ABSTRACT: Obesity is a risk factor for infertility and adverse reproductive outcomes. Adipose tissue is an important endocrine gland that secretes a host of endocrine factors, called adipokines, which modulate diverse physiologic processes including appetite, metabolism, cardiovascular function, immunity and reproduction. Altered adipokine expression in obese individuals has been implicated in the pathogenesis of a host of health disorders including diabetes and cardiovascular disease. It remains unclear whether adipokines play a significant role in the pathogenesis of adverse reproductive outcomes in obese individuals and, if so, whether the adipokines are acting directly or indirectly on the peripheral reproductive tissues. Many groups have demonstrated that receptors for the adipokines leptin and adiponectin are expressed in peripheral reproductive tissues and that these adipokines are likely, therefore, to exert direct effects on these tissues. Many groups have tested for direct effects of leptin and adiponectin on reproductive tissues including the testis, ovary, uterus, placenta and egg/embryo. The hypothesis that decreased fertility potential or adverse reproductive outcomes may result, at least in part, from defects in adipokine signaling within reproductive tissues has also been tested. Here, we present a critical analysis of published studies with respect to two adipokines, leptin and adiponectin, for which significant data have been generated. Our evaluation reveals significant inconsistencies and methodological limitations regarding the direct effects of these adipokines on peripheral reproductive tissues. We also observe a pervasive failure to account for in vivo data that challenge observations made in vitro. Overall, while leptin and adiponectin may directly modulate peripheral reproductive tissues, existing data suggest that these effects are minor and non-essential to human or mouse reproductive function. Current evidence suggests that direct effects of leptin or adiponectin on peripheral reproductive tissues are unlikely to factor significantly in the adverse reproductive outcomes observed in obese individuals.

Key words: leptin / adiponectin / reproduction / obesity / adipokine

Altered caloric intake during gestation impairs the health of offspring

Throughout the course of human history our survival has depended upon satisfactory harvesting of food to avoid starvation. The long-term effects of caloric restriction, including adverse health effects on offspring conceived and gestated during a time of maternal starvation, have been studied. Though not the focus of this review, caloric restriction in pregnant humans is well recognized to contribute to adult obesity and associated diseases in the offspring (Tamashiro and Moran, 2010). Babies born small for gestational age secondary to maternal caloric restriction were found, as adults, to have increased incidence of obesity, diabetes and hypertension (Hales and Barker, 1992; Painter et al., 2005).

Effects of gestational caloric restriction on adult disease: animal data

Interestingly, caloric restriction in mice has been observed to enhance ovarian oocyte counts and to prolong female reproductive function when compared with mice that are not calorie-restricted (Selesniemi et al., 2008). Predictably, caloric restriction in mice leads to a wide array of endocrine changes in virtually all endocrine organs including hypothalamus, pituitary, thyroid, adrenal, liver, pancreas, gut, gonads and adipose tissue (Martin et al., 2008). Given the widespread endocrine changes associated with caloric restriction, it is not surprising that the mechanisms by which caloric restriction preserves or replenishes oocytes in mice are not known. Ultimately, calorie-restricted mice (and
humans) demonstrate suppression of the hypothalamic-pituitary axis leading to anovulation. It is not known whether the hypogonadotropic-hypogonadism in calorie-restricted mice plays a role in preserving ovarian reserve. Whether caloric restriction prolongs human reproductive potential remains unknown.

Notably, the ovulatory defects observed in calorie-restricted mice and sheep can be corrected with central administration of leptin, arguing that direct ovarian effects of leptin are not required for correction of the hypothalamic–pituitary–gonadal axis (Nagatani et al., 1998; Henry et al., 2001). So, although leptin administration has been shown to regulate the hypothalamic–pituitary–gonadal axis and pubertal development, a role for leptin in directly regulating peripheral reproductive organs remains to be established (Donato et al., 2011; Bellefontaine et al., 2014).

Adverse health effects of neonatal caloric excess

Mouse studies suggest that the early neonatal nutritional environment also affects hypothalamic programming, acting at the level of the arcuate nucleus. Leptin appears to play a neurotrophic role during the critical neonatal period of hypothalamic development and neonates of diet-induced obese rats have been shown to display abnormal organization of hypothalamic pathways that modulate neuronal responses to leptin (Bouret et al., 2004, 2008). Further, both over- and under- nutrition of mouse pups during lactation is associated with perturbations in hypothalamic development, which can impair reproductive function in later adulthood (Castellano et al., 2011; Caron et al., 2012).

Adverse health and reproductive outcomes associated with obesity

In most of the developed and developing world, the problem of maternal over-nutrition has surpassed caloric restriction as a risk factor for metabolic diseases in affected offspring. Paradoxically, as observed for starved babies with stunted intrauterine growth, babies born from obese mothers are also predisposed to the development of obesity and associated health disorders (Guo and Jen, 1995; Ghosh et al., 2001; Khan et al., 2003; Armitage et al., 2004; Boney et al., 2005). Mechanistic studies regarding these observations are in their infancy. We do not fully understand which factors, acting on what tissues/cells, and at what time(s) during human development (i.e. during gametogenesis, antenatal, or post-natal periods), are most responsible for gestational programming of adult disease. Current data indicate neuroendocrine re-programming of the hypothalamus and other central pathways that regulate appetite and metabolism, as well as peripheral (i.e. adipocyte) reprogramming, possibly involving leptin, insulin, and glucocorticoid pathways that are perturbed during fetal development (Tamashiro et al., 2009; Spencer, 2012; Correa-Branco et al., 2015).

The prevalence of obesity, both nationally and globally, has increased dramatically over the past three decades (Stevens et al., 2012). Currently, approximately one-third of adults and 17% of children in the USA are obese (defined as body mass index >30 kg/m²) (Ogden et al., 2013, 2014). Obesity increases the risk of important health problems including hypertension, type 2 diabetes, atherosclerosis, some cancers and all-cause mortality (Grundy, 2004a, b; Burke et al., 2008; Prospective Studies Collaboration et al., 2009; Gonzalez and Riboli, 2010; Danaei et al., 2011; Zheng et al., 2011; Flegal et al., 2013; Landsberg et al., 2013). In addition, obesity is a risk factor for a wide array of gestational and perinatal complications that include gestational diabetes, gestational hypertension, pre-eclampsia, thromboembolism, antepartum hospitalization, Cesarean delivery, instrumental delivery, preterm delivery, shoulder dystocia, post-partum re-hospitalization, large-for-gestational age, isolated and combined congenital anomalies, miscarriage, intrauterine fetal demise, neonatal death, and neonatal admission to the neonatal intensive care unit (Cnattingius and Stephansson, 2002; Myles et al., 2002; Weiss et al., 2004; Cnattingius et al., 2005, 2012, 2013; Nohr et al., 2007; Stothard et al., 2009; Blomberg and Kallen, 2010; McDonald et al., 2010; Pandey et al., 2010; Rankin et al., 2010; Liston and Davies, 2011; Pinborg et al., 2011; Sohlberg et al., 2012; El-Chaar et al., 2013). The pathogenesis underlying many of the adverse reproductive outcomes in obese individuals remains incompletely understood.

Adverse effects of obesity on male reproductive function

In men, there is a strong relationship between increasing BMI and reduced testosterone, sex hormone binding globulin, and free testosterone levels as well as elevated estradiol levels (Du Plessis et al., 2010; MacDonald et al., 2010; Palmer et al., 2012). A slim majority of studies suggests that obesity is associated with reduced sperm concentrations, but this is an inconsistent association (MacDonald et al., 2010; Sermon-dade et al., 2013). There is no consensus as to whether obesity impairs sperm motility or morphology (Palmer et al., 2012). Further, it remains controversial whether male obesity is a risk factor for infertility or for reduced success rates in couples pursuing assisted reproductive technologies (Du Plessis et al., 2010; Palmer et al., 2012).

Adverse effects of obesity on female reproductive function

There is emerging evidence that obesity contributes to female infertility and to reduced success with assisted reproductive technologies (Mulders et al., 2003a, b; Maheshwari et al., 2007). While study results have been inconsistent and study designs heterogeneous, female obesity has been associated with 25–50% reductions in pregnancy rates after in vitro fertilization (IVF) (Styne-Gross et al., 2005; Bellver et al., 2007, 2010; Robker, 2008; Robker et al., 2009; Brewer and Balen, 2010; Luke et al., 2011a, b). For patients undergoing IVF, excess weight (particularly morbid obesity) is associated with increased gonadotrophin requirements, higher cancelation rates, decreased follicle development and oocyte numbers, lower mean embryo grades, fewer embryos to cryopreserve, lower implantation rates, and lower live birth rates (Maheshwari et al., 2007; Metwally et al., 2007, 2008; Bellver et al., 2010; Petersen et al., 2013). Similar associations have been demonstrated in anovulatory obese women undergoing ovulation induction with gonadotrophins, wherein obesity is associated with elevated rates of cycle cancelation, reduced response to gonadotrophins, and lower rates of ovulation and pregnancy (Mulders et al., 2003a). The detrimental effects of obesity on IVF success rates have been attributed to problems of oocyte/embryo quality (i.e. the ‘seed’) (Robker, 2008; Igosheva et al., 2010; Wu et al., 2010; Luke et al., 2011b; Poston et al., 2011; Junghem et al., 2013) as well as problems of endometrial...
function (i.e. the ‘soil’) (Bellver et al., 2007, 2010, 2011; Minge et al., 2008). The underlying mechanisms by which obesity impairs ovarian/embryonic/endometrial function remain incompletely understood. Importantly, the majority of obese men and women are of normal fertility potential and experience unremarkable pregnancies and deliveries. Based upon this observation, obesity appears to be a modifier of human fertility and reproductive outcomes but is not universally associated with adverse outcomes.

Fetal programming of adult diseases

Most concerning is the emerging evidence (from animal and human data) that maternal and/or paternal obesity may ‘program’ offspring for increased risk of adult disorders including obesity, diabetes and atherosclerosis, while also reducing the reproductive potential of these offspring (Eriksson et al., 2001; Barker, 2005, 2007; Huang et al., 2007; Samuelsson et al., 2008; Muhlhauser and Smith, 2009; Drake and Reynolds, 2010; Ng et al., 2010; Aceti et al., 2012; Cnattingsius et al., 2012; Fernandez et al., 2012; Kim et al., 2012; Barker and Thornburg, 2013a, b; Taylor et al., 2014). If confirmed, these findings would indicate long-term and inter-generational health and reproductive consequences stemming from the increased prevalence of obesity. Whether these risks are reversed after weight loss remains unknown and cannot be assumed (Grayson et al., 2013).

Studies in animal model systems have begun to shed light on mechanisms by which obesity impairs reproductive function. Several strains of mice are susceptible to diet-induced obesity (DIO) and obese mice generate blastocysts with reduced survival rates and abnormal embryonic cellular differentiation when compared with lean mice (Igosheva et al., 2010; Junghem and Moyle, 2010; Junghem et al., 2010; Luzzo et al., 2012). Additionally, embryos from diet-induced obese mice display evidence of oxidative stress with increased generation of reactive oxygen species (ROS) in vivo and subsequent failure to support blastocyst formation (Igosheva et al., 2010; Poston et al., 2011). Collectively, these studies have implicated endoplasmic reticulum stress, mitochondrial dysfunction, lipotoxicity, and insulin resistance as mediators of reproductive dysfunction in rodents and possibly in obese women (Minge et al., 2008; Wu et al., 2010; Junghem et al., 2011).

Are there direct effects of adipokines on the peripheral reproductive tissues?

Direct effects of adipokines on the peripheral reproductive tissues have not been shown to contribute to the pathogenesis of reproductive dysfunction in obesity. That said, numerous studies have demonstrated expression of adipokine receptors, either at the mRNA or protein levels, in each of the reproductive tissues. Additional work, generally in vitro, has indicated adipokine responsiveness in peripheral reproductive tissues. These studies, though sometimes inconsistent and/or incomplete, have sought to bolster the hypothesis that adipokines exert direct effects on the peripheral reproductive tissues and that alterations of adipokine expression in obesity might contribute to the adverse reproductive outcomes in this population. Below, we provide a critical appraisal of existing data regarding the direct effects of two adipokines on peripheral reproductive tissues (summarized in Table 1).

The altered physiology of obesity: systemic adipokine effects

In addition to storing energy in the form of lipids, adipose tissue functions as an endocrine organ that helps to regulate appetite, metabolism and the hypothalamic–pituitary–gonadal (HPG) axis (Weisberg et al., 2003; Xu et al., 2003; Ahima et al., 2006; Halberg et al., 2008). The HPG endocrine axis is, in turn, essential for pubertal development and is the central regulator of reproductive processes in both sexes (Ouchi et al., 2011; Tena-Sempere, 2012). Adipose tissue is composed of lipid-laden adipocytes, fibroblast-like pre-adipocytes, and stromavascular cells including fibroblasts, vascular endothelial cells and immune cells (principally macrophages and various T cell populations, but also NK cells, neutrophils, mast cells, and B cells). Ultimately, obesity leads to increased adipose tissue infiltration with M1 macrophages and increased inflammatory activation of these cells (Ferrante, 2007; Odegaard and Chawla, 2008, 2011). Collectively, the adipose depot secretes adipokines which exert local and systemic effects and which contribute to a chronic, low grade, inflammatory state (Weisberg et al., 2003; Xu et al., 2003; Grundy, 2004a, b). Obese individuals demonstrate local and systemic increases of most adipokines including tumor necrosis factor alpha (TNFα), monocyte chemoattractant protein-1 (MCP-1), leptin, retinol binding protein-4 (RBP4), interleukin-6 (IL-6), visfatin, resistin, and plasminogen activator inhibitor-1 (PAI-1) (Ouchi et al., 2011; Michalakis et al., 2013; Navarro and Kaiser, 2013). Obesity is associated with reduced expression of the anti-inflammatory proteins adiponectin and secreted frizzled-related protein 5 (Pajvani et al., 2003; Tilg and Wolf, 2005).

In addition to generating the classical adipokines, adipose tissue generates and/or metabolizes autocrine/endocrine factors including growth factors, angiopoietins, steroid hormones, prostaglandins, and retinoids (Trayhurn and Wood, 2007; Waki and Tontonoz, 2007). In aggregate, these adipose tissue-derived factors have been implicated in the regulation of appetite, energy balance, immunity, angiogenesis, blood pressure, lipid metabolism, hemostasis and insulin sensitivity; altered expression of these factors in obese individuals has been implicated in the pathogenesis of diabetes, hypertension and atherosclerosis (Grundy, 2004a; b; Ouchi et al., 2011). In mouse models of obesity, for example, the genetic or pharmacologic inhibition of TNFα, iNos, MCP-1, or PAI-1 activities each attenuates obesity-induced insulin resistance (Uysal et al., 1997; Ruan and Lodish, 2004; Kamei et al., 2006; Kanda et al., 2006; Ferrante, 2007; Tamura et al., 2008). This review will consider the evidence supporting direct effects of adipokines on peripheral reproductive organs, namely the ovary or testis, the uterus, the embryo, and the placenta. The central nervous system effects of adipokines, which are essential for normal reproductive and metabolic function, have been reviewed elsewhere and will not be addressed in this review (Ahima, 2005; Michalakis et al., 2013; Navarro and Kaiser, 2013).

We recognize that established effects of obesity on reproductive organs are incompletely understood, are complex, and may include direct effects of lipids, oxidative stress, insulin/glucose metabolism, hypoxia, and altered tissue temperature (related to altered distribution of fat). Adipokines might modulate reproductive processes indirectly,
### Table I: Summary of the proposed direct effects of leptin and adiponectin on reproductive tissues; critical analysis of the supporting data (see text for details).

<table>
<thead>
<tr>
<th>Adiponectin and its receptors</th>
<th>General considerations: Adiponectin null mice are viable and exhibit normal fertility. In most studies, genetic deletion of the receptors for adiponectin (AdipoR1, AdipoR2, or both) is not associated with subfertility. The observation that adiponectin null male and female mice can produce viable adiponectin null offspring indicates that the ovary, testis, uterus, placenta, and embryo can each function in the absence of adiponectin signaling. Maternal adiponectin levels are inversely correlated with birthweight though causation and mechanism underlying this observation remain to be established. Plasma levels of adiponectin generally range from 5 to 20 μg/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed targets</td>
<td>Proposed direct effects</td>
</tr>
<tr>
<td>Oocyte/embryo</td>
<td>Adiponectin receptor expression changes with follicle development and oocyte growth, adiponectin enhances egg maturation and ovulation, adiponectin and its receptors are expressed in preimplantation embryos, possible role in implantation, elevated levels in mid-secretory phase, chronic infusion of adiponectin in pregnant mice produced intrauterine growth restriction</td>
</tr>
<tr>
<td>Endometrium</td>
<td>Adiponectin and its receptors are expressed in human endometrium and transcript levels appear to peak during the mid-luteal phase, possible role in implantation</td>
</tr>
<tr>
<td>Testis</td>
<td>Adiponectin treatment of testicular tissue/cells had variable effects on testosterone production, AdipoR2 null mice demonstrated atrophic seminiferous tubules with aspermia (lack of semen) and enlarged brains, but displayed normal testosterone levels</td>
</tr>
<tr>
<td>Placenta</td>
<td>Placenta principally expresses AdipoR2 but receptor expression may depend upon gestational age; treatment of placental cells/cell lines with adiponectin altered cytokine and steroid hormone synthesis, inhibited proliferation, impaired insulin signaling, and stimulated trophoblast invasion in vitro</td>
</tr>
</tbody>
</table>

### Most relevant references

- Ledoux et al. (2006), Chabrolle et al. (2007a, b), Lagaly et al. (2008), Chabrolle et al. (2009), Pierre et al. (2009) and Maillard et al. (2010)
- Campos et al. (2008), Maillard et al. (2010), Palin et al. (2012) and Richards et al. (2012)
- Takemura et al. (2006)
- Bursell et al. (2007), Caminos et al. (2008) and Martin (2014)
- Wang et al. (2010) and Rosario et al. (2012)
<table>
<thead>
<tr>
<th>Proposed targets</th>
<th>Proposed direct effects</th>
<th>Problems and inconsistencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theca/granulosa</td>
<td>Leptin is produced in the ovary and regulates cellular proliferation and steroidogenesis</td>
<td>Leptin null and leptin receptor null ovaries are fully functional and generate offspring when transplanted into wild-type recipient mice, leptin null mice are fertile when stimulated with gonadotrophins, many studies with inconsistent demonstration of receptor expression at the protein level, highly inconsistent effects of ligand with respect to steroidogenesis depending upon the model system being studied, the physiologic significance of modest effects on steroidogenesis remains speculative, in most clinical studies follicular fluid leptin levels parallel serum levels (suggesting negligible local production of ligand) and levels do not correlate with fertility outcomes after in vitro fertilization. Hummel (1957), Hummel et al. (1966), Batt (1972), Swerdloff et al. (1976), Swerdloff et al. (1978), Chehab et al. (1996), Cioffi et al. (1997), Chehab et al. (2002), Pineda et al. (2010) and Zhang et al. (2012)</td>
</tr>
<tr>
<td>Oocyte/embryo</td>
<td>Leptin variably enhances or inhibits oocyte/embryo maturation and development</td>
<td>Leptin receptor null and leptin null eggs can generate normal pregnancies and offspring, highly inconsistent reports regarding effects of leptin on egg/embryo maturation in vitro. Karlsson et al. (1997), Zachow and Magoffin (1997), Agarwal et al. (1999), Zachow et al. (1999), Duggal et al. (2000), Spicer et al. (2000), Ghizzoni et al. (2001), Duggal et al. (2002a, b), Sirotkin et al. (2005), Ricci et al. (2006), Nicklin et al. (2007) and Karamouti et al. (2008) de Luca et al. (2005)</td>
</tr>
<tr>
<td>Endometrium</td>
<td>Leptin is expressed in the endometrium and variably enhances or impairs embryo implantation</td>
<td>Leptin receptor null mice can have normal pregnancies and deliveries once hormonally induced to conceive, one human with defective/absent leptin receptors has had a child. A paucity of evidence supporting local leptin production. Masuzaki et al. (1997), Mounzih et al. (1998), Malik et al. (2001), Ramos et al. (2005), Farooqi et al. (2007), Magarinos et al. (2007), Perez-Perez et al. (2009) and Dubern and Clement (2012)</td>
</tr>
<tr>
<td>Testis</td>
<td>Leptin modulates hCG-stimulated androgen production</td>
<td>Conflicting reports regarding Ob-Rb receptor protein expression in testis, inconsistent dose effects in rodent studies of steroidogenesis, modest effect sizes. Fei et al. (1997), Caprio et al. (1999), Tena-Sempere et al. (1999) and Herrid et al. (2008a, b)</td>
</tr>
<tr>
<td>Placenta</td>
<td>Leptin modulates trophoblast proliferation and invasion in vitro</td>
<td>Studies with leptin null mating pairs indicate that embryonic, placental, and maternal leptin signaling are dispensable for reproduction after ovulation has been induced, leptin receptor null and leptin null eggs can generate normal pregnancies/placentae and offspring, leptin null human fetuses have unremarkable placentation, gestations and deliveries. Masuzaki et al. (1997), Mounzih et al. (1998), Malik et al. (2001), Ramos et al. (2005), Farooqi et al. (2007), Magarinos et al. (2007), Perez-Perez et al. (2009) and Dubern and Clement (2012)</td>
</tr>
</tbody>
</table>
by modulating some or all of these processes (i.e. glucose metabolism). Further, adipokines could directly affect peripheral reproductive tissues or modulate the functions of resident immune cells or vascular endothelial cells. This review focuses on data supporting (or rejecting) the hypothesis that obesity-associated changes in local and systemic adipokine levels directly impact the function of peripheral reproductive tissues and negatively impact reproductive outcomes in obese individuals. We conclude that two signature adipokines, leptin and adiponectin, do not significantly contribute to the physiology of reproduction via direct effects on the peripheral reproductive tissues and are unlikely to contribute to adverse reproductive outcomes in obese individuals via direct effects on these target tissues.

**Adiponectin: overview**

Adiponectin is the most abundantly secreted protein from white adipose tissue and is a recently described ‘beneficial’ adipokine whose levels are reduced in obesity. Adiponectin is detected in serum as a low molecular weight (trimeric) complex and as higher molecular weight (hexameric and multimeric) complexes, each with variable biological activities depending upon the target tissue (Arita et al., 1999; Yang et al., 2001; Pajvani et al., 2003). Females have 2–3 fold higher circulating levels of adiponectin than males (Pajvani et al., 2003). Adiponectin improves insulin sensitivity, inhibits hepatic gluconeogenesis, and inhibits vascular inflammation (Berg et al., 2001; Combs et al., 2001; Yamauchi et al., 2001, 2002; Stefan and Stumvoll, 2002; Berg and Scherer, 2005; Awazawa et al., 2011; Okamoto, 2011; Okamoto et al., 2013; Yamauchi and Kadowaki, 2013; Comninos et al., 2014). Adiponectin levels are lower in obese individuals but increase with weight loss (Arita et al., 1999; Yang et al., 2001). Circulating adiponectin levels are inversely correlated with hyperinsulinemia and insulin resistance (Stefan and Stumvoll, 2002).

**Adiponectin receptors in peripheral reproductive tissues**

Adiponectin exerts its actions mainly through two receptors, AdipoR1 and AdipoR2, as well as possibly via the T-cadherin receptor (Hug et al., 2004; Yamauchi et al., 2007). Adiponectin has been shown to exert anti-inflammatory effects in macrophages and adipocytes by inhibiting nuclear factor kappa B (NFκB) transcriptional responses (Ajuwon and Spurlock, 2005). In contrast, adiponectin appears to exert pro-inflammatory responses in synovial joints and gut epithelial cells (Fayad et al., 2007). In addition to cell type-specific responses, the dose and form of adiponectin exposure (monomeric versus multimeric, full length versus globular) may influence its net effects (Yamauchi et al., 2003; Hug et al., 2004). Whether adiponectin has pro- or anti-inflammatory effects on peripheral reproductive tissues remains to be established.

One or both of the adiponectin receptors is present in all peripheral reproductive tissues including the ovaries, oviduct, endometrium, testes, and placenta (Caminos et al., 2005; Michalakis and Segars, 2010). Adiponectin’s role in peripheral reproductive tissues remains unclear. Adiponectin null mice are viable and, when fertility outcomes are mentioned, appear to exhibit normal fertility (Kubota et al., 2002; Ma et al., 2002; Maeda et al., 2002; Nawrocki et al., 2006; Qiao et al., 2012). Likewise, genetic deletion of the receptors for adiponectin (Adipor1, Adipor2, or both) was not associated with subfertility in one study (Yamauchi et al., 2007) but loss of AdipoR2 was associated with male subfertility in another study (Lindgren et al., 2013). The fact that adiponectin null male and female mice can produce viable adiponectin null offspring indicates that the ovary, testis, uterus, placenta, and embryo can each function in the absence of adiponectin signaling (Qiao et al., 2012). Together, these data support the conclusion that adiponectin signaling is non-essential to normal mouse reproduction. That said, lack of maternal or fetal adiponectin expression did produce a host of metabolic changes in the offspring indicating non-redundant functions of this hormone with respect to mouse metabolism (Qiao et al., 2012). Maternal adiponectin does not cross the placenta so any effects of maternal adiponectin on fetal development are likely to be indirect.

Because circulating adiponectin levels are naturally quite high (5–20 μg/ml) it has been difficult to genetically engineer mice that systematically over-express the hormone. Meanwhile, female transgenic mice engineered to over-express a mutated adiponectin (lacking 13 Gly-X-Y repeats in the collagenous domain of the protein) were infertile, suggesting a modulatory role for the protein with respect to reproduction (Combs et al., 2004). The mechanisms underlying this infertility remain largely unexplored and ‘off-target’ effects of the over-expressed and mutated protein cannot be excluded. The adiponectin over-expressing mice were obese and demonstrated elevated levels of prolactin; their infertility may thus have resulted from central and/or peripheral effects of adiponectin (Combs et al., 2004).

**Adiponectin effects on ovarian function**

Evidence supporting a peripheral response to adiponectin includes expression of the hormone and/or its receptors in ovarian granulosa and theca cells of chickens, pigs, cows, rats, and humans. Here adiponectin appears to modulate granulosa cell and theca cell steroidogenesis albeit with inconsistent findings (i.e. variably enhancing, suppressing, or neutral with respect to steroidogenesis, depending upon the model systems being tested) (Ledoux et al., 2006; Chabrolle et al., 2007a, b, 2009; Lagaly et al., 2008; Pierre et al., 2009; Maillard et al., 2010). Human cumulus granulosa cells express AdipoR1 and AdipoR2 proteins, but little if any adiponectin (Richards et al., 2012). AdipoR1 and AdipoR2 expression appear to be enhanced by hCG in rat granulosa cells (Chabrolle et al., 2007b). In gonadotrophin-stimulated cycles in humans, LH was shown to enhance follicular fluid concentrations of adiponectin (Gutman et al., 2009). In aggregate, these data indicate potential endocrine, paracrine, and autocrine roles for adiponectin in modulating ovarian function. That said, given the broad inconsistencies in the literature (complicated by diverse model species, cell systems, culture milieus (i.e. co-stimulation with luteinizing hormone or insulin), and variable adiponectin source/dosage/time course), the net effects of adiponectin on ovarian cell physiology remain controversial and of uncertain clinical significance. Recall that adiponectin signaling is not essential for mouse reproduction (Kubota et al., 2002; Ma et al., 2002; Maeda et al., 2002; Nawrocki et al., 2006; Qiao et al., 2012).

Hypoadiponectinemia has been suggested to play a role in the pathogenesis of the Polycystic Ovary Syndrome (PCOS) with some authors suggesting disordered ovarian response to adiponectin in affected patients (Sieminska et al., 2004; Escobar-Morreale et al., 2006; Campos et al., 2012).
Leptin and adiponectin in reproductive tissues

2008; Toulis et al., 2009). To date, experimental data supporting a direct role for adiponectin on ovarian function have been limited to animal and in vitro studies. Whether adiponectin represents an important and direct regulator of ovarian function in lean or obese individuals remains to be established.

While AdipoR1 and AdipoR2 have been detected in primary follicles and antral follicles, treatment of oocyte-cumulus complexes with adiponectin in vitro did not affect embryo development in bovine studies (Maillard et al., 2010; Palin et al., 2012) but enhanced embryonic development in mouse studies (Richards et al., 2012). A role for adiponectin in modulating early oocyte/embryo development remains incompletely studied.

Adiponectin effects on uterine function

Adiponectin and its receptors are expressed in human endometrium and transcript levels appear to peak during the mid-luteal phase (Takemura et al., 2006). Human endometrial stromal or epithelial cells treated with supraphysiologic (50 μg/ml) dosages of adiponectin demonstrated altered phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and reduced production of Interleukin-6, Interleukin-8, and Monocyte chemotactic protein-1 (MCP1) in response to Interleukin-1β co-stimulation (Takemura et al., 2006). Further investigation is needed to determine the role(s) of adiponectin signaling on endometrial function in health and disease.

Adiponectin effects on placental function

While initial reports indicated adiponectin expression in human placenta (Caminos et al., 2005; Chen et al., 2006), recent studies suggest that the human placenta does not express significant quantities of adiponectin, expresses transcripts for AdipoR1 and AdipoR2, but principally expresses AdipoR2 at the protein level (Corbetta et al., 2005; McDonald and Wolfe, 2009; Tie et al., 2009). Fetal adiponectin is expressed in muscle and gut, and is detectable in fetal umbilical cord serum by 24 weeks of gestation (Wang et al., 2010). Treatment of placental cytotrophoblasts with adiponectin modulated steroidogenic and cytokine gene expression (Lappas et al., 2005; McDonald and Wolfe, 2009). Further, chronic infusion of adiponectin in pregnant mice produced intrauterine growth restriction and neonatal umbilical cord levels were inversely correlated with birthweight in humans (Wang et al., 2010; Rosario et al., 2012). Whether, and by what mechanism, the inverse association between gestational exposure to adiponectin and intrauterine growth plays a role in the pathogenesis of macrosomia or fetal growth restriction in humans remains to be established.

Adiponectin effects on testicular function

Adiponectin receptors are expressed in human testes including the Leydig cells but also in the epididymis, and spermatozoa, and the ligand may be expressed in Leydig cells (Caminos et al., 2008; Martin, 2014). Adiponectin treatment of rat testicular tissue reduced testosterone production but did not modulate anti-Mullerian hormone (AMH) transcript levels (Caminos et al., 2008). AdipoR2 null mice demonstrated atrophic seminiferous tubules with aspermatia (lack of semen) and enlarged brains, but displayed normal testosterone levels; whether these testicular defects reflect central or peripheral responses to the loss of AdipoR2 signaling remains unknown (Bjursell et al., 2007). The significance of these effects with respect to male fertility in lean or obese individuals remains unclear. Thus, while adiponectin signaling appears to be present in male gonadal tissue, the extent to which this signaling contributes to normal testicular function and fertility potential in lean or obese individuals, remains unclear.

Adiponectin: summary of effects on peripheral reproductive tissues

In summary, there is agreement that one or both of the receptors for adiponectin is expressed at the protein level in human endometrium, ovary, testis and placenta. Whether via autocrine, paracrine, or endocrine pathways, it is also certain that these tissues are exposed to adiponectin ligand. Far less is understood regarding adiponectin signaling in the function of the reproductive organs or the feto-placental unit in lean or obese individuals. Mouse data suggest that there is no requirement for adiponectin signaling in normal reproduction although excessive exposure to adiponectin may impair reproduction in female mice (Table I). Emerging data suggest a role for adiponectin in modulating peripheral reproductive tissues and reproductive outcomes, most notably affecting fetal growth during gestation (Qiao et al., 2012). Understanding the specific effects of adiponectin, whether insulin sensitizing or anti-inflammatory, on peripheral reproductive tissues will require additional investigation. The extent to which altered adiponectin tone directly modulates peripheral reproductive/embryonic tissues or contributes to adverse reproductive performance in obesity (or after caloric restriction) remains unclear.

Leptin: overview

Leptin is the 167 amino acid protein encoded by the LEP (a.k.a. OB) gene. Leptin regulates food intake, energy expenditure, fat storage, immune function and insulin signaling (Ahima et al., 2006). Leptin is expressed principally in adipocytes and, to a lesser extent, in the intestines, skeletal muscle, placental syncytiotrophoblasts, and possibly the ovary (Hassink et al., 1997; Masuzaki et al., 1997; Perez-Perez et al., 2009). Peripheral leptin levels correlate with fat mass and body mass index (BMI); levels range from means of 7.5 ng/ml in lean individuals to 31.3 ng/ml in obese individuals (Considine et al., 1996; Carlsson et al., 1997). Leptin receptors are present centrally in several hypothalamic nuclei (including the arcuate nucleus) where leptin plays essential roles in regulating appetite, metabolism, pubertal development and gonadotrophin secretion (Tartaglia et al., 1995; Chehab et al., 2002).

The reproductive phenotype of leptin null mice

Leptin null and leptin receptor null mice exhibit low gonadotrophin concentrations, immature reproductive organs, and impaired sexual
maturation which is restored in leptin null mice with exogenous administration of leptin (Hummel, 1957; Batt, 1972; Swerdloff et al., 1976, 1978; Mounzih et al., 1997; Chehab et al., 2002). Leptin and leptin receptor gene defects are rare causes of human obesity and cannot explain delayed puberty, oligo-anovulation, or subfertility in most human populations (Dubern and Clement, 2012).

When leptin deficient male mice are diet-restricted (i.e. lean) some remain fertile, in contrast to lean leptin deficient females which are uniformly infertile (Lane and Dickie, 1954; Swerdloff et al., 1976). We can therefore conclude that leptin signaling, both central and peripheral, is not strictly required for male mouse reproductive function.

**Peripheral leptin is not required for ovarian function or establishment of pregnancy**

Once ovulatory dysfunction is overcome in obese or lean leptin null female mice (i.e. via LH+FSH stimulation of leptin null females), normal pregnancy and parturition can proceed (Lane, 1959; Batt, 1972; Yokoyama et al., 1995; Chehab et al., 1996, 2002). Likewise, early studies revealed that leptin null and leptin receptor null ovaries are fully functional when transplanted into wild-type recipient mice, demonstrating that intraovarian leptin production and signaling is dispensable for female mouse reproduction and that ovaries devoid of leptin signaling respond normally to gonadotrophins (Hummel, 1957; Hummel et al., 1966; Batt, 1972; Swerdloff et al., 1976, 1978; Chehab et al., 1996, 2002; Pineda et al., 2010; Zhang et al., 2012) (Table I).

Elegant studies have formally tested pregnancy outcomes in which no embryonic, placental, or maternal leptin signaling is present after conception: leptin null females and males were mated (while replete with exogenous leptin), followed by early withdrawal of exogenous leptin treatments. Absence of leptin did not impair reproductive success (though the pregnant mice became more obese), indicating that neither fat-derived nor placenta-derived leptin is required to sustain mouse embryonic development once embryo implantation has occurred (Mounzih et al., 1998). These observations contrast with reports suggesting that leptin is required for embryo implantation but not thereafter (Malik et al., 2001; Ramos et al., 2005). Some of the reported responses to leptin treatment could represent off target effects (i.e. Leukemia Inhibitory Factor receptor effects) of the leptin receptor blocking peptides employed; studies for which detailed molecular analyses including proof of specificity were not presented (Gonzalez and Leavis, 2003; Ramos et al., 2005).

Meanwhile, compelling human and mouse data demonstrate convincingly that peripheral leptin signaling is not required for embryo implantation. Recall that the db/db mouse, which is incapable of transmitting leptin signaling, is developmentally normal in utero and after birth, indicating that placental/embryonic leptin signaling is not required for normal placentaion or development in mice (though it is essential for body weight management and pubertal development). When leptin receptor signaling was restored only in the neurons of leptin receptor deficient mice (db/db), fertility was restored: these data indicate that peripheral leptin receptor signaling (i.e. ovarian, uterine) is not essential to mouse reproduction (de Luca et al., 2005). Likewise, ablation of agouti-related protein expressing neurons, or of global neuropeptide Y expression, or of global neuropeptide Y4 receptor expression, each partially restored fertility in leptin null mice, indicating that peripheral leptin signaling is unlikely to be required for fertility in male or female mice (Erickson et al., 1996; Sainsbury et al., 2002; Wu et al., 2012).

**Leptin acts centrally to modulate reproduction: leptin deficient humans**

Humans that are leptin or leptin receptor deficient demonstrate the expected metabolic and endocrine defects, as well as T cell immune defects, but reveal otherwise normal gestational and post-natal development (Farooqi et al., 2007; Dubern and Clement, 2012). A handful of leptin receptor mutations/truncations have now been described in humans, each of which demonstrates delayed puberty and clinical hypogonadism of variable severity (Farooqi et al., 2007). Together, these human data indicate that placental leptin production and placental leptin signaling cascades are not essential for normal establishment and progression of pregnancy. In addition, one woman with defective leptin receptors (lacking the transmembrane and intracellular signaling domains of the receptor), while unusual in that she underwent pubertal development, albeit delayed, was able to conceive spontaneously and to deliver a healthy baby, raising considerable questions about the requirements for leptin signaling in normal ovarian and endometrial functions (Nizard et al., 2012).

Together, the preponderance of evidence indicates that the neuroendocrine effects of leptin account for the fertility of leptin null mice (lep−/−) and leptin receptor null mice (lep−/− /−) and humans. Such observations do not, however, preclude the hormone from modulating the functions of peripheral reproductive tissues in lean or obese humans. Below, we address the evidence that leptin directly regulates ovarian function, oocyte maturation, embryo development, embryo implantation and placental function.

**Leptin effects on ovarian function**

Serum leptin levels vary with the phases of the menstrual cycle and peak in the luteal phase (Hardie et al., 1997). While early reports suggested that leptin is expressed in the ovary, oocyte and early embryo, these studies focused on mRNA expression and were non-quantitative (Cioffi et al., 1997). Where immunofluorescent/immunohistochemical staining was performed on reproductive tissues, there remained no way to determine the source of the leptin that was detected and important experimental controls were lacking (Antczak et al., 1997; Cioffi et al., 1997; Loffler et al., 2001; Basak and Dutta Roy, 2012). Thus, while it remains possible that leptin is generated in the ovary/corpus luteum, no recent studies have elaborated on the clinical significance, if any, of ovary-derived leptin. In most studies, follicular fluid leptin levels parallel serum levels and no consistent association has been drawn between these levels and in vitro fertilization (IVF) success rates (Dorn et al., 2003; Chen et al., 2004; Wunder et al., 2005; Hill et al., 2007; Takikawa et al., 2010; Almom et al., 2011).

What are the data supporting a role for leptin in modulating ovarian function? Leptin has been shown to exert effects on ovarian theca and granulosa cells as well as on cells in the oviduct, the endometrium, and the developing embryo. Specifically, leptin has been shown to modulate steroidogenesis and to influence embryo development, though many
studies employed supraphysiologic leptin dosing, findings were broadly inconsistent, and the magnitude of many of the measured changes was often marginal (Karlsson et al., 1997; Zachow and Magoffin, 1997; Agarwal et al., 1999; Zachow et al., 1999; Duggal et al., 2000, 2002a, b; Spicer et al., 2000; Ghizzi et al., 2007; Sirotkin et al., 2005; Ricci et al., 2006; Nicklin et al., 2007; Karamouti et al., 2008). The importance of these leptin effects with respect to human reproductive performance remains uncertain. While enhancing our understanding of steroid hormone synthesis remains intellectually worthwhile, infertility, per se, as well as adverse reproductive outcomes, have not been linked to mild impairments in steroidogenesis. The reader is reminded that studies in leptin and leptin receptor null mice have indicated that adipose tissue/ovarian/embryonic and placental effects of leptin are not essential for normal mouse embryologic/gestational development (Mounzih et al., 1998; Zhang et al., 2012). Further, successful donor egg cycles in oophorectomized women indicate that any granulosa/theca cell-derived leptin (Cioffi et al., 1997) is similarly dispensable for sustaining human pregnancies when supplemental estrogen and progesterone are provided to oocyte/embryo recipients. Apart from estrogen and progesterone, no ovarian products (adipokine or otherwise) are required to initiate or sustain normal human embryo implantation, placentation, and pregnancy.

**Leptin effects on placental function**

Meanwhile, leptin is produced by the placenta and leptin levels rise during pregnancy (Masuzaki et al., 1997). Supraphysiologic leptin concentrations applied to choriocarcinoma cells stimulated their proliferation and survival, suggesting autocrine effects of placentally-derived leptin (Masuzaki et al., 1997; Magarinos et al., 2007; Perez-Perez et al., 2009). Similarly, supraphysiologic concentrations of leptin (100 ng/ml) enhanced placental cytokine production (Lappas et al., 2005). The physiologic significance of placental leptin production remains unclear in light of the observation that leptin signaling is not required for normal intrauterine development in humans or mice (Mounzih et al., 1998; Zhang et al., 2012). It has been proposed that placental leptin modulates maternal appetite and metabolism during pregnancy, just as adipose tissue-derived leptin does outside of gestation (Mounzih et al., 1998).

**Leptin effects on testicular function**

Leptin and leptin receptor transcripts have been detected in testicular cells suggesting the potential for leptin to directly modulate testicular functions: unfortunately, no consensus exists regarding whether full-length leptin receptors (i.e. the Ob-Rb receptors capable of leptin signal transduction) are expressed in the testis at the protein level (Tena-Sempere et al., 1999; Herrid et al., 2008a, b). Whereas leptin receptor isoforms can be detected in many tissues by RT–PCR, no receptor mRNA isoforms are detectable in testis by Northern blot analysis (Fei et al., 1997). Although it was observed that 30–90 min (but not 24 h) leptin treatments of rat Leydig cells modulated hCG-stimulated androgen production, this remains an effect without clear clinical significance (Caprio et al., 1999), particularly in light of conflicting reports regarding receptor expression in these cells (Herrid et al., 2008a). Similar effects were observed in isolated rat testes, though modest in magnitude, and without a discernable dose response (Tena-Sempere et al., 1999).

**Leptin: summary of effects on peripheral reproductive tissues**

What, then, can be concluded from the vast number of studies demonstrating leptin responsiveness in ovarian, endometrial, embryonic, placental, and testicular tissues? The majority of studies are constrained by significant methodologic limitations: most employ supraphysiologic leptin dosing, demonstrate inconsistent dose responses to leptin treatment, and neglect to demonstrate leptin production or full length leptin receptor (OB-Rb) expression at the protein level (by Western blot or by well-controlled immunohistochemistry). Whether there is functional redundancy in leptin signaling, or whether leptin performs non-essential functions in placental and embryonic development remains to be fully established. Redundancy of ‘thirsty’ gene functions is suggested by the disappointing results of clinical trials in which leptin administration was tested for weight loss in obese individuals: leptin supplementation is, apparently, unable to overcome the host of adaptations collectively engineered to protect and enhance caloric intake (Heymsfield et al., 1999; Hukshorn et al., 2002). In summary, direct leptin signaling cascades in the testis, ovary, uterus, placenta, and embryo appear to be non-essential to mouse and human reproductive function (Gonzalez and Leavis, 2001; Tanaka and Umesaki, 2008; Dos Santos et al., 2012; Dubern and Clement, 2012).

**Conclusions**

Because the placenta and fetus are both of fetal origin, any adipokine receptor null mice that are viable argue for non-essential roles for that adipokine in early embryo development, later embryo implantation and placentation, and final fetal development. Similarly, fertile adipokine null or receptor null mice indicate the non-essential role of that adipokine with regard to sperm production as well as follicle recruitment, ovulation, fertilization, and endometrial embryo receptivity. Based upon the reproductive phenotypes of leptin null and leptin receptor null mice, which can be made fertile if pubertal development and gonadotrophin levels are restored (which is possible via a host of central nervous system genetic or pharmacologic manipulations, and by gonadal transplantation), we conclude that leptin production from the placenta or embryo, and leptin signaling in the ovary, testis, placenta, uterus, and fetus are not required for mouse reproduction. Humans that lack leptin or the leptin receptor are similarly viable and at least one woman lacking functional leptin receptors was able to reproduce. While leptin may directly modulate peripheral reproductive tissues, current evidence indicates that the principal effects of leptin with respect to reproductive performance are indirect effects.

Similar reasoning can be applied to adiponectin and its receptors: while the peripheral reproductive tissues are bathed in the adiponectin ligand and many peripheral reproductive tissues express adiponectin receptors, no compelling evidence indicates that direct effects of adiponectin are essential for normal mouse reproduction. While adiponectin may directly modulate peripheral reproductive tissues, current evidence
indicates that the principal effects of adiponectin with respect to reproductive performance are likely to be indirect effects.

Although the majority of obese women will have favorable reproductive outcomes, emerging evidence in animal models and humans indicates a significantly elevated risk of subfertility and adverse perinatal, perinatal, and post-natal outcomes among obese women and their offspring as compared with lean counterparts. The reproductive problems associated with obesity are real; the pathogenesis of these problems remains poorly understood. Because obesity leads to perturbations in adipokine expression, and adipokine receptors are expressed in reproductive tissues, there has been a biologically plausible rationale for testing the effects of adipokines on peripheral reproductive tissues.

With respect to the highly tested adipokines leptin and adiponectin, many of the studies have been sub-optimally designed, executed, and interpreted (lacking suitable positive and negative control cells/tissues, physiologic hormone dosages, dose response and time course analyses, bona fide antibodies, protein loading controls, and quantitative methods at the mRNA and protein levels). Results from many studies have been inconsistent and sometimes contradictory. A cursory review of the literature perpetuates an overly exuberant conclusion that leptin and adiponectin are associated with obesity are real; the pathogenesis of these problems remains poorly understood. Because obesity leads to perturbations in adipokine expression, and adipokine receptors are expressed in reproductive tissues, there has been a biologically plausible rationale for testing the effects of adipokines on peripheral reproductive tissues.

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Authors’ roles
This manuscript was researched, written, discussed and edited by all three authors (J.F.K., R.S. and C.B.K.); the Table was created by J.F.K. and C.B.K.

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Conflict of interest
None declared.

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