopausal symptoms in Chinese women. *Maturitas* 2012;**73**:354–360.

Lim SO, Gu JM, Kim MS, Kim HS, Park YN, Park CK, Cho JW, Park YM, Jung G. Epigenetic changes induced by reactive oxygen species in hepatocellular carcinoma: methylation of the E-cadherin promoter. *Gastroenterology* 2008; **135**:2128–2140, 2140 e1–8.

Lim SS, Norman RJ, Davies MJ, Moran LJ. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. *Obes Rev* 2013;**14**:95–109.

Lomniczi A, Loche A, Castellano JM, Ronnekleiv OK, Bosch M, Kaidar G, Knoll JG, Wright H, Pfeifer GP, Ojeda SR. Epigenetic control of female puberty. *Nat Neurosci* 2013;**16**:281–289.

Lorincz AM, Sukumar S. Molecular links between obesity and breast cancer. *Endocr Relat Cancer* 2006;**13**:279–292.

Luhn P, Dallal CM, Weiss JM, Black A, Huang WY, Lacey JV Jr, Hayes RB, Stanczyk FZ, Wentzensen N, Brinton LA. Circulating adipokine levels and endometrial cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev* 2013;**22**:1304–1312.

Maillard V, Uzbekova S, Guignot F, Perreau C, Rame C, Coyral-Castel S, Dupont J. Effect of adiponectin on bovine granulosa cell steroidogenesis, oocyte maturation and embryo development. *Reprod Biol Endocrinol* 2010;**8**:23.

Makker K, Agarwal A, Sharma R. Oxidative stress & male infertility. *Indian J Med Res* 2009;**129**:357–367.

Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. *PLoS One* 2013;**8**:e55387.

Marti A, Martinez-Gonzalez MA, Martinez JA. Interaction between genes and lifestyle factors on obesity. *Proc Nutr Soc* 2008;**67**:1–8.

Mateescu B, Batista L, Cardon M, Gruosso T, de Feraudy Y, Mariani O, Nicolas A, Meyniel JP, Cottu P, Sastre-Garau X *et al.* miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response. *Nat Med* 2011; **17**:1627–1635.

Milagro FI, Campion J, Cordero P, Goyenechea E, Gomez-Uriz AM, Abete I, Zulet MA, Martinez JA. A dual epigenomic approach for the search of obesity biomarkers: DNA methylation in relation to diet-induced weight loss. *FASEB J* 2011; **25**:1378–1389.

Milagro FI, Gomez-Abellan P, Campion J, Martinez JA, Ordovas JM, Garaulet M. CLOCK, PER2 and BMAL1 DNA methylation: association with obesity and metabolic syndrome characteristics and monounsaturated fat intake. *Chronobiol Int* 2012;**29**:1180–1194.

Milagro FI, Mansego ML, De Miguel C, Martinez JA. Dietary factors, epigenetic modifications and obesity outcomes: progresses and perspectives. *Mol Aspects Med* 2013;**34**:782–812.

Minge CE, Bennett BD, Norman RJ, Robker RL. Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone reverses the adverse effects of diet-induced obesity on oocyte quality. *Endocrinology* 2008;**149**:2646–2656.

Mohr BA, Bhasin S, Link CL, O'Donnell AB, McKinlay JB. The effect of changes in adiposity on testosterone levels in older men: longitudinal results from the Massachusetts Male Aging Study. *Eur J Endocrinol* 2006;**155**:443–452.

Morelli A, Sarchielli E, Comeglio P, Filippi S, Vignozzi L, Marini M, Rastrelli G, Maneschi E, Cellai I, Persani L *et al*. Metabolic syndrome induces inflammation and impairs gonadotropin-releasing hormone neurons in the preoptic area of the hypothalamus in rabbits. *Mol Cell Endocrinol* 2014;**382**:107–119.

Nestler JE, Jakubowicz DJ. Decreases in ovarian cytochrome P450c17 alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. *N Engl J Med* 1996;**335**:617–623.

Nitert MD, Dayeh T, Volkov P, Elgzyri T, Hall E, Nilsson E, Yang BT, Lang S, Parikh H, Wessman Y *et al*. Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. *Diabetes* 2012; **61**:3322–3332.

Ordovas JM, Smith CE. Epigenetics and cardiovascular disease. *Nat Rev Cardiol* 2010; **7**:510–519.

Osborne CK, Bolan G, Monaco ME, Lippman ME. Hormone responsive human breast cancer in long-term tissue culture: effect of insulin. *Proc Natl Acad Sci USA* 1976; **73**:4536–4540.

Pembrey M, Saffery R, Bygren LO. Human transgenerational responses to early-life experience: potential impact on development, health and biomedical research. *J Med Genet* 2014;**51**:563–572.

Pinilla L, Seoane LM, Gonzalez L, Carro E, Aguilar E, Casanueva FF, Dieguez C. Regulation of serum leptin levels by gonadal function in rats. *Eur J Endocrinol* 1999; **140**:468–473.

Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 2008;**8**:915–928.

Portela A, Esteller M. Epigenetic modifications and human disease. *Nat Biotechnol* 2010; **28**:1057–1068.

Qu F, Wang FF, Yin R, Ding GL, El-Prince M, Gao Q, Shi BW, Pan HH, Huang YT, Jin M *et al.* A molecular mechanism underlying ovarian dysfunction of polycystic ovary syndrome: hyperandrogenism induces epigenetic alterations in the granulosa cells. *J Mol Med (Berl)* 2012;**90**:911–923.

Rajender S, Avery K, Agarwal A. Epigenetics, spermatogenesis and male infertility. *Mutat Res* 2010;**727**:62–71.

Rajendran R, Garva R, Krstic-Demonacos M, Demonacos C. Sirtuins: molecular traffic lights in the crossroad of oxidative stress, chromatin remodeling, and transcription. *J Biomed Biotechnol* 2011;**2011**:368276.

Reeves GK, Pirie K, Beral V, Green J, Spencer E, Bull D. Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study. *BMJ* 2007; **335**:1134.

Renehan AG, Frystyk J, Flyvbjerg A. Obesity and cancer risk: the role of the insulin-IGF axis. *Trends Endocrinol Metab* 2006;**17**:328–336.

Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008;**371**:569–578.

Richardson ME, Bleiziffer A, Tuttelmann F, Gromoll J, Wilkinson MF. Epigenetic regulation of the RHOX homeobox gene cluster and its association with human male infertility. *Hum Mol Genet* 2014;**23**:12–23.

Robker RL, Akison LK, Bennett BD, Thrupp PN, Chura LR, Russell DL, Lane M, Norman RJ. Obese women exhibit differences in ovarian metabolites, hormones, and gene expression compared with moderate-weight women. *J Clin Endocrinol Metab* 2009;**94**:1533–1540.

Roca-Rivada A, Alonso J, Al-Massadi O, Castelao C, Peinado JR, Seoane LM, Casanueva FF, Pardo M. Secretome analysis of rat adipose tissues shows location-specific roles for each depot type. *J Proteomics* 2011;**74**:1068–1079.

Rodriguez-Paredes M, Esteller M. Cancer epigenetics reaches mainstream oncology. *Nat Med* 2011;**17**:330–339.

Rodriguez C, Freedland SJ, Deka A, Jacobs EJ, McCullough ML, Patel AV, Thun MJ, Calle EE. Body mass index, weight change, and risk of prostate cancer in the Cancer Prevention Study II Nutrition Cohort. *Cancer Epidemiol Biomarkers Prev* 2007;**16**:63–69.

Ronn T, Volkov P, Davegardh C, Dayeh T, Hall E, Olsson AH, Nilsson E, Tornberg A, Dekker Nitert M, Eriksson KF *et al*. A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. *PLoS Genet* 2013; **9**:e1003572.

Russell SH, Small CJ, Stanley SA, Franks S, Ghatei MA, Bloom SR. The *in vitro* role of tumour necrosis factor-alpha and interleukin-6 in the hypothalamic-pituitary gonadal axis. *J Neuroendocrinol* 2001;**13**:296–301.

Sanchez-Garrido MA, Tena-Sempere M. Metabolic control of puberty: roles of leptin and kisspeptins. *Horm Behav* 2013;**64**:187–194.

Sanchez-Mut JV, Aso E, Panayotis N, Lott I, Dierssen M, Rabano A, Urdinguio RG, Fernandez AF, Astudillo A, Martin-Subero JI *et al*. DNA methylation map of mouse and human brain identifies target genes in Alzheimer's disease. *Brain* 2013; **136**:3018–3027.

Sanchez-Vega F, Gotea V, Petrykowska HM, Margolin G, Krivak TC, Deloia JA, Bell DW, Elnitski L. Recurrent patterns of DNA methylation in the ZNF154, CASP8, and VHL promoters across a wide spectrum of human solid epithelial tumors and cancer cell lines. *Epigenetics* 2013;**8**:1355–1372.

Semaan SJ, Kauffman AS. Emerging concepts on the epigenetic and transcriptional regulation of the Kiss1 gene. *Int J Dev Neurosci* 2013;**31**:452–462.

Sermondade N, Faure C, Fezeu L, Shayeb AG, Bonde JP, Jensen TK, Van Wely M, Cao J, Martini AC, Eskandar M *et al*. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. *Hum Reprod Update* 2013;**19**:221–231.

Shen HR, Qiu LH, Zhang ZQ, Qin YY, Cao C, Di W. Genome-wide methylated DNA immunoprecipitation analysis of patients with polycystic ovary syndrome. *PLoS One* 2013;**8**:e64801.

Simone NL, Soule BP, Ly D, Saleh AD, Savage JE, Degraff W, Cook J, Harris CC, Gius D, Mitchell JB. Ionizing radiation-induced oxidative stress alters miRNA expression. *PLoS One* 2009;**4**:e6377.

Skinner MK, Guerrero-Bosagna C. Role of CpG deserts in the epigenetic transgenerational inheritance of differential DNA methylation regions. *BMC Genomics* 2014;**15**:692.

Skinner MK, Manikkam M, Guerrero-Bosagna C. Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends Endocrinol Metab* 2010; **21**:214–222.

Skinner MK, Manikkam M, Tracey R, Guerrero-Bosagna C, Haque M, Nilsson EE. Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. *BMC Med* 2013;**11**:228.

Taby R, Issa JP. Cancer epigenetics. *CA Cancer J Clin* 2010;**60**:376–392.

Tena-Sempere M, Pinilla L, Gonzalez LC, Dieguez C, Casanueva FF, Aguilar E. Leptin inhibits testosterone secretion from adult rat testis *in vitro*. *J Endocrinol* 1999; **161**:211–218.

Tena-Sempere M, Barreiro ML, Gonzalez LC, Gaytan F, Zhang FP, Caminos JE, Pinilla L, Casanueva FF, Dieguez C, Aguilar E. Novel expression and functional role of ghrelin in rat testis. *Endocrinology* 2002;**143**:717–725.

Tosi F, Negri C, Perrone F, Dorizzi R, Castello R, Bonora E, Moghetti P. Hyperinsulinemia amplifies GnRH agonist stimulated ovarian steroid secretion in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2012; **97**:1712–1719.

Tracey R, Manikkam M, Guerrero-Bosagna C, Skinner MK. Hydrocarbons (jet fuel JP-8) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. *Reprod Toxicol* 2013;**36**:104–116.

Travison TG, Araujo AB, Kupelian V, O'Donnell AB, McKinlay JB. The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. *J Clin Endocrinol Metab* 2007;**92**:549–555.

Trikudanathan S, Pedley A, Massaro JM, Hoffmann U, Seely EW, Murabito JM, Fox CS. Association of female reproductive factors with body composition: the Framingham Heart Study. *J Clin Endocrinol Metab* 2013;**98**:236–244.

Tropea A, Tiberi F, Minici F, Orlando M, Gangale MF, Romani F, Miceli F, Catino S, Mancuso S, Sanguinetti M *et al*. Ghrelin affects the release of luteolytic and luteotropic factors in human luteal cells. *J Clin Endocrinol Metab* 2007; **92**:3239–3245.

Ulmer H, Bjorge T, Concin H, Lukanova A, Manjer J, Hallmans G, Borena W, Haggstrom C, Engeland A, Almquist M *et al*. Metabolic risk factors and cervical cancer in the metabolic syndrome and cancer project (Me-Can). *Gynecol Oncol* 2012;**125**:330–335.

Urdinguio RG, Sanchez-Mut JV, Esteller M. Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. *Lancet Neurol* 2009;**8**:1056–1072.

van der Steeg JW, Steures P, Eijkemans MJ, Habbema JD, Hompes PG, Burggraaff JM, Oosterhuis GJ, Bossuyt PM, van der Veen F, Mol BW. Obesity affects spontaneous pregnancy chances in subfertile, ovulatory women. *Hum Reprod* 2008;**23**:324–328.

van Dorp W, Blijdorp K, Laven JS, Pieters R, Visser JA, van der Lely AJ, Neggers SJ, van den Heuvel-Eibrink MM. Decreased ovarian function is associated with obesity in very long-term female survivors of childhood cancer. *Eur J Endocrinol* 2013; **168**:905–912.

Vendramini V, Cedenho AP, Miraglia SM, Spaine DM. Reproductive function of the male obese Zucker rats: alteration in sperm production and sperm DNA damage. *Reprod Sci* 2014;**21**:221–229.

Viani I, Vottero A, Tassi F, Cremonini G, Sartori C, Bernasconi S, Ferrari B, Ghizzoni L. Ghrelin inhibits steroid biosynthesis by cultured granulosa-lutein cells. *J Clin Endocrinol Metab* 2008;**93**:1476–1481.

Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes (Lond)* 2006;**30**:400–418.

Vincent HK, Innes KE, Vincent KR. Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. *Diabetes Obes Metab* 2007; **9**:813–839.

Wachsman JT. DNA methylation and the association between genetic and epigenetic changes: relation to carcinogenesis. *Mutat Res* 1997;**375**:1–8.

Webb PM. Obesity and gynecologic cancer etiology and survival. *Am Soc Clin Oncol Educ Book* 2013;**33**:e222–e228.

Weichenhan D, Plass C. The evolving epigenome. *Hum Mol Genet* 2013;**22**:R1–R6.

Wu FC, Tajar A, Pye SR, Silman AJ, Finn JD, O'Neill TW, Bartfai G, Casanueva F, Forti G, Giwercman A *et al*. 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.

Wu C, Ding X, Li H, Zhu C, Xiong C. Genome-wide promoter methylation profile of human testis and epididymis: identified from cell-free seminal DNA. *BMC Genomics* 2013;**14**:288.

Xu N, Kwon S, Abbott DH, Geller DH, Dumesic DA, Azziz R, Guo X, Goodarzi MO. Epigenetic mechanism underlying the development of polycystic ovary syndrome (PCOS)-like phenotypes in prenatally androgenized rhesus monkeys. *PLoS One* 2011;**6**:e27286.

Xu X, Su S, Barnes VA, De Miguel C, Pollock J, Ownby D, Shi H, Zhu H, Snieder H, Wang X. A genome-wide methylation study on obesity: differential variability and differential methylation. *Epigenetics* 2013;**8**:522–533.

Yang HP, Wentzensen N, Trabert B, Gierach GL, Felix AS, Gunter MJ, Hollenbeck A, Park Y, Sherman ME, Brinton LA. Endometrial cancer risk factors by 2 main histologic subtypes: the NIH-AARP Diet and Health Study. *Am J Epidemiol* 2012; **177**:142–151.

Zaina S, Heyn H, Carmona FJ, Varol N, Sayols S, Condom E, Ramirez-Ruz J, Gomez A, Goncalves I, Moran S *et al*. A DNA methylation map of human atherosclerosis. *Circ Cardiovasc Genet* 2014;**7**:692–700.

Zou C, Shao J. Role of adipocytokines in obesity-associated insulin resistance. *J Nutr Biochem* 2008;**19**:277–286.