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Leptin and its potential interest in assisted reproduction cycles

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TABLE OF CONTENTS

- Introduction
- Methods
- Leptin: the link between energy homeostasis and fertility
  - History of the discovery of leptin
  - Leptin gene, protein and its receptors
  - Leptin-induced signal transduction pathways
  - Mutations on leptin and leptin receptor genes
  - Roles of leptin in food intake control
  - Obesity and mechanisms of leptin resistance
- Role of leptin in female reproductive function
  - Evidence from animal models
  - Clinical observations in humans
  - Action of leptin on the central nervous system
  - Variation of serum leptin levels during the menstrual cycle
  - Actions of leptin on female reproductive organs
- Current knowledge of leptin evolution during ovarian stimulation and clinical interest in IVF cycles
  - Serum leptin measurements in IVF cycles
  - Leptin measurement in follicular fluid in IVF cycles
  - Comments on heterogeneity in study designs and end-points
- Conclusion and prospects

BACKGROUND: Leptin, an adipose hormone, has been shown to control energy homeostasis and food intake, and exert many actions on female reproductive function. Consequently, this adipokine is a pivotal factor in studies conducted on animal models and humans to decipher the mechanisms behind the infertility often observed in obese women.

METHODS: A systematic PubMed search was conducted on all articles, published up to January 2015 and related to leptin and its actions on energy balance and reproduction, using the following key words: leptin, reproduction, infertility, IVF and controlled ovarian stimulation. The available literature was reviewed in order to provide an overview of the current knowledge on the physiological roles of leptin, its involvement in female reproductive function and its potential interest as a prognostic marker in IVF cycles.

† These two authors contributed equally to this work and should be considered co-last authors.

RESULTS: Animal and human studies show that leptin communicates nutritional status to the central nervous system and emerging evidence has demonstrated that leptin is involved in the control of reproductive functions by acting both directly on the ovaries and indirectly on the central nervous system. With respect to the clinical use of leptin as a biomarker in IVF cycles, a systematic review of the literature suggested its potential interest as a predictor of IVF outcome, as high serum and/or follicular fluid leptin concentrations have correlated negatively with cycle outcome. However, these preliminary results remain to be confirmed.

CONCLUSION: Leptin regulates energy balance and female reproductive function, mainly through its action on hypothalamic-pituitary-ovarian function, whose molecular and cellular aspects are progressively being deciphered. Preliminary studies evaluating leptin as a biomarker in human IVF seem promising but need further confirmation.

Key words: leptin / reproduction / obesity / IVF / ovarian stimulation

Introduction

Leptin is a hormone synthesized by white adipose tissue and is part of the adipokine family, which also includes resistin, adiponectin, visfatin, omentin and vaspin. It is involved in the regulation of food intake and energy homeostasis by acting on the central nervous system (CNS). The leptin gene, or *ob* obesity gene, was cloned and sequenced in 1994. Mutations in this gene lead to morbid obesity and diabetes in humans and animals (Ingalls *et al.*, 1950; Coleman, 1978; Montague *et al.*, 1997). Several leptin receptors have been located in many organs, and particularly in the brain. The serum leptin level is correlated with body fat mass quantity and distribution, providing a peripheral signal to the CNS on the adequacy of nutritional status for reproductive function (Frisch and McArthur, 1974). Moreover, this hormone is involved in the control of many physiological functions, such as growth, metabolism and reproduction. In the case of female fertility, leptin is a key regulator and stimulator of the hypothalamic–pituitary–gonadal axis, through its direct action on the CNS leading to the regulation of gonadotrophin-releasing hormone (GnRH) secretion in the hypothalamus (Yu *et al.*, 1997; Quennell *et al.*, 2009; Roa and Herbison, 2012). Leptin positively influences the reproductive system from the onset of puberty to pregnancy, establishing a close link between energy homeostasis and fertility. However, animal studies have clearly shown that excessive leptin secretion may have adverse effects on female fertility. Observations made in obese women of reproductive age partly have confirmed this hypothesis, as the prevalence of infertility is higher in this subgroup of women than in the general population (Comninos *et al.*, 2014). Although the physiological and pathophysiological consequences of this interconnection between leptin and ovarian physiology in humans are now extensively studied, particularly in assisted reproductive technology (ART) cycles, a small number of studies have tried to evaluate the interest for measuring the serum and/or follicular leptin levels in infertile women undergoing IVF cycles, but have conflicting results (Anifandis *et al.*, 2005b; Gürbüz *et al.*, 2005).

This review of the literature presents an overview of the current knowledge of the role of leptin in female reproductive function, and its potential application in ART.

Methods

We first conducted a systematic PubMed search of all articles related to leptin and its action on energy homeostasis and natural or assisted reproduction and published up to January 2015. Concerning the sections 'Leptin, the link between energy homeostasis and fertility' and 'Role of leptin in female reproductive function', we selected articles using the following keywords (either alone or in combination): leptin, adipokines, obesity, appetite regulation, food intake control, mutation, leptin receptor, signal transduction pathways, leptin resistance, central nervous system, puberty, reproduction, menstrual cycle, hypothalamus, gonadal axis, hormones (GnRH, FSH, LH), gonadotrophins, hypogonadism, neuropeptides, fertility, infertility, ovary, gonadal steroid hormones, estrogens, ovulation, ovarian follicles and embryo. All animal and human studies based on fundamental research or clinical observations were included.

We also conducted a systematic review on all articles dealing with leptin in infertile women and its correlation with IVF cycle outcome. We performed a systematic search of the literature available up to January 2015 using the Pubmed database which, at first, was specifically conducted on all articles with the following keywords: leptin and IVF and ovarian stimulation, and was completed by an articles search including the following keywords in combination with leptin: ICSI, agonist protocol, antagonist protocol, ovarian response, leptin kinetic, serum leptin level, follicular fluid, polycystic ovary syndrome, obesity, gonadal steroid hormones, gonadotrophins, estrogens, ovarian follicles, oocyte quality, embryo/blastocyst, embryo quality, pregnancy and cycle outcome. For this search, only full-length articles dealing with clinical observations in humans, i.e. analysis of the variation of serum leptin levels during ovarian stimulation and/or evaluation of the relationship between serum or follicular fluid leptin levels and IVF cycle characteristics (ovarian response, oocyte quality, embryo quality) and outcomes (pregnancy), were included. Studies investigating the correlation between IVF cycle characteristics and a ratio between leptin and other markers were excluded. Studies were also excluded from the analysis when the leptin measurement was not realized in serum or follicular fluid. Studies reporting the variation of leptin during natural cycle or stimulated cycles with timed intercourse or intrauterine insemination were not included in the analysis. Studies which exclusively investigated selected populations such as women with PCOS or oocyte recipients were also discarded. The primary outcome measure was pregnancy rate. Other outcome measures analysed were the type of biological fluid analysed (i.e. serum or follicular fluid), dose of FSH administered during controlled ovarian stimulation, serum estradiol concentration, number of retrieved oocytes, number of mature oocytes, fertilization rate and embryo quality. No statistical tests were carried out with these data.

One author, A.C., selected the included papers from the references, and the inclusion of studies was confirmed by T.F., D.M. and P.B. In total, over 100 references were analysed and cited in this article.

Leptin: the link between energy homeostasis and fertility

History of the discovery of leptin

The leptin gene and protein were discovered in 1994, but many previous studies suggested the existence of a satiety factor. In 1950, a genetic

mutation, named *ob*, was identified and linked with obesity after the observation of severe obesity in *ob/ob* mice (Ingalls *et al.*, 1950). Homozygous *ob/ob* mice presented with several symptoms: bulimia, hyperinsulinism, hyperglycaemia, adipose tissue gain, altered thermoregulation and hypogonadism. In 1994, the *ob* gene mutation was located on the leptin gene (Zhang *et al.*, 1994).

In 1959, an experiment conducted on parabiotic mice enabled the discovery of a factor regulating weight by acting on the hypothalamus (Hervey, 1959). This circulating factor, named the satiety factor, was absent in *ob/ob* mice. In 1995, administration of this factor, leptin, in cerebral ventricles of *ob/ob* mice caused weight loss and reduction of daily food intake (Campfield *et al.*, 1995). Zhang *et al.* demonstrated leptin secretion in adipocytes (Zhang *et al.*, 1994), paving the way for several experiments aiming at deciphering the role of leptin in obesity and female reproduction.

Another recessive mutation in mice, named *db*, responsible for diabetes, was identified (Coleman, 1978). Homozygous *db/db* mice presented with similar symptoms to *ob/ob* mice. No effect was observed in *db/db* mice after administering leptin in the CNS, demonstrating that the *db* mutation affected the leptin receptor or signal transduction (Campfield *et al.*, 1995).

All these data were in accordance with the hypothesis of a circulating protein acting directly on the CNS to regulate food intake and body weight.

Leptin gene, protein and its receptors

In 1994, Zhang *et al.* discovered leptin and cloned its mouse and human genes (Zhang *et al.*, 1994). The human leptin gene, also called the obesity gene, is located on chromosome 7 and ranges over 20 kb (Isse *et al.*, 1995). It is composed of three exons separated by two introns, and its coding sequence is present in exons 2 and 3. Gene transcription results in a messenger RNA (mRNA) of 4.5 kb that is translated into a protein of 167 amino acids (16 kDa) (Zhang *et al.*, 1994).

The promoter sequence of the leptin gene ranges over 3 kb and contains several binding sites for transcription factors, such as Sp-1, CRE (cAMP Response Element), GRE (Glucocorticoid Response Element), ERE (Oestrogen Response Element) and the CCAAT box linking CCAAT/Enhancer Binding Protein α factor (C/EBPα) (Gong *et al.*, 1996; O'Neil *et al.*, 2001). Furthermore, promoter methylation, an epigenetic mechanism involved in leptin gene regulation, is inversely correlated with leptin gene expression (Marchi *et al.*, 2011).

The dimensions of the leptin protein are 20 Å × 25 Å × 45 Å, and its structure is formed by four antiparallel α helices (A, B, C and D), connected by two links and by a small loop composed of one helix (E). These observations classify leptin in the long-chain helical cytokine family, including IL-6, IL-11, IL-12, LIF (Leukaemia Inhibitory Factor), G-CSF (Granulocyte-Colony-Stimulating Factor), CNTF (Ciliary Neuro-Trophic Factor) and oncostatin M (Zhang *et al.*, 1997).

In 1995, the leptin receptor gene, named OB-R, was identified and cloned in mice and humans (Tartaglia *et al.*, 1995). The human leptin receptor protein comprises 1165 amino acids and is separated into three domains: extracellular, transmembrane and intracellular. Alternative splicing results in six forms of human receptors (OB-Ra, OB-Rb, OB-Rc, OB-Rd, OB-Re and OB-Rf) divided into three categories: short (OB-Ra, OB-Rc, OB-Rd, OB-Rf), long (OB-Rb) and soluble (OB-Re) forms. Differences between these classes of receptors are linked with the size of the intracellular domain and with the tissue expression level. OB-Rb, the functional leptin receptor form, is expressed in many human brain structures such as hypothalamic nuclei and the cerebellum (Couce *et al.*, 1997; Savioz *et al.*, 1997). Leptin receptors are also present in peripheral tissues such as adipose tissue and ovaries.

Leptin-induced signal transduction pathways

The binding of leptin with its receptor OB-Rb activates many signal transduction pathways, depending on the location of the receptor. The cytoplasmic domain contains a box1 domain which interacts with a Janus protein tyrosine kinase, JAK, leading to the activation of the JAK/STAT pathway which is the main transduction mechanism identified. More specifically, leptin binding with OB-Rb leads to the formation of a homodimer-activated receptor and enables recruitment and binding of JAK2 with the intracellular box1 domain. This results in JAK2 autophosphorylation, which will in return phosphorylate OB-Rb and STAT3 (Signal Transducers and Activators of Transcription). This transcription factor will then translocate into the cell nucleus in a dimeric form and regulate transcription of leptin target genes. Another transcription factor called SOCS-3 is also recruited and can exert negative feedback on the JAK/STAT pathway (Frühbeck, 2006; Robertson *et al.*, 2008). Furthermore, leptin can also activate MAPK/ERK and insulin signalling pathways after JAK2 activation (Morton *et al.*, 2006). These various transduction pathways indicate that leptin regulates a variety of diverse and complex processes. Leptin-induced signal transduction pathways and its target genes are summarized in Fig. 1.

Mutations on leptin and leptin receptor genes

Mutations of the leptin gene and its receptor have been described in mice and humans. Two mouse models carrying specific mutations have enabled the study of the physiopathology of leptin. Firstly, the *ob* mutation in the leptin gene is responsible for the production of a truncated and inactive protein. Secondly, the *db* mutation concerning the gene encoding for leptin receptor, leads to a lack of effect of leptin on target cells. In these two models, mice develop hyperphagia, obesity and type 2 diabetes, suggestive of morbid obesity in humans (Ingalls *et al.*, 1950; Coleman, 1978; Zhang *et al.*, 1994).

In humans, only a few mutations of leptin or leptin receptor genes have been identified (Montague *et al.*, 1997; Karvonen *et al.*, 1998; Strobel *et al.*, 1998; Farooqi *et al.*, 2007; Mazen *et al.*, 2009; Niv-Spector *et al.*, 2010). Some mutations of the leptin gene are associated with low serum leptin concentrations. However, these mutations remain rare, and have not been shown to be involved in most major cases of obesity. It should be noted that patients with a homozygous mutation of leptin receptor gene present with less severe symptoms than those having a homozygous mutation of the leptin gene. With these observations in mice and humans, a strong association between leptin and obesity has been demonstrated.

Roles of leptin in food intake control

Leptin plays a pivotal role in weight control, as it indicates the amount of fat mass to the CNS. Several mechanisms regulating leptin expression have been described. First of all, the leptin gene promoter can be methylated, repressing its expression. The methylation pattern of the leptin gene is tissue-dependent (Marchi *et al.*, 2011). Secondly, leptin synthesis is regulated by hormonal pathways. In animals and humans, insulin,

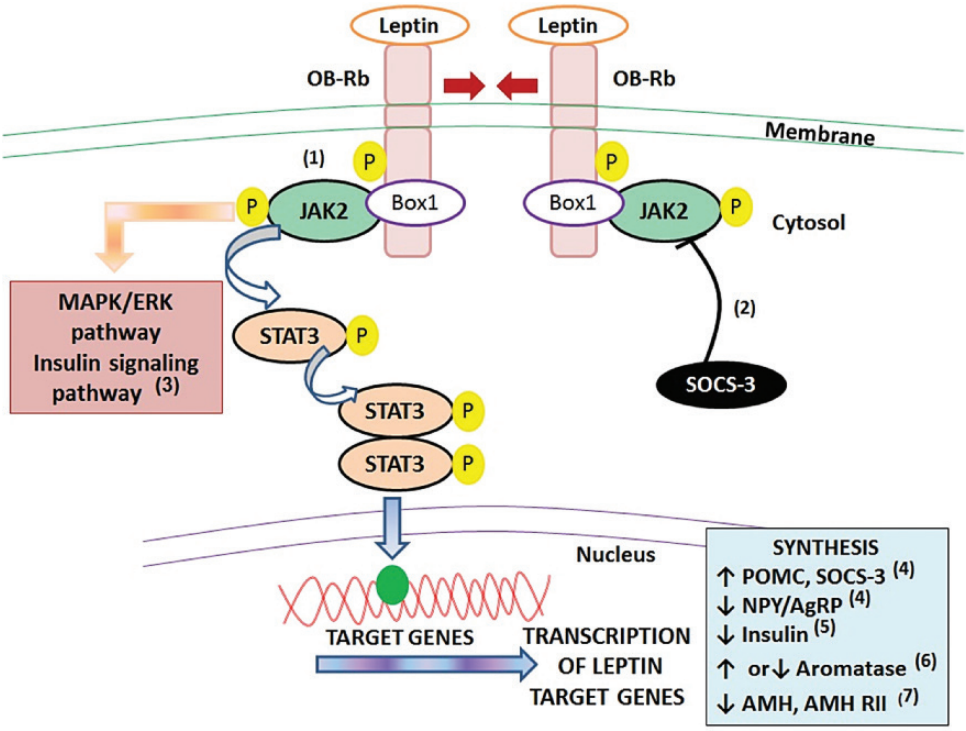

Figure 1 Signalling pathways activated by leptin in humans. AMH RII: anti-Mullerian hormone receptor, type II; JAK2: janus kinase 2; STAT: signal transducers and activators of transcription; POMC: pro-opiomelanocortin; SOCS-3: suppressor of cytokine signalling 3; NPY: neuropeptide Y; AgRP: agouti-related peptide; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase. (1) (Robertson *et al.*, 2008) (2) (Frühbeck, 2006); (3) (Morton *et al.*, 2006); (4) (Stephens *et al.*, 1995); (5) (Kieffer and Habener, 2000); (6) (Kitawaki *et al.*, 1999); (7) (Merhi *et al.*, 2013).

glucocorticoids and estradiol have been identified as inducers of leptin gene expression (De Vos *et al.*, 1995; Saladin *et al.*, 1995; Kolaczynski *et al.*, 1996; Miller *et al.*, 1996; Pedersen *et al.*, 1996; Shimizu *et al.*, 1997).

Leptin circulates in the blood in free form and bound with its soluble receptor, OB-Re. In healthy subjects, the bound leptin form represents the main circulating form, whereas free leptin is the only biologically active form. Consequently, OB-Re regulates the availability of the free bioactive form (Huang *et al.*, 2001).

In humans, OB-Rb is strongly expressed in the hypothalamus, especially in nuclei governing appetite and metabolism, such as the arcuate nucleus (Couce *et al.*, 1997; Savioz *et al.*, 1997). Two neuronal populations involved in this regulation have been described in this nucleus: neurons co-expressing AgRP and NPY (orexigenic peptides), and POMC neurons (precursor of α- melanocyte stimulating factor (α-MSH) an anorexigenic peptide). Leptin acts on these two neuronal populations. Through the JAK/STAT pathway, leptin inhibits AgRP/NPY synthesis and activates POMC synthesis in neurons, leading to an anorexigenic effect and an inhibition of food intake (Stephens *et al.*, 1995). α-MSH, released by POMC neurons, exercises its anorexigenic role by linking with its central receptor. In rats, the analysis of leptin receptor cDNA demonstrates the presence of alternative 5′ untranslated regions, including the presence of estrogen response element. This suggests a possible regulation by estrogen of food intake (Lindell *et al.*, 2001).

The insulin signalling pathway is also necessary for the expression of anorexigenic effects of leptin (Xu *et al.*, 2005). Insulin and leptin exercise the same action on POMC neurons by stimulating PI3K, leading to an anorexigenic effect. However, leptin and insulin have opposite effects on AgRP neurons.

Obesity and mechanisms of leptin resistance

Leptin is mainly synthesized by adipose tissue. Given that the serum leptin level is positively correlated with BMI (Body Mass Index), serum total and free leptin levels are higher in obese than in normo-weight patients (Considine *et al.*, 1995; Maffei *et al.*, 1995). Despite this increase, it does not enable control of food intake and regulation of body weight. These observations suggest that some leptin resistance mechanisms exist in obese patients, for which several hypotheses have been suggested. The decrease of the cerebrospinal-fluid/serum leptin ratio in obesity suggests saturation of the leptin transport mechanism in the brain, which could contribute to leptin resistance syndrome (Caro *et al.*, 1996; Kastin *et al.*, 1999). Moreover, it has been shown that the chronic increase in leptin in the CNS leads to decreased expression of its receptors and affects its transduction signal (Zhang and Scarpace, 2006). As mentioned previously, SOCS-3 is an inhibitor of the JAK/STAT pathway recruited after leptin binds with its receptor. An excessive SOCS-3 activity in obese patients has also been suggested as a potential leptin resistance mechanism (Bjørbaek *et al.*, 1998).

Studies have suggested that leptin could be the link between obesity and diabetes and consequently leptin has been associated with insulin resistance. Insulin is adipogenic, increases body fat mass, and stimulates the expression and secretion of leptin. In turn, leptin inhibits pancreatic β-cell

functions through direct actions and indirectly through central neural pathways. In obese women, leptin resistance does not enable the negative feedback on insulin secretion. It is suggested that adipoinsular axis dysregulation may contribute to obesity and the development of hyperinsulinaemia (Kieffer and Habener, 2000).

Some pharmacological experiments have also provided evidence for the responsibility of leptin resistance in obese patients. Interestingly, metformin, a well-known insulin-sensitiser, has been shown in obese rats to act on the CNS by potentiating hypothalamic expression of OB-Rb (Aubert *et al.*, 2011). Moreover, a peripheral mechanism of metformin has been discovered in mice. Metformin directly inhibits leptin secretion in adipocytes in a dose-dependent manner, contributing to the anorexigenic effect of metformin (Klein *et al.*, 2004). These two actions explain the action of metformin by limiting leptin resistance.

In conclusion, leptin resistance mechanisms in obese patients prevents leptin from exerting its regulating effects on food intake, thus worsening the physiopathology of obesity and its metabolic complications.

Role of leptin in female reproductive function

Evidence from animal models

At the interface of energy metabolism and fertility, leptin is the most extensively studied factor to explain the association between obesity and infertility. In 1950, the association between female obesity and infertility was noted in leptin-deficient *ob/ob* mice (Ingalls *et al.*, 1950). In 1963, Kennedy and Mitra observed that the triggering of puberty in rats was correlated with body weight rather than age (Kennedy and Mitra, 1963). In order to decipher leptin impact on female reproductive function, many studies have focused on female *ob/ob* mice deficient in active leptin. It was soon reported that these mice remained prepubertal and presented with hypogonadotropic hypogonadism (Swerdloff *et al.*, 1976). This hormonal deficiency was confirmed by the restoration of fertility after GnRH or gonadotrophin administration. Based on these observations, the role of leptin as a gonadotropic axis stimulator was suspected. In prepubertal female mice, injecting leptin resulted in an earlier onset of reproductive function and earlier maturation of the reproductive tract than in controls (Chehab *et al.*, 1997). The same treatment administered to female *ob/ob* mice resulted in elevated serum gonadotrophin levels, uterine development, larger ovaries, a higher number of ovarian follicles and finally restored cycles and gestations (Chehab *et al.*, 1996). These results have not been observed after an isolated weight loss in these leptin-deficient mice, highlighting the essential role of leptin in the CNS for normal reproductive function.

Clinical observations in humans

In humans, some clinical situations illustrate the interplay between leptin and the hypothalamic–pituitary–gonadal axis. Firstly, mutations in human leptin and its receptor genes have also been shown to be responsible for hypogonadotropic hypogonadism (Strobel *et al.*, 1998; Farooqi *et al.*, 2007). In 1974, Frisch and Mc Arthur demonstrated that a minimal amount of fat mass was required for the onset of puberty and maintenance of women's menstrual cycles (Frisch and McArthur, 1974). Moreover, patients suffering from anorexia nervosa present a dramatically diminished serum leptin concentration and usually present with central amenorrhoea (Licinio, 1997). Interestingly, gonadotropic axis function can be restored after recombinant human leptin treatment in the case of leptin gene mutations (Farooqi *et al.*, 1999). A minimal leptin threshold seems to be necessary for the onset of puberty and the continuity of menstrual cycles. Thus, anorexic patients recover regular menstrual cycles after the serum leptin level rises above 2 ng/ml (Mantzoros *et al.*, 2000).

Action of leptin on the central nervous system

In rats, leptin administration in the hypothalamus stimulates GnRH secretion and subsequent LH secretion (Yu *et al.*, 1997). While OB-Rb mRNA has been identified in GnRH neurons in mice (Magni *et al.*, 1999), leptin receptors are not present in human GnRH neurons, suggesting an indirect action of leptin. Indeed, the action of leptin is mediated through neuronal mediators called α-MSH, AgRP and neuropeptide Y, synthesized by neurons located in the arcuate nucleus and projecting towards GnRH neurons (Leranth *et al.*, 1988; Kalra and Kalra, 1996; Couce *et al.*, 1997; Savioz *et al.*, 1997; Quennell *et al.*, 2009; Roa and Herbison, 2012).

Leptin also acts on another family of neuropeptides (Smith *et al.*, 2006) called kisspeptins, which regulate reproductive function (Gottsch *et al.*, 2004; Navarro *et al.*, 2005). These neuropeptides are coded by the Kiss1 gene and bind with their receptor, GPR54, belonging to the G-protein-coupled receptor family. Kisspeptins appear to be involved in the positive and negative feedback effects of estradiol on GnRH release (Smith *et al.*, 2005). Moreover, the stimulation of Kiss1 expression mRNA in the anteroventral periventricular (AVPV) nucleus is necessary for the initiation of ovulatory LH peaks (Smith *et al.*, 2006; Dhillo *et al.*, 2007). Recently, is has been discovered that kisspeptin neurons of the arcuate nucleus co-express two others neurotransmitters, neurokinin B and dynorphin (Oakley *et al.*, 2009). These neurons, called KNDy neurons, have been hypothesized to regulate the pattern of GnRH release as a self-activating and self-inhibiting network (Navarro *et al.*, 2009; Lehman *et al.*, 2010). In fact, neurokinin B is described as an activator of the KNDy network, resulting in GnRH release, whereas dynorphin inhibits KNDy neurons after stimulation by neurokinin B (Navarro *et al.*, 2009; Lehman *et al.*, 2010). Moreover, arcuate KNDy neurons have been proposed to mediate estradiol negative feedback on gonadotrophin secretion (Rance, 2009; Mittelman-Smith *et al.*, 2012). According to various studies, leptin action on kisspeptins and KDNy neurons could be essential (Smith *et al.*, 2006; Louis *et al.*, 2011). Interestingly, experiments on pubertal mice suggest a negative effect of high serum leptin levels on the gonadotropic axis by blocking the kisspeptin pathway (Ahn *et al.*, 2012). This observation may explain the negative effect of obesity on the female reproductive system. However, further studies are needed to confirm this hypothesis.

These interactions between leptin and the CNS are summarized in Fig. 2. Despite the identification of these neural networks, the exact mechanisms of the action of leptin on GnRH and kisspeptin neurons have not been completely deciphered to date.

Variation of serum leptin levels during the menstrual cycle

In healthy women during the menstrual cycle, the serum leptin level reaches its nadir in the morning and its peak at night (Riad-Gabriel *et al.*, 1998). Leptin has physiological fluctuations during the menstrual cycle. In normo-ovulatory and normo-weight women, the serum leptin level has been shown to be lower during the early follicular phase, followed by an approximately 50% increase at the end of follicular phase.

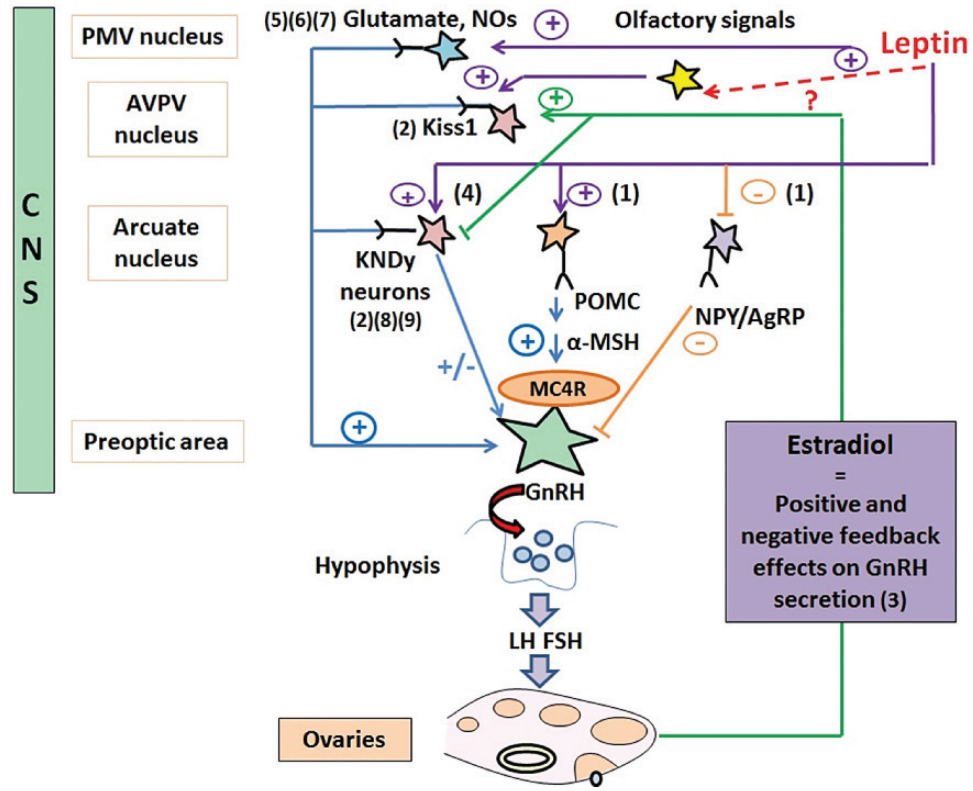

Figure 2 Interactions between leptin and neural gonadotropic axis networks. (the red dotted lines illustrate unknown neural networks.) CNS: central nervous system; PMV: ventral premammillary; AVPV: anteroventral periventricular; Kiss1: kisspeptin 1; KNDy neurons: kisspeptin/neurokinin B/dynorphin neurons; POMC: pro-opiomelanocortin; NOs: nitric oxide synthase; NPY: neuropeptide Y; AgRP: agouti-related peptide; a-MSH: α-melanocyte stimulating factor; MC4R: melanocortin 4 receptor; GnRH: gonadotrophin-releasing hormone; LH: luteinising hormone; FSH: follicle-stimulating hormone. Colour code for neuronal activity: purple: NPY/AgRP neurons; orange: POMC neurons; pink: Kiss1 and KNDy neurons; blue: glutamate and Nos neurons; yellow: unknown neurons. [1] (Leranth *et al.*, 1988; Kalra and Kalra, 1996; Quennell *et al.*, 2009; Roa and Herbison, 2012); [2] (Gottsch *et al.*, 2004; Navarro *et al.*, 2005); [3] (Smith *et al.*, 2005); [4] (Smith *et al.*, 2006); [5] (Leshan *et al.*, 2009); [6] (Ratra and Elias, 2014); [7] (Leshan and Pfaff, 2014); [8] (Lehman *et al.*, 2010); [9] (Navarro *et al.*, 2009).

The plateau observed during the pre-ovulatory LH surge is followed by a decrease in the midluteal phase (Riad-Gabriel *et al.*, 1998). Leptin and LH secretions are synchronous at night, especially as the LH surge approaches, suggesting that leptin could regulate LH secretion (Licinio *et al.*, 1997; Teirmaa *et al.*, 1998). Conversely, the serum leptin level does not significantly vary further as menopause occurs (Riad-Gabriel *et al.*, 1998).

The hypothesis of a positive effect of estradiol binding with its adipose receptor on leptin secretion has been suggested to explain variations of leptin levels throughout the cycle (Pedersen *et al.*, 1996; Mannucci *et al.*, 1998). Studies have also highlighted that the presence of an ERE on leptin and leptin receptor gene promoters could explain the interaction between both hormones (Lindell *et al.*, 2001; O'Neil *et al.*, 2001). This hypothesis is further supported by the positive correlation between serum leptin and estradiol concentrations observed during controlled ovarian stimulation (Stock *et al.*, 1999; Geber *et al.*, 2012).

Actions of leptin on female reproductive organs

In rats, leptin receptors are expressed in ovarian tissue (Zamorano *et al.*, 1997). In humans, both short and long forms of leptin receptors are present in pre-ovulatory follicles, ovarian theca cells, granulosa cells and oocytes (Cioffi *et al.*, 1997; Karlsson *et al.*, 1997; Agarwal *et al.*, 1999).

Several actions of leptin have been described throughout folliculogenesis. The pre-ovulatory follicle produces estradiol through a paracrine interaction between theca and granulosa cells. For this steroidogenesis, estradiol production by granulosa cells is stimulated by FSH and Insulin-like Growth Factor 1 (IGF-1) and LH acts on theca cells which produce androgens. Estrogens synthesized by granulosa cells originate from the action of aromatase on androgens.

Kitawaki *et al.* have evaluated the effects of leptin on estradiol synthesis in human pre-ovulatory follicles. Luteinised granulosa cells were cultured under different leptin concentrations, in association with FSH and/or IGF-1. Low leptin levels stimulated aromatase activity and subsequently estradiol synthesis by granulosa cells. This action was also potentiated by FSH and/or IGF-1. Aromatase expression and activity were maximal for a low leptin concentration, and decreased when the leptin level was more elevated (Kitawaki *et al.*, 1999).

Another study investigated the effects of leptin on granulosa and theca cells, with leptin concentrations ranging from 0 to 100 ng/ml (Agarwal *et al.*, 1999). Leptin concentrations $\geq$50 ng/ml led to an inhibition of

estradiol synthesis by granulosa cells and inhibited positive effect of IGF-1 on androstenedione synthesis by theca cells.

The different effects of leptin on steroidogenesis in granulosa cells are summarized in Fig. 3. These mechanisms may contribute to impaired development of the dominant follicle in the natural cycle (Agarwal *et al.*, 1999).

Furthermore, leptin seems to impact the expression and action of anti-Mullerian hormone (AMH), a hormone secreted by growing pre-antral and small antral follicles. Indeed, a recent study established that leptin suppresses AMH and AMH receptor mRNA expression in luteinised granulosa cells of women undergoing IVF (Merhi *et al.*, 2013). The main limitation of this study is that results may not fully extrapolate to non-luteinised granulosa cells. Moreover, further studies are necessary to clarify this action on AMH signalling and to understand the role of leptin in ovarian dysfunction. Well-known leptin actions on AMH signalling are summarized in Fig. 3.

In the case of PCOS, which is a complex endocrine and metabolic disorder, insulin resistance and visceral adiposity are frequently associated, suggesting an essential role of adipose tissue in this dysovulatory infertility. This association may be explained by the secretion of several mediators by adipocytes, such as leptin, potentiating insulin resistance (Escobar-Morreale *et al.*, 2014). In fact, a study investigating the expression profiles of adipose tissue in morbidly obese women with or without PCOS reported differences in gene expression profiles between both groups, particularly in the expression of genes encoding certain components of physiopathological mechanisms such as insulin resistance (Cortón *et al.*, 2007). The interaction between obesity and PCOS seems to correspond to a vicious circle combining hyperandrogenism with the development of abdominal adiposity that induces insulin resistance. In ovaries, hyperinsulinaemia acts synergistically with luteinising hormone to stimulate the synthesis of androgens by ovarian theca cells *in vitro* (Dunaif, 1997). Finally, high ovarian insulin concentrations stop follicular maturation. In order to develop PCOS, women must have an intrinsic steroidogenesis defect with elevated androgen secretions by ovarian theca cells. Despite the association between PCOS and obesity, serum leptin variations between PCOS and non-PCOS groups are subject to debate. Some studies note that serum leptin concentrations are not influenced by the presence of PCOS, whereas others found higher serum leptin levels in PCOS women than in control women, with a similar BMI (Chapman *et al.*, 1997; Takeuchi and Tsutsumi, 2000; Wang *et al.*, 2011; Chakrabarti, 2013).

In respect of endometrial receptivity as an effect of steroidogenesis, some studies have shown that progesterone synthesis in human granulosa cells is not influenced by leptin concentrations (Agarwal *et al.*, 1999; Kitawaki *et al.*, 1999), whereas Lin *et al.* more recently demonstrated a leptin-induced inhibition of progesterone synthesis (Lin *et al.*, 2009). Recently, the effects of different doses of leptin on the expression of proteins involved in progesterone synthesis, such as steroidogenic acute regulatory protein (StAR) and 3β-hydroxysteroid dehydrogenase (3βHSD), were studied in rats. The targets and effects of leptin differ in a dose-dependent manner; at physiological levels, leptin seems to increase synthesis of proteins involved in progesterone synthesis whereas this is inhibited at higher concentrations (Karamouti *et al.*, 2009; Bilbao *et al.*, 2013).

Many effects of leptin in regulating ovarian function have been identified but complete signalling pathways involved in the ovary have not been fully established to date. The effects of leptin currently described on the ovary are summarized in Fig. 3.

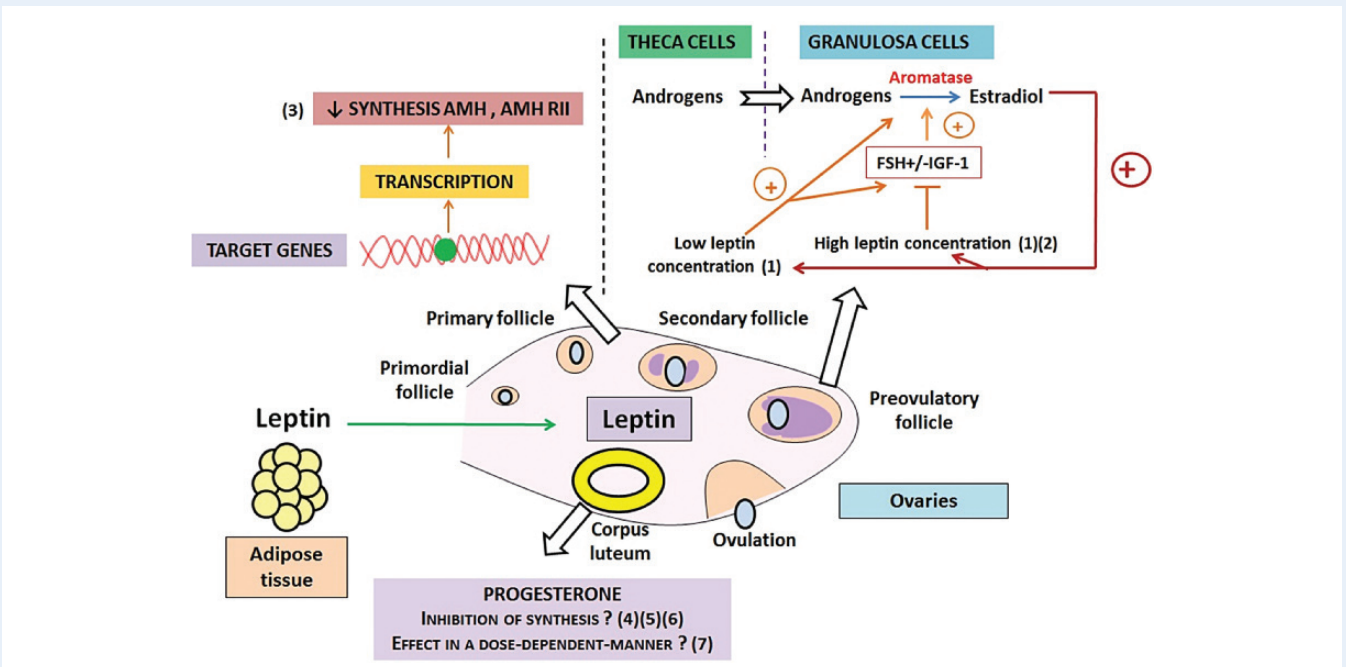

Figure 3 Peripheral interactions of leptin on the human ovary. AMH: anti-Mullerian hormone, FSH: follicle-stimulating hormone; IGF-1: insulin-like growth factor 1. (1) (Kitawaki *et al.*, 1999); (2) (Agarwal *et al.*, 1999); (3) (Merhi *et al.*, 2013); (4) (Kitawaki *et al.*, 2000); (5) (Agarwal *et al.*, 1999); (6) (Lin *et al.*, 2009); (7) (Bilbao *et al.*, 2013).

An effect of leptin on endometrial receptivity can be suspected in humans, but remains to be confirmed. Indeed, leptin receptors and particularly OB-Rb have been identified in the endometrium, suggesting an endometrial effect of leptin (Cioffi *et al.*, 1997; Kitawaki *et al.*, 2000). Their level of expression was correlated with serum leptin levels during the menstrual cycle; during the follicular phase, the expression of leptin receptors was low, further increasing during the luteal phase (Kitawaki *et al.*, 2000). Leptin is also expressed by the human endometrium, suggesting an autocrine/paracrine effect and a role during the implantation process. Low endometrial expression of leptin has been reported in patients with implantation failures or miscarriages (Laird *et al.*, 2001; Santos *et al.*, 2012). Moreover, an association between low endometrial thickness and a deficiency in OB-Rb mRNA expression has been reported in infertile patients without ovulation abnormalities (Alfer *et al.*, 2000). An additional putative mechanism correlating leptin excess and altered endometrial receptivity is the dysregulation of expression of ion channels, recently emerging as key factors in regulating endometrial receptivity. Indeed, a very recent study demonstrated that high serum leptin levels in obese PCOS patients were associated with down-regulated expression of epithelial Na+ channel via STAT3 signal pathway activation in the endometrium (Lin *et al.*, 2015).

These data highlight the possible role of leptin receptors in endometrial function, and could account for some cases of female infertility with implantation failures. Thus, the hypothesis that leptin may contribute to the preparation of the endometrium to sustain blastocyst implantation can be suggested.

Although the human embryo starts to synthesize leptin from the blastocyst stage, leptin receptor mRNA has been detected at all developmental stages of the embryo. Thus, the hypothesis of a leptin-based mechanism regulating the embryo-endometrium dialogue at the preimplantation stage has been suggested. Embryonic leptin receptors are activated, contributing to early embryonic development. At the blastocyst stage, the embryo itself expresses leptin, potentiating its own development, enhancing embryo-endometrium interaction and finally favouring implantation and placentation (Cervero *et al.*, 2004). In mice, effects of leptin on the in-vitro development of preimplantation embryos were determined in the presence of various concentrations of human recombinant leptin and development was observed. At a concentration of 10 ng/ml, leptin had an effect only on proportion of embryos developing to blastocysts and to hatched blastocysts. At a concentration of 100 ng/ml, leptin exerted an inhibitory effect on all stages of embryo development. These results provide that leptin has a concentration and development stage-dependent effect on early mouse embryo development and that this hormone can regulate implantation (Herrid *et al.*, 2006).

In humans, the syncytiotrophoblast expresses leptin and leptin receptors. Indeed both leptin and OB-Rb transcripts have been detected in placenta, from early pregnancy to delivery (Señarís *et al.*, 1997; Henson *et al.*, 1998). Leptin promotes the proliferation and survival of trophoblast cells by an autocrine action and an anti-apoptotic effect (Maymó *et al.*, 2011; Toro *et al.*, 2014). The positive effect of HCG on leptin gene expression in placenta was recently reported (Maymó *et al.*, 2012). This can be linked with the variation of serum leptin levels observed throughout pregnancy. Indeed, the serum leptin level progressively increases during the first weeks of pregnancy to reach a maximum at the 28th week (24 ± 11 ng/ml). Afterwards, the serum leptin level starts to decrease and reaches a minimum at delivery (15 ± 10 ng/ml) (Stock *et al.*, 1999). These results suggest that leptin may not only play a key role between the endometrium and embryo at the time of implantation, but that it is also necessary for physiological placental function, finally allowing the maintenance of fetal development and normal pregnancy.

However, as high serum leptin levels inhibit progesterone production (Lin *et al.*, 2009), this pivotal role of leptin on embryo implantation might not be systematically positive and the fine pro/anti-implantation balance exerted by leptin remains to be clarified.

Current knowledge of leptin evolution during ovarian stimulation and clinical interest in IVF cycles

As leptin is a pivotal molecular actor involved in ovarian physiology, an interest in its measurement in serum as a biological predictive tool in the monitoring of ovarian stimulation, has been assessed, especially for obese women who experience a higher prevalence of infertility than normal weight women (Clark *et al.*, 1998; Fedorcsák *et al.*, 2004; Dokras *et al.*, 2006; Bellver *et al.*, 2010) and higher basal serum leptin levels. Some studies have evaluated the correlation between leptin and ovarian stimulation characteristics and/or IVF cycle outcome, but their conclusions were different, mainly because of a vast heterogeneity in their design and end-points: ovarian stimulation protocol, timing of blood sample, definition of pregnancy, etc. Comparing these data is thus difficult because of the lack of standardization. Therefore, we conducted a systematic review on all articles dealing with leptin in infertile women and its correlation with IVF cycle outcomes, in order to provide a comprehensive review of the available data, evidence and still unanswered questions on this promising topic. The method used for this systematic review of the literature is presented in the 'Methods' section.

A total of 17 and 16 studies reported the association between serum or follicular fluid leptin concentrations, respectively, and IVF cycle characteristics and/or outcome. About 30 studies had been excluded according to above cited exclusion criteria. We have separated the interpretation of results, concerning leptin level analyses in serum or in follicular fluid. Details of the included studies are provided in Tables I and II for serum and follicular leptin analyses, respectively.

Serum leptin measurements in IVF cycles

Serum leptin levels during ovarian stimulation

Among the 17 studies dealing with serum leptin in IVF cycles, 10 reported the dynamics of serum leptin level during ovarian stimulation (Bützow *et al.*, 1999; Unkila-Kallio *et al.*, 2001; Tsai *et al.*, 2002; Ayustawati *et al.*, 2004; Anifandis *et al.*, 2005b; Gürbüz *et al.*, 2005; Wunder *et al.*, 2005; Hill *et al.*, 2007; Chakrabarti *et al.*, 2012; Ergenoğlu *et al.*, 2012). These studies were different in terms of female age, with the maximum age at inclusion ranging from 36 (Chakrabarti *et al.*, 2012) to 42 years (Wunder *et al.*, 2005; Hill *et al.*, 2007). The frequency and timing of blood collection for serum leptin analysis were also very heterogeneous among studies, with studies making the first assessment before the onset of the IVF cycle (Gürbüz *et al.*, 2005), during ovarian suppression (Bützow *et al.*, 1999; Hill *et al.*, 2007) or during ovarian stimulation at various moments (Tsai *et al.*, 2002; Ayustawati *et al.*, 2004; Anifandis

Table I Principal characteristics of the studies reported on serum leptin analysis in IVF cycles. Studies are listed in chronological order.

Study	Methodology/ participants	Exclusion criteria	Ovarian stimulation protocol	Methods, outcome measures	Results	Comments, limitations
Bützow 1999	Prospective study, $n = 52$ Age: 34 ± 0.6 years BMI: 24 ± 0.5 kg/m^2	None	Long agonist	– Serum leptin at 4 times: ovarian suppression, hCG day, OPU day and 16 days after ET – IVF cycle characteristics and cycle outcome: serum levels of FSH, estradiol and LH at the 4 times, total dose of FSH, number of follicles, number of retrieved oocytes, pregnancy test 16 days after ET	– 60% increase of serum leptin between ovarian suppression and hCG day (10.9 ± 1.1 ng/ml to 15.7 ± 1.5 ng/ml respectively) – No significant correlation between serum leptin and FSH, estradiol or LH at the four time points – Negative association between the relative serum leptin increase and the ovarian response to hyperstimulation, as revealed by the numbers of follicles ($P < 0.05$) and oocytes retrieved ($P < 0.01$)	– Correlation with pregnancy outcome was not reported
Mantzoros 2000	Prospective study, $n = 103$	Smoking women PCOS	Long agonist	– Serum leptin on hCG day – IVF cycle characteristics and cycle outcome: total dose of gonadotrophins, duration of stimulation, serum estradiol, number of oocytes retrieved, fertilization rate, number of transferred embryos, live birth rate	– No significant correlation between serum leptin levels and number of oocytes retrieved, amount of gonadotrophins administered and serum estradiol concentrations – Serum leptin concentration lower in ART success group compared with ART failure group but without statistical significance	– BMI and age global ranges were not indicated
Brannian 2001	Prospective and retrospective study, $n = 139$	Age > 39 years PCOS	Long agonist	– Serum leptin on stimulation day 1 – IVF cycle characteristics and cycle outcome: peak estradiol level, number of oocytes retrieved, number of oocytes mature, fertilization and cleavage rates, embryo development and blastulation rate, clinical pregnancy rate	– Significant correlation between serum leptin and pregnancy rate – Low leptin/BMI ratio significantly associated with higher embryo quality – Serum leptin to BMI ratio more strongly correlated with IVF success than leptin alone. A leptin/BMI ratio ≥ 0.7 seems to be predictive of poorer pregnancy success	– BMI global range were not indicated – Various embryo transfer strategies were included – No information was provided concerning age, BMI, IVF stimulation characteristics between pregnancy and failure groups

Continued

Table I *Continued*

Study	Methodology/ participants	Exclusion criteria	Ovarian stimulation protocol	Methods, outcome measures	Results	Comments, limitations
Unkila-kallio 2001	Prospective study, $n = 66$ Age [23–41 years] BMI: 23 ± 0.4 kg/m^2	None	Long agonist	– Serum leptin at 6 times: mid-luteal phase before pituitary down-regulation, ovarian suppression, 1–2 days before hCG injection, OPU, 8 and 14 days after oocyte retrieval – IVF cycle characteristics and cycle outcome: basal FSH, duration of stimulation, total dose of gonadotrophins, number of oocytes retrieved, peak estradiol level, clinical pregnancy rate, miscarriage rate, delivery rate	– Significant decrease of leptin concentration from the mid-luteal phase to ovarian suppression – Significant increase of leptin level during ovarian stimulation, regardless of BMI, this rise being maximum at the time of oocyte retrieval (76 ± 8%) – No correlation between leptin and ovarian response to stimulation – Significantly different leptin concentration at 12 days after ET according to cycle outcome (failure, pregnancy, miscarriage) – Significant correlation between the rise in leptin levels and peak estradiol concentration, but only in pregnant patients	– Various embryo transfer strategies
Tsai 2002	Prospective study, $n = 50$ Age ≤ 40 years	PCOS, Basal FSH ≥ 10 UI/l or E2 ≥ 80 pg/ml Smoking women	Long agonist	– Serum leptin level on stimulation day 2, hCG and OPU day – IVF stimulation characteristics and cycle outcome: total dose of gonadotrophins, serum estradiol level, number of follicles, number of oocytes retrieved, fertilization rate, clinical pregnancy rate	– No significant correlation between serum leptin and serum estradiol levels – Serum leptin levels on hCG day were significantly lower in pregnancy group than in failure group	
Ayustawati 2004	Prospective study, $n = 66$ Age [29–38 years] BMI [17.9–28.3 kg/m^2]	severe OHSS	Long agonist	– Serum leptin at 5 times: on stimulation day 1, hCG day, 7, 14 and 21 days after oocyte retrieval – IVF outcome: clinical pregnancy	– No significant correlation between leptin concentration and estradiol level – Twofold increase of leptin concentration from the 1st day of stimulation to hCG day – Leptin concentrations lower in ART success group compared with ART failure group but without statistical significance apart from results obtained 7 days after oocyte retrieval	– Patients in ART success group were significantly younger than those in ART failure group – No indication on embryo transfer strategy
Nikolettos 2004	Prospective study, $n = 95$ Age: 32.3 ± 4.6 years BMI: 25.1 ± 3.94 kg/m^2	PCOS Basal FSH ≥ 10 IU/l	Long agonist	– Serum leptin level on OPU day – IVF cycle characteristics and cycle outcome: total dose of gonadotrophins, peak estradiol level, number of retrieved oocytes, number of mature oocytes, fertilization rate, clinical pregnancy	– Similar leptin concentrations between pregnant and non-pregnant women	

Asimakopoulos 2005	Prospective study, $n = 17$ Age [32.3 ± 5.0 years] BMI [21.39 ± 26.13 kg/m^2]	basal FSH > 10 IU/l	Long agonist	– Serum leptin level on OPU day – IVF cycle characteristics and cycle outcome: total dose of gonadotrophins, peak estradiol level, number of oocytes retrieved, number of mature oocytes, number of 2PN oocytes, clinical pregnancy	– No difference in leptin levels between pregnant and non-pregnant women	– No indication on embryo transfer strategy
Anifandis 2005 (1)	Prospective study, $n = 200$ Basal FSH levels < 8.5 IU/l	PCOS OHSS	Long agonist	– Serum leptin level on hCG day – IVF cycle characteristics and cycle outcome: ovarian response to hyperstimulation, peak estradiol level, total dose of gonadotrophins, duration of stimulation, number of retrieved oocytes, number of mature oocytes, fertilization rate, number of transferred embryos, embryo quality and pregnancy test 15 days after ET	– Negative association between leptin levels of women with peak estradiol levels 3001–4000 pg/ml and good-quality embryos – Negative correlation between estradiol and leptin concentrations in women with peak estradiol levels above 3000 pg/ml – Pregnancy success higher for women with serum estradiol and serum leptin levels comprised between [1001–2000 pg/ml] and [46–49 ± 8.4 ng/ml] respectively – Association of both parameters yields higher prognostic IVF value than each one considered alone	– BMI and age global ranges not indicated – Number of retrieved oocytes, number of mature oocytes were different between groups
Anifandis 2005 (2)	Prospective study, $n = 100$ Basal FSH levels < 8.5 IU/l	PCOS OHSS	Long agonist	– Serum leptin at 4 times: on stimulation day 6, hCG day, OPU day, and 15 days after ET – IVF cycle characteristics and cycle outcome: ovarian response to hyperstimulation, serum estradiol levels, total dose of gonadotrophins, duration of stimulation, number of retrieved oocytes, number of mature oocytes, fertilization rate, number of transferred embryos, embryo quality and pregnancy test 15 days after ET	– No correlation between leptin and estradiol concentrations – Significant increase leptin levels during the IVF cycle (44.09 ± 3.54 to 52.10 ± 4.27 ng/ml) – Serum leptin concentration on OPU day is the most predictive of cycle outcome – Elevated leptin levels associated with reduced ovarian stimulation, embryo quality and pregnancy success	– BMI and age global ranges were not indicated – Number of mature oocytes and fertilization rate were different between the two groups
Gürbüz 2005	Prospective study, $n = 65$ Age: 32.7 ± 5.3 years BMI: 25.3 ± 3.6 kg/m^2	PCOS endometriosis	Long agonist	– Serum leptin on the 3rd day of the cycle before IVF and OPU day – IVF cycle characteristics and cycle outcome: estradiol, FSH, LH concentrations on the day of oocyte retrieval, total dose of gonadotrophins – Pregnancy: not defined	– 66.4% increase of serum leptin between the cycle before IVF and OPU day (14.3 ± 12.4 to 23.8 ± 20.9 ng/ml) – No correlation between serum leptin levels and those of estradiol, FSH or LH on the day of OPU – Negative correlations between increase of leptin levels and estradiol levels and the number of retrieved oocytes – No correlation between pregnancy rate and leptin level or increase	– Definition of pregnancy was not given

Continued

Table I *Continued*

Study	Methodology/ participants	Exclusion criteria	Ovarian stimulation protocol	Methods, outcome measures	Results	Comments, limitations
Wunder 2005	Prospective study, $n = 162$	Age > 42 years	Long agonist	– Serum leptin at 3 times: at the beginning of stimulation, hCG day or the one before, OPU day – IVF cycle characteristics: number of oocytes retrieved, number of transferred embryos and embryo quality (cumulative embryo score) – Pregnancy: not clearly defined. Probably biochemical pregnancy rate.	– No correlation between leptin levels and estradiol concentrations, embryo quality or pregnancy rate	– BMI and age ranges were not indicated – Various embryo transfer strategies were included – Age, number of retrieved oocytes and cumulative embryo score were higher in the pregnancy group than in failure group
Hill 2007	Prospective study, $n = 10$ Age: 33.7 ± 2.8 years BMI: 27.2 ± 5.2 kg/m^2	FSH ≥ 12 IU/l Age > 42 years	Long agonist	– Serum leptin at 6 times: IVF baseline date, on stimulation day 6, hCG and hCG + 1 day after, 7 and 14 days after OPU – IVF cycle characteristics and cycle outcomes: number of follicles, oocytes retrieved and embryos, basal serum FSH-LH-estradiol, peak estradiol level, total dose of gonadotrophins, cancellation rate, miscarriage rate, clinical pregnancy rate and live birth rate	– 82% increase of serum leptin levels during the IVF cycle (19.3 ± 13 ng/ml to 35.2 ± 27.2 ng/ml), but no correlation with pregnancy outcome – No correlation between serum leptin levels and IVF cycle characteristics and outcome	– Small sample size – Mean of total FSH dose administered was not mentioned – No indication was given on embryo transfer strategy
Asimakopoulos 2009	Prospective study, $n = 77$ Age [32.8 ± 3.7 years]	basal FSH > 10 IU/l	Antagonist	– Serum leptin on hCG day – IVF cycle characteristics and cycle outcome: total dose of gonadotrophins, peak estradiol concentration, number of retrieved and mature oocytes, fertilization rate, cumulative embryo score and clinical pregnancy	– No difference in serum leptin levels between pregnant and non-pregnant women – No correlation between serum leptin concentration and IVF cycle characteristics and outcome	– BMI global range was not indicated
Almog 2011	Prospective study, $n = 63$ Age: 32.7 ± 4.7 years	None	Short agonist	– Serum leptin on OPU day – IVF cycle characteristics and cycle outcome: number of retrieved oocytes, fertilization rate, number of transferred embryos – Pregnancy: not defined	– No difference in serum leptin concentrations between HMG and rFSH protocols – No correlation between serum leptin levels and number of retrieved oocytes, fertilization rate, pregnancy rate	– BMI global range was not indicated – Definition of pregnancy was not given
Ergenoglu 2012	Prospective study, $n = 55$ Age [20–40 years]	PCOS	Short agonist or antagonist	– Serum leptin at 5 times: on stimulation day 1 and 3, hCG day, OPU day and 12 days after ET – IVF cycle characteristics and cycle outcome: gonadotrophin dose, number of retrieved oocytes, fertilization rate, number of transferred embryos and pregnancy test 14 days after ET	– No difference in serum leptin levels between short agonist group and antagonist group and between pregnant and non-pregnant women	– BMI global range was not indicated – The number of transferred embryos significantly was higher in pregnant women compared with non-pregnant women

Chakrabarti 2012	Prospective study, $n = 18$ Age [24–36 years] BMI: 24.52 ± 0.73 kg/m^2	None	Long agonist	– Serum leptin at 3 times: on hCG day, OPU day and 16 days after ET – IVF cycle characteristics and cycle outcome: number of follicles, number of oocytes retrieved, fertilization and cleavage rates, embryo development and clinical pregnancy – No difference in the number of retrieved oocytes, fertilization rate, cleavage and embryo development according to serum leptin levels – No patient with > 60% leptin increase achieved pregnancy – Negative correlation between leptin levels and endometrial thickness

BMI: body mass index; ET: embryo transfer; IVF: *in vitro* fertilization; OHSS: ovarian hyperstimulation syndrome; OPU: ovum pick up; PCOS: polycystic ovarian syndrome; rFSH: recombinant FSH.
Anifandis 2005 (1): Anifandis *et al.*, (2005a); Anifandis 2005 (2): Anifandis *et al.*, (2005b).

et al., 2005b; Wunder *et al.*, 2005; Ergenoğlu *et al.*, 2012). The last time point of leptin serum analysis also ranged from oocyte retrieval (Tsai *et al.*, 2002; Gürbüz *et al.*, 2005; Wunder *et al.*, 2005) to pregnancy test 15 days after embryo transfer (Bützow *et al.*, 1999; Unkila-Kallio *et al.*, 2001; Ayustawati *et al.*, 2004; Anifandis *et al.*, 2005b; Hill *et al.*, 2007; Ergenoğlu *et al.*, 2012). It is important to note that the time of day of blood collection and fasting state were not precised in some studies, although serum leptin levels may be influenced by meals (Chapelot *et al.*, 2000) and has a diurnal variation (Saad *et al.*, 1998). More importantly, the differences observed in terms of mean female BMI between studies also questions the possibility of providing a relevant synthesis on leptin levels in IVF cycles, as serum leptin levels are directly correlated to BMI. Considering all these differences, it is difficult to draw any firm conclusion from this review of the available literature on serum leptin levels and variation throughout ovarian stimulation, and any interpretation should be cautious. However, the overall conclusions raised by the authors were concordant, showing a significant elevation of serum leptin levels during ovarian stimulation (ranging from +30% to +82%). One study did not allow the comparison between leptin levels at the beginning and at the end of ovarian stimulation (Chakrabarti *et al.*, 2012). However, the circulating leptin levels were compared between the days of HCG triggering day and oocyte retrieval in this study, interestingly showing that an increase in the serum leptin level of more than 60% between HCG and oocyte retrieval was associated with a failure to achieve term pregnancy.

This increase of serum leptin level during ovarian stimulation along with rising serum estradiol concentration is consistent with other previous reports (Strowitzki *et al.*, 1998; Zhao *et al.*, 2000). This increase in serum leptin concentration during ovarian stimulation reported in most studies may explain, at least in part, the need for higher gonadotrophin doses in obese patients undergoing controlled ovarian stimulation (Imani *et al.*, 2002). Given that the protocol used for ovarian stimulation varied among these 10 studies (long and short agonist, antagonist) with various effects on estradiol concentration, its relative influence on leptin kinetics could be questioned. The study conducted by Ergenoglu *et al.* could help in addressing this issue, as it evaluated serum leptin levels between patients treated with a short agonist protocol or a antagonist protocol (Ergenoğlu *et al.*, 2012). No difference were observed between the groups at any time point.

Apart from these 10 studies reporting leptin kinetic during ovarian stimulation, the 7 remaining studies only reported serum leptin level at one specific time point, such as stimulation day 1 (Brannian *et al.*, 2001), hCG triggering day (Mantzoros *et al.*, 2000; Anifandis *et al.*, 2005a; Asimakopoulos *et al.*, 2009) or OPU day (Nikolettos *et al.*, 2004; Asimakopoulos *et al.*, 2005; Almog *et al.*, 2011).

Serum leptin and IVF cycle characteristics

Besides these reports on the evolution of serum leptin during ovarian stimulation, 14 studies included in the analysis attempted to study the association between serum leptin and IVF cycle characteristics.

Ovarian response to ovarian stimulation. Firstly, all these 14 studies evaluated the association between serum leptin and ovarian response to ovarian stimulation, i.e. serum estradiol concentration, number of antral follicles growing during monitoring and/or number of oocytes retrieved.

Table II Principal characteristics of the studies reported on follicular fluid leptin in IVF cycles. Studies are listed in chronological order

Study	Methodology/ participants	Exclusion criteria	Ovarian stimulation protocol	Methods, outcome measures	Results	Comments, limitations
Mantzoros 2000	Prospective study, $n = 103$	Smoking women PCOS	Long agonist	– Follicular fluid samples: only fluid from the first aspiration of the dominant follicle was saved – IVF stimulation characteristics and cycle outcome: dose of FSH administered, number of stimulation days, estradiol concentrations, number of oocytes retrieved and fertilized, number of embryos transferred, live birth rate	– Lower FF leptin concentrations in pregnant women than in non-pregnant, even after adjusting for BMI and age ($P = 0.017$) – No significant correlation found between FF leptin levels and number of oocytes retrieved, amount of gonadotrophins administered and serum estradiol concentrations – FF and serum leptin concentrations were highly correlated	– Only fluid from the first aspiration of the dominant follicle was used
Welt 2003	Prospective study, $n = 30$ Age [24–45 years]	None	Long agonist	– FF samples collection: pool of all FF recovered in each patient – IVF stimulation characteristics and cycle outcome: number of follicles, dose of FSH administered, oocyte grade, oocyte stage, fertilization, embryo grade	– FF leptin concentrations were correlated with FF estradiol levels – No correlation between FF leptin level and oocyte grade, stage, fertilization and embryo grade	– All FF were pooled – Pregnancy outcome was not analysed in this study
Nikolettos 2004	Prospective study, $n = 95$ Age: 32.3 ± 4.6 years BMI: 25.1 ± 3.94 kg/m^2	PCOS Basal FSH $\geq$ 10 IU/l	Long agonist	– FF samples collection: total FF pool of each women – IVF stimulation characteristics and cycle outcome: total amount of administered gonadotrophins, estradiol at the day of hCG administration, number of retrieved oocytes, number of mature oocytes and fertilization rate, clinical pregnancy rate	– FF leptin levels were significantly higher in non-pregnant women compared with pregnant women	– All FF were pooled
Anifandis 2005 (1)	Prospective study, $n = 200$ Basal FSH $<$ 8.5 IU/l	PCOS OHSS	Long agonist	– IVF stimulation characteristics and cycle outcome: response to rFSH administration, serum estradiol level on the day of hCG administration, number of rFSH ampoules administered, number of stimulation days, number of retrieved oocytes, number of mature oocytes, fertilization rate, number of transferred embryos, embryo quality, biochemical pregnancy rate (serum β-hCG determination 15 days after ET)	– FF leptin levels correlated negatively with good-quality embryos and positively with poor-quality embryos in women with peak estradiol levels 3001–4000 pg/ml – Pregnancy success rate was maximal when serum estradiol and FF leptin levels were between 1001–2000 pg/ml and 52 ± 9.8 ng/ml respectively	– Method of FF collection was not explained

Anifandis 2005 (2)	Prospective study, $n = 100$ Basal FSH levels < 8.5 IU/l	PCOS OHSS	Long agonist	– FF samples collection: pool of all FF recovered in each patient – IVF stimulation characteristics and cycle outcome: response to rFSH administration, serum estradiol concentrations during IVF treatment, number of rFSH ampoules administered, number of stimulation days, number of retrieved oocytes, number of mature oocytes, fertilization rate, number of transferred embryos, embryo quality, biochemical pregnancy rate (serum β-hCG determination 15 days after ET)	– FF leptin levels had a positive correlation with serum leptin concentrations – FF leptin levels were significantly lower in pregnant patients than in non-pregnant ones – FF leptin levels were significantly lower in patients with good embryo quality than in patients with poor embryo quality	– The number of mature oocytes retrieved and the fertilization rate were different between the two groups – All FF were pooled
Asimakopoulos 2005	Prospective study, $n = 17$ Age [32.3 $\pm$ 5.0 years] BMI [21.39 $\pm$ 26.13 kg/m^2]	Low responders, basal FSH $>$ 10 IU/l	Long agonist	– FF sample collection: not clear. Probable pool of all FF – IVF stimulation characteristics and cycle outcome: total amount of administered FSH, estradiol at the day of hCG administration, number of oocytes retrieved, number of mature oocytes and number of 2PN oocytes, clinical pregnancy rate	– Non-pregnant patients had threefold higher FF total leptin levels than pregnant ones	– FF sample collection unclear – No indication given on embryo transfer strategy
Wunder 2005	Prospective study, $n = 162$, Normal basal hormonal values	Age $>$ 42 years	Long agonist	– FF samples collection: pool of all FF recovered in each patient – IVF stimulation characteristics and cycle outcome: number of oocytes retrieved, number of transferred embryos and cumulative embryo score	– No correlation between FF leptin levels and estradiol concentrations, embryo quality or pregnancy rate	– All FF were pooled – Age, number of retrieved oocytes and cumulative embryo score were higher in the pregnancy group than in the non-pregnancy group – Pregnancy outcome not defined
Hill 2007	Prospective study, $n = 10$ Age: 33.7 $\pm$ 2.8 years Prospective study BMI: 27.2 $\pm$ 5.2 kg/m^2	FSH $\geq$ 12 mIU/ml Age $>$ 42 years	Long agonist	– FF sample collection: the largest follicle was aspired – IVF stimulation characteristics and cycle outcome: number of follicles, oocytes retrieved, and embryos, basal hormonal status, serum estradiol level on hCG day, ampules of gonadotrophins administered, cycle cancellation rate, biochemical pregnancy rate, clinical pregnancy rate, live birth rate and pregnancy loss	– FF and serum leptin concentrations were significantly correlated throughout the cycle – No correlation were found between FF leptin levels and IVF stimulation characteristics	– Small sample size ($n = 10$) – FF leptin concentration was evaluated only in one follicle. – No indication on embryo transfer strategy
Asimakopoulos 2008	Prospective study, $n = 43$ Age [28–32 years]	FSH $\geq$ 10 mIU/ml	Antagonist	– FF sample collection: the first two to four follicles having a diameter of approximately 20 mm were punctured separately. Only the fluid from follicles with mature oocytes were stored individually – IVF outcome characteristics: number of mature oocytes, fertilization outcome of the mature oocytes, embryo quality	– No association between leptin level in individual FF and the successful fertilization of the oocytes derived from the same follicles – FF leptin levels were significantly correlated with embryo quality (embryo score) ($r = 0.276$, $P = 0.011$)	– Pregnancy outcome was not analysed in this study

Continued

Table II *Continued*

Study	Methodology/ participants	Exclusion criteria	Ovarian stimulation protocol	Methods, outcome measures	Results	Comments, limitations
Asimakopoulos 2009	Prospective study, $n = 77$ Age $= 32.8 \pm 3.7$ years	basal FSH > 10 IU	Antagonist	– FF sample collection: pool of FF coming from follicles with mature oocytes – IVF stimulation characteristics and cycle outcome: total dose FSH administered, serum estradiol concentration on hCG day, number of mature oocytes retrieved, fertilization rate, cumulative embryo score, estradiol-progesterone-testosterone-LH serum concentrations on hCG day, clinical pregnancy rate	– Positive correlation between FF and serum leptin concentrations – Positive correlation between BMI and FF and serum leptin concentrations respectively – No correlation between FF leptin concentrations and IVF stimulation characteristics – FF leptin levels did not differ between pregnant and non-pregnant women	– All FF were pooled
Takikawa 2010	Prospective study, $n = 46$	Age > 42 years BMI > 29 kg/m^2	Long agonist	– FF sample collection: only first mature follicle – IVF stimulation characteristics and cycle outcome: total dose of gonadotrophin, peak serum estradiol levels, number of retrieved oocytes, fertilization rate, number of transferred embryos, clinical pregnancy rate	– No significant difference in FF leptin concentrations between pregnant and non-pregnant women	– FF leptin concentration was evaluated only in one follicle. – Number of mature oocytes was significantly higher in pregnant women group compared with non-pregnant – Various embryo transfer strategies
Almog 2011	Prospective study, $n = 63$ Age: 32.7 ± 4.7 years	None	Short agonist	– FF sample collection: : pool of all FF recovered in each patient – IVF stimulation characteristics and cycle outcome: number of retrieved oocytes, fertilization rate, number of transferred embryos	– No difference in FF leptin levels according to the type of gonadotrophin used for COS – No correlation was found between FF leptin levels and number of retrieved oocytes, fertilization rate and pregnancy rate	– All FF were pooled – BMI global range not indicated – Pregnancy outcome was not analysed in this study
Chakrabarti 2012	Prospective study, $n = 18$ Age [24–36 years]	None	Long agonist	– FF sample collection: pool of all FF recovered in each patient – IVF stimulation characteristics and cycle outcome: number of follicles, number of oocytes retrieved, gradation of oocytes, fertilization and cleavage rates, embryo development, clinical pregnancy rate	– Positive correlation between serum and FF leptin – FF leptin levels had no impact on oocyte maturity, fertilization rate and embryo development	– All FF were pooled
Ergenoglu 2012	Prospective study, $n = 55$ Age [20–40 years]	PCOS	Short agonist or antagonist	– FF sample collection: only one FF sample, without any additional information – IVF stimulation characteristics and cycle outcome: gonadotrophin dose, number of retrieved oocytes, fertilization rate, number of transferred embryo(s), biochemical pregnancy rate (hCG levels ≥ 50 IU/l 14 days after ET)	– No difference in FF leptin levels between short agonist group and antagonist group and between pregnant and non-pregnant women	– No precise indication on FF sample collection – The number of transferred embryos was significantly higher in pregnant women compared with non-pregnant women

Chang 2014	Prospective study, $n = 67$ Age: 35.0 ± 3.4 years BMI: 22.7 ± 3.1 kg/m^2	PCOS severe endometriosis hypothalamic amenorrhoea history of ovarian surgery	Antagonist	– FF sample collection: single dominant follicle punctured for each ovary – IVF stimulation characteristics and cycle outcome: oocyte stage, fertilization, embryo grade, embryo quality score	– No statistically significant correlation between FF leptin level and fertilization or embryo quality – FF leptin concentration was evaluated only in one follicle. – Pregnancy outcome was not analysed in this study
Llaneza-Suarez 2014	Prospective study, $n = 130$ Age [26–40 years]	PCOS	Antagonist	– FF sample collection: only the first follicle aspirated during OPU – IVF stimulation characteristics and cycle outcome: number of follicles, number of retrieved oocytes, number of mature and fertilized oocytes, number of transferred embryos, clinical pregnancy rate	– Significant negative correlation between FF leptin levels and the number of fertilized oocytes – FF leptin concentration was significantly lower in women ending with a live birth (11.5 ± 4.6 ng/ml) compared with women who did not become pregnant or did not have a live birth (16.8 ± 6.2 ng/ml) – ROC analysis for FF leptin determined a cut-off value of 16.0 ng/ml below which a live birth would be predicted with a sensitivity of 78.3% and a specificity of 54.2% – Various embryo transfer strategies, with significantly different number of transferred embryos between women ending with a live birth and women who did not – FF leptin concentration was evaluated only in one follicle. The oocyte originating from this follicle did not systematically lead to one of the embryos transferred – Limited specificity and sensitivity of the proposed threshold for the prediction of live birth

BMI: body mass index; ET: embryo transfer; FF: follicular fluid; IVF: *in vitro* fertilization; OHSS: ovarian hyperstimulation syndrome; PCOS: polycystic ovarian syndrome; rFSH: recombinant FSH.
Anifandis 2005 (1): Anifandis *et al.*, (2005a); Anifandis 2005 (2): Anifandis *et al.*, (2005b).

None of these studies found a significant relationship between the total dose of FSH administered during ovarian stimulation and serum leptin levels (Mantzoros *et al.*, 2000; Nikolettos *et al.*, 2004; Anifandis *et al.*, 2005b; Hill *et al.*, 2007; Asimakopoulos *et al.*, 2009). Concerning the influence of the type of gonadotrophins used for ovarian stimulation, (i.e. human menopausal gonadotrophin or recombinant follicle-stimulating hormones) on leptin levels, both were compared in the study conducted by Almog *et al.*, with no difference observed in serum leptin levels according to the type of gonadotrophins used (Almog *et al.*, 2011).

The 12 studies evaluating the association between serum leptin levels and peak serum estradiol levels yielded conflicting results (Bützow *et al.*, 1999; Mantzoros *et al.*, 2000; Unkila-Kallio *et al.*, 2001; Tsai *et al.*, 2002; Ayustawati *et al.*, 2004; Nikolettos *et al.*, 2004; Anifandis *et al.*, 2005a, b; Gürbüz *et al.*, 2005; Wunder *et al.*, 2005; Hill *et al.*, 2007; Asimakopoulos *et al.*, 2009). Indeed, while 9 studies did not retrieve any correlation between peak serum estradiol and serum leptin level after ovarian stimulation (Bützow *et al.*, 1999; Tsai *et al.*, 2002; Ayustawati *et al.*, 2004; Nikolettos *et al.*, 2004; Anifandis *et al.*, 2005b; Wunder *et al.*, 2005; Hill *et al.*, 2007; Asimakopoulos *et al.*, 2009; Mantzoros *et al.*, 2011), other studies reported a significant negative correlation between the increase in leptin levels and estradiol levels (Anifandis *et al.*, 2005a; Gürbüz *et al.*, 2005). These last authors suggested that high serum leptin levels might inhibit estradiol production through direct or indirect mechanisms. Interestingly, the study by Unkila-kallio *et al.* (2001) demonstrated that the significantly positive correlation found between the increase in serum leptin levels during ovarian stimulation and maximal estradiol levels in women achieving pregnancy could not be found in women failing to achieve pregnancy after IVF cycle (Unkila-Kallio *et al.*, 2001). On the other hand, Anifandis *et al.* reported that a negative correlation between serum estradiol levels and serum leptin concentrations could only be observed in women with a peak estradiol level above 3000 pg/ml (Anifandis *et al.*, 2005a).

The correlation between serum leptin and the number of oocytes retrieved was reported in 9 studies (Bützow *et al.*, 1999; Mantzoros *et al.*, 2000; Nikolettos *et al.*, 2004; Anifandis *et al.*, 2005b; Gürbüz *et al.*, 2005; Hill *et al.*, 2007; Asimakopoulos *et al.*, 2009; Almog *et al.*, 2011; Chakrabarti *et al.*, 2012), once again with contradictory conclusions. There were 3 studies that concluded that a serum leptin increase was negatively associated with the number of oocytes retrieved (Bützow *et al.*, 1999; Anifandis *et al.*, 2005b; Gürbüz *et al.*, 2005), while others did not find any significant correlation between these parameters (Mantzoros *et al.*, 2000; Nikolettos *et al.*, 2004; Hill *et al.*, 2007; Asimakopoulos *et al.*, 2009; Almog *et al.*, 2011; Chakrabarti *et al.*, 2012). Of these 9 studies, 3 also evaluated the correlation between serum leptin levels and the number of mature follicles obtained during ovarian stimulation. Unsurprisingly, their conclusions were comparable to what was found for the number of oocyte retrieved (Bützow *et al.*, 1999; Unkila-Kallio *et al.*, 2001; Hill *et al.*, 2007). Oocyte maturity was not reported to be impacted by the serum leptin concentration (Nikolettos *et al.*, 2004; Asimakopoulos *et al.*, 2009).

Whether elevated leptin levels are associated with reduced ovarian response to ovarian stimulation remains to be clarified.

Embryonic parameters. Secondly, 11 studies evaluated the association between serum leptin levels and embryonic parameters, i.e. fertilization rate and embryo development (Mantzoros *et al.*, 2000; Brannian *et al.*, 2001; Tsai *et al.*, 2002; Nikolettos *et al.*, 2004; Anifandis *et al.*, 2005a,

b; Wunder *et al.*, 2005; Asimakopoulos *et al.*, 2009; Almog *et al.*, 2011; Chakrabarti *et al.*, 2012; Ergenoğlu *et al.*, 2012). All these studies concluded that the fertilization rate was not impacted by serum leptin concentrations. Concerning embryo development and quality, conflicting results were reported. While some authors reported a negative correlation between serum leptin levels and embryonic parameters (Brannian *et al.*, 2001), others did not identify any significant association between these parameters (Wunder *et al.*, 2005; Asimakopoulos *et al.*, 2009; Chakrabarti *et al.*, 2012). Once again, the heterogeneity observed between studies prevents any final conclusions on such an association.

Serum leptin and IVF cycle outcome

Among the 17 studies included in this review, 16 evaluated the correlation between serum leptin levels and pregnancy outcome in IVF cycle, with divergent conclusions. First, one of the main difficulties in comparing these results was the different timing of blood sample collection. Indeed, serum leptin levels were measured at various stages of ovarian stimulation up to the day of HCG triggering, oocyte retrieval, embryo transfer or pregnancy test. Another difficulty lies in the various end-points used for pregnancy outcome, ranging from positive pregnancy test to live birth. Overall, the majority of these studies did not report any significant association between serum leptin levels and pregnancy outcome (Mantzoros *et al.*, 2000; Ayustawati *et al.*, 2004; Nikolettos *et al.*, 2004; Asimakopoulos *et al.*, 2005, 2009; Gürbüz *et al.*, 2005; Wunder *et al.*, 2005; Hill *et al.*, 2007; Almog *et al.*, 2011; Ergenoğlu *et al.*, 2012). However, 6 studies reported a negative correlation between serum leptin levels and pregnancy outcome (Brannian *et al.*, 2001; Unkila-Kallio *et al.*, 2001; Tsai *et al.*, 2002; Anifandis *et al.*, 2005a, b; Chakrabarti *et al.*, 2012). Grouping studies according to similar leptin assessment timing and outcome also did not allow us to draw any firm conclusion. The above mentioned heterogeneity between studies in terms of leptin assessment timing, pregnancy definition population studied and embryo transfer strategies highlights the numerous biases preventing any relevant clinical interpretation of these data. Moreover, pregnancy outcome not only depends on embryonic parameters, but also on the endometrium, the latter being also potentially impacted by leptin (see above). Interestingly, one study focused on leptin dynamics rather than on a single time point to study the association with pregnancy (Chakrabarti *et al.*, 2012). Although their report that an increase in serum leptin level of more than 60% between the day of HCG and oocyte retrieval was associated with a failure to achieve term pregnancy deserves confirmation, this dynamic approach might be of interest to study the relationship between leptin and IVF cycle outcome.

Leptin measurement in follicular fluid in IVF cycles

In relation to the effects of leptin on folliculogenesis, 16 studies have focused on leptin levels in follicular fluid in women undergoing IVF cycles, seeking to evaluate the potential association between follicular fluid leptin and IVF cycle characteristics and IVF outcomes (Table II). Comparable to the studies conducted in serum, these studies yielded discrepant results. The main difference between these studies, was the methodology of follicular fluid collection, thus representing a considerable bias. Indeed, whereas some studies measured follicular fluid originating from all follicles punctured and pooled (Welt *et al.*, 2003; Nikolettos *et al.*, 2004; Anifandis *et al.*, 2005b; Wunder *et al.*, 2005; Asimakopoulos *et al.*, 2009; Chakrabarti *et al.*, 2012), others included follicular fluid from only one follicle (Mantzoros *et al.*, 2000; Hill *et al.*, 2007; Takikawa *et al.*, 2010; Ergenoğlu *et al.*, 2012; Chang *et al.*, 2014; Llaneza-Suarez *et al.*, 2014) or fluid from a specific subgroups of follicles (Asimakopoulos *et al.*, 2008, 2009). This issue should be considered when interpreting the results of these studies. Indeed, it cannot be excluded that follicular fluid composition, including hormonal levels, significantly differs between individual follicles as a result of the follicular microenvironment. Both methodologies, i.e. pooled or isolated follicular fluid, could thus lead to a huge bias, limiting the possibility of correlating leptin follicular concentration with oocyte quality, fertilization rate or subsequent embryo quality. A relevant study design could eventually consist in aspirating each mature follicle individually, measure the leptin concentration, and following the embryo development for each cumulus-oocyte complex, eventually up to embryo transfer and implantation. Although quite difficult to set up from an organizational point of view, such a study would allow a more accurate assessment of follicular microenvironment, and especially leptin follicular concentration, with regard to cycle outcome.

Follicular fluid leptin levels during ovarian stimulation

As it could be postulated that follicular hormonal composition might more directly affect follicular growth and maturation than serum circulating hormones, all of the 16 studies evaluated the correlation between follicular fluid leptin and ovarian response to ovarian stimulation (Table II). None of these studies established any association between follicular fluid leptin level and ovarian response to ovarian stimulation, except one which reported a positive correlation between follicular fluid leptin and follicular fluid estradiol concentrations (Welt *et al.*, 2003). This last result appears quite surprising with regard to in-vitro human studies which concluded that high leptin concentrations in the ovary may suppress estradiol production and negatively interact with the development of dominant follicles and oocyte maturation (Agarwal *et al.*, 1999; Kitawaki *et al.*, 1999).

Follicular fluid leptin and IVF cycle characteristics

Several studies also evaluated the correlation between follicular fluid leptin levels and embryonic parameters. The majority of these reported the absence of correlation between leptin and fertilization rate or embryo development (Mantzoros *et al.*, 2000; Welt *et al.*, 2003; Nikolettos *et al.*, 2004; Asimakopoulos *et al.*, 2005; Wunder *et al.*, 2005; Hill *et al.*, 2007; Takikawa *et al.*, 2010; Almog *et al.*, 2011; Chakrabarti *et al.*, 2012; Ergenoğlu *et al.*, 2012; Chang *et al.*, 2014), while others found a significant negative correlation between follicular fluid leptin and embryo quality (Anifandis *et al.*, 2005a, b; Asimakopoulos *et al.*, 2008; Llaneza-Suarez *et al.*, 2014). Interestingly, studies conducted by Asimakopoulos *et al.* only included individual follicular fluid from which mature oocytes could be retrieved, theoretically leading to a better analysis of the correlation between follicular fluid leptin concentration and oocyte maturity, fertilization and embryo development (Asimakopoulos *et al.*, 2008, 2009).

Follicular fluid leptin and IVF cycle outcome

Among these 16 studies, the potential association between follicular fluid leptin and IVF cycle outcome was analysed in 12 studies. Once again, there was a huge discrepancy among the results reported. Indeed, while some authors did not report any association between follicular fluid leptin level and pregnancy outcome (Wunder *et al.*, 2005; Hill

et al., 2007; Asimakopoulos et al., 2009; Takikawa et al., 2010; Almog et al., 2011; Ergenoğlu et al., 2012), some others suggested a negative impact of high follicular fluid leptin concentration on cycle outcome (Mantzoros et al., 2000; Nikolettos et al., 2004; Anifandis et al., 2005a, b; Asimakopoulos et al., 2005; Llaneza-Suarez et al., 2014), with women failing to achieve pregnancy having 1.4 to 3 fold higher follicular fluid leptin levels than pregnant women (Nikolettos et al., 2004; Anifandis et al., 2005b; Asimakopoulos et al., 2005; Llaneza-Suarez et al., 2014). Comparably to other variables, the vast heterogeneity observed between these studies in terms of patients included, design (pooled or individual follicular fluid) and methods makes it impossible to draw firm conclusions on whether follicular fluid leptin should be used as a prognostic marker of success in IVF cycles.

In addition to these studies conducted in women undergoing IVF, some other authors focused on specific subgroups of infertile patients. Plati et al. (2010) compared follicular fluid leptin concentrations in polycystic ovary syndrome (PCOS) patients undergoing controlled ovarian stimulation and in controls matched by age and BMI (Plati et al., 2010). They reported that follicular fluid leptin levels were slightly but significantly lower in PCOS women than in controls, whereas serum leptin concentrations (Plati et al., 2010; Svendsen et al., 2012) and pregnancy rates were not different between both groups. The mechanism underlying this apparent difference in leptin synthesis by granulosa cells in PCOS women remains to be elucidated.

Other studies have exclusively analysed different adipokines such as adiponectin, in addition to leptin. Adiponectin, secreted exclusively by adipocytes, in contrast to leptin, is reduced in obese women and is increased in low BMI patients (Hu et al., 1996). Li et al. (2012) chose to evaluate the predictive interest of the follicular leptin to adiponectin (L/A) ratio for oocyte quality and embryo development in IVF cycles. The follicular fluid L/A ratio, which was not surprisingly correlated with BMI, was found to be positively associated with successful cleavage and blastulation (Li et al., 2012). However, the results of this small pilot study have not been confirmed by other teams to date.

Comments on heterogeneity in study designs and end-points

We observed a vast heterogeneity in terms of design and end-points among these selected studies (Tables I and II). Firstly, the inclusion criteria of the population studied varied significantly. For example, the mean age or the age range of female patients were very heterogeneous among studies. Female BMI range varied too, although it was sometimes not even reported in some articles. Exclusion criteria were also heterogeneous, as some studies excluded patients with PCOS or poor ovarian reserve or smokers, while others did not. Concerning ovarian stimulation, the ovarian stimulation protocol (long agonist/short agonist/antagonist) and/or type of gonadotrophins were also not similar between studies.

The main difficulty in the comparison of these results concerned leptin serum measurements. Indeed, the timing and the number of blood samples analysed varied considerably between studies, with some studies measuring leptin at one or two points during the IVF cycle, while others measured leptin level at up to six different time points throughout the IVF cycle. It is important to note that some studies did neither give information on the hours of blood collection nor on the respect of a fasting state.

The differences in follicular fluid sample collection methodology also represented an important bias. Some studies measured follicular fluid sourced from all follicles punctured and pooled, while other isolated one or more follicle(s) fluid from specific subgroups of follicles. Various embryo transfer strategies, with one to three embryo(s) transferred at cleavage or blastocyst stage, were used and should thus also be taken into consideration. Concerning pregnancy, a positive pregnancy test 15 days after embryo transfer was used as end-point in some studies, while others reported clinical pregnancy. Successful outcomes rely on many factors and comparisons between studies need to be made with precautions.

Finally, although some studies suggested that leptin may negatively impact IVF outcome by impairing various stages of ovarian and endometrial physiology, the precise role of leptin in the poorer results generally observed in obese patients during controlled ovarian stimulation needs to be elucidated and it would be interesting to evaluate the relationship between free bioactive leptin and parameters of the IVF cycle.

Conclusion and prospects

Leptin, synthesized by adipose tissue, is a hormone which controls energy homeostasis by acting on the CNS as a satiety factor. This adipose factor is also deeply involved in female reproductive system regulation, by acting both on the gonadotropic axis and directly on the ovaries. High serum leptin levels seem to exert negative effects on female reproductive function, thus partly explaining the physiopathology of infertility frequently observed in obese women. However, the studies evaluating an interest in measuring serum or follicular leptin concentrations in IVF cycles have yielded conflicting results, mainly due to the lack of standardization in studies, making firm and relevant interpretation impossible. Further investigations are necessary to improve the knowledge on the role of leptin in female reproductive physiology and to evaluate the actions of leptin in infertile women undergoing IVF, especially for overweight/obese women.

Authors' roles

A.C. conducted the review of the literature and wrote the first draft of the paper. T.F. and D.M. supervised the work, helped in performing the review of the literature and corrected the paper. H.C., P.B. and M.G.D. critically reviewed the manuscript. All authors approved the final version of the manuscript.

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Conflict of interest

All authors declare that they have no conflict of interest.

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