Review

Antonio Pérez-Pérez, Flora Sánchez-Jiménez, Julieta Maymó, José L. Dueñas, Cecilia Varone and Víctor Sánchez-Margalet*

Role of leptin in female reproduction

Abstract: Reproductive function is dependent on energy resources. The role of weight, body composition, fat distribution and the effect of diet have been largely investigated in experimental female animals as well as in women. Any alteration in diet and/or weight may induce abnormalities in timing of sexual maturation and fertility. However, the cellular mechanisms involved in the fine coordination of energy balance and reproduction are largely unknown. The brain and hypothalamic structures receive endocrine and/or metabolic signals providing information on the nutritional status and the degree of fat stores. Adipose tissue acts both as a store of energy and as an active endocrine organ, secreting a large number of biologically important molecules termed adipokines. Adipokines have been shown to be involved in regulation of the reproductive functions. The first adipokine described was leptin. Extensive research over the last 10 years has shown that leptin is not only an adipose tissue-derived messenger of the amount of energy stores to the brain, but also a crucial hormone/cytokine for a number of diverse physiological processes, such as inflammation, angiogenesis, hematopoiesis, immune function, and most importantly, reproduction. Leptin plays an integral role in the normal physiology of the reproductive system with complex interactions at all levels of the hypothalamic-pituitary gonadal (HPG) axis. In addition, leptin is also produced by placenta, where it plays an important autocrine function. Observational studies have demonstrated that states of leptin excess, deficiency, or resistance can be associated with abnormal reproductive function. This review focuses on the leptin action in female reproduction.

Keywords: infertility; leptin; reproduction.

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***Corresponding author: Víctor Sánchez-Margalet,** Department of Medical Biochemistry and Molecular Biology, School of Medicine, UGC Clinical Biochemistry, Virgen Macarena University Hospital, Dr. Fedriani 3 Avenue, Seville 41071, Spain, E-mail: margalet@us.es
Antonio Pérez-Pérez and Flora Sánchez-Jiménez: Department of Medical Biochemistry and Molecular Biology, School of Medicine, UGC Clinical Biochemistry, Virgen Macarena University Hospital, Seville, Spain
Julieta Maymó and Cecilia Varone: Facultad de Ciencias Exactas y Naturales, Department of Biological Chemistry, Universidad de Buenos Aires, Argentina
José L. Dueñas: Obstetrics and Gynecology Service, Virgen Macarena University Hospital, Seville, Spain

Introduction

Reproductive function, as other physiological functions, depends on the energy reserves stored as fat in adipose tissue. The large energy requirement of a hypothetical pregnancy in the future was the original rationale for explaining the disruption of reproductive function by low fat stores in the present. The teleological nature of this argument compelled investigators to search for an endocrine signal that conveys information to the brain about the size of fat stores [1]. In 1994, leptin was the first adipokine claimed to be the 'missing link' between fat and reproduction. Leptin is a 16 kDa peptide hormone secreted mainly from adipose tissue which plays an integral role in the regulation of body weight and energy expenditure [2]. Plasma levels of leptin are correlated with the degree of obesity and are regulated by feeding and fasting. At present, leptin is considered to be a multifunctional hormone that regulates not only body weight homeostasis, but also thermogenesis, angiogenesis, hematopoiesis, osteogenesis, chondrogenesis, neuroendocrine, and immune functions, as well as arterial pressure control [3–6]. These actions of leptin are consistent with its production by various tissues and organs, such as the stomach, skeletal muscle, pituitary cells and the placenta [7]. Compelling evidence has also implicated leptin in reproductive functions, such as the regulation of ovarian function, oocyte maturation, embryo development as well as implantation and placentation [8, 9]. This influence of leptin on human reproductive function was indicated by observed associations of leptin or leptin-receptor deficiency with impaired reproductive development [10–12].

The leptin receptor (LEPR), product of the diabetes (db) gene, is a member of the class I cytokine receptor superfamily, with six known isoforms. LEPRb is primarily found in the hypothalamus and is involved in satiety response [13]. There are three other membrane bound LEPR isoforms LEPRa, LEPRc, LEPRd and LEPRf (short forms) which vary from the full length LepRb depending on the length of the intracellular domain. Their function also varies depending on the tissue in which they are localized and the length of the cytoplasmic tail. The short isoform LEPRa has a role in the leptin transport across the blood-brain barrier [14]. Other functions include leptin cellular internalization and signaling through the MAPK pathway [15, 16]. The fifth LEPR isoform is a soluble form (LEPRe). This form does not contain the transmembrane domain or intracellular domains. In vitro studies have shown that a human-derived LEPRe isoform can occur by post-translational modification by proteolytic cleavage [17].

The LEPRb, which contains a long intracellular domain, is the only isoform with two of the protein motifs necessary for activation of the Janus kinase 2 and signal transducers and activators of transcription (JAK-STAT) pathway [18], the major signaling mechanism activated by the LEPR. Activation of JAK-2 stimulates the phosphorylation of multiple residues (Tyrosine 985, Tyrosine 1138, and Tyrosine 1077) on the intracellular domain of LEPRb. Phosphorylation of each of these residues leads to the recruitment of a distinct set of downstream signaling molecules. By example, phosphorylated Tyr985 recruits the SH2-containing tyrosine phosphatase 2(SHP2), which presents the first step in the activation of the extracellular signal-regulated kinase (MAPK) cascade [19]. Additionally, phosphorylated Tyr985 also recruits SOCS3, a negative regulator of leptin action. Phosphorylation of tyrosine residue 1138 mainly recruits the transcription factor signal transducer and activator of transcription 3 (STAT3), which upon subsequent, JAK-2-dependent, phosphorylation translocates to the nucleus to regulate specific gene expression. It has been reported that hypothalamic leptin control of reproduction is regulated by signals independent of STAT3 signaling [20]. Finally, the phosphorylation of Tyr1077, the major phosphorylation site mediating leptin's effects on reproduction, promotes the recruitment and transcriptional activation of STAT5 [19] and is required for ongoing appropriate function of the female reproductive function [21].

Other intracellular signaling pathways have been reported to be stimulated by leptin including activation of phosphatidylinositolkinase-3 (PI3K) and the mammalian target of Rapamycin (mTOR) and inhibition of the AMP-dependent protein kinase (AMPK) [22]. Therefore, leptin signaling via LEPRb Tyr1077 might serve as an important mechanism by which leptin modulates endocrine function, linking body adiposity and the reproductive axis.

Research has demonstrated that leptin plays an integral role in the normal physiology of the reproductive system with complex interactions at all levels of the hypothalamic-pituitary gonadal axis (HPG) (stimulatory effects at the hypothalamus and pituitary and inhibitory actions at the gonads). Thus, leptin serves as a putative signal that links metabolic status with the reproductive axis. The intent of this review is to examine the biological role of leptin with emphasis on its actions in female reproduction. The effects of obesity on pregnancy rates and complications, together with the effects on delivery and fetal morbidities and mortality will not be included in this article, and readers should therefore refer to recent reviews on these issues [23, 24].

Role of leptin in the regulation of gonadotrophs secretion

Gonadotropin-releasing hormone (GnRH) cells of the hypothalamus are the primary regulators of the reproductive axis, regulating puberty and ovulation. Most of the GnRH-producing cells in the brain reside in the preoptic area of the hypothalamus. GnRH is secreted into the hypophyseal portal blood vessels and controls secretion of the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The hypothesis that leptin plays an important role in regulating GnRH secretion, and ultimately in reproduction, stems from several findings. The *ob*/*ob* mouse, lacking a functional leptin gene, is infertile and has atrophic reproductive organs [25]. Treatment with leptin rejuvenates the reproductive system in *ob*/*ob* mice, leading to growth and function of the reproductive organs and fertility [25] via secretion of gonadotropins [25, 26]. In human, patients lacking leptin protein [12] or functional LEPRs [11] do not attain pubertal maturity and have low serum levels of follicle-stimulating hormone and LH. The initiation of the menstrual cycle and the function of the reproductive system in women suggest the existence of a critical threshold leptin concentration. Moreover, the LEPR is expressed abundantly within the hypothalamus [2, 27–29], mainly present in arcuate and ventromedial hypothalamic nuclei controlling both sexual behavior and food intake [28, 30]. In the hypothalamus, leptin has a direct stimulatory effect on the HPG axis by accelerating GnRH secretion in the arcuate hypothalamic neurons in a dose-dependent manner. However, GnRH neurons in the preoptic area do not express LEPR-b, indicating that leptin

indirectly regulates these cells by acting on interneurons upstream of GnRH neurons. In fact, GnRH cell bodies are not affected by leptin directly [31] and other neuropeptides (target for leptin), such as NPY [32, 33] and kisspeptin [30, 34–36] and proopiomelanocortin (POMC) [35] could mediate the action of leptin. In addition to the stimulatory effect on the HPG axis at the hypothalamic level, leptin has direct effects on the anterior pituitary as well [37]. Almost 90% of the gonadotropes in the pars tuberalis (i.e., the portion of the pituitary in close proximity with the primary plexus of the hypophyseal portal system) and 30% of the gonadotropes in the pars distalis express LEPR [38]. Results from pituitary tissue culture studies demonstrated that leptin induces a dose-related increase in LH, FSH, and prolactin (PRL) release [39] via nitric oxide synthase activation in the gonadotropes [40]. In fact, several reports demonstrated that inhibition of LH secretion by restricted-feeding was reversed with leptin treatment, demonstrating a positive association between LH secretion and leptin [41–43]. Moreover, as much as 20%–25% of anterior pituitary cells, predominantly folliculostellate cells and corticotropes, express leptin, which may serve to regulate pituitary cell growth and differentiation in addition to its effect on LH/FSH secretion [44]. In addition, leptin regulates gonadotropin secretion via the regulation of GnRH function [45], which, could be mediated through kisspeptin neurons indirectly via its action at NPY and (or) POMC cell bodies.

As leptin has been shown to be influenced by steroid hormones and can stimulate LH release, it has been hypothesized that leptin acts as a permissive factor in the development of puberty [46]. In leptin-deficient (*ob/ob*) mice leptin administration accelerates sexual maturation and puberty in normal female mice [47, 48]. In fact, the rise in leptin levels may be the earliest signal of the initiation of puberty and may contribute to activation of the HPG axis, resulting in increased sex steroid production and subsequent activation of the GH/IGF-I axis. Rodents and humans with LEPR deficiency have hypothalamic hypogonadism, resulting in delayed pubertal development and infertility too. In these, atrophic uterine and ovarian size, abnormal estrous cyclicity and impaired mammary gland morphology and function have been reported [11, 49, 50]. Moreover, synchronicity of LH, estradiol, and leptin rhythmicity has been demonstrated during the mid-to-late follicular phase of the menstrual cycle in healthy women [51]. Taken together, these studies indicate that leptin either regulates or contributes significantly to the regulation of LH secretion [52–54].

In summary, leptin augments secretion of gonadotropin hormones, which are essential for initiation and maintenance of normal reproductive function, by acting centrally at the hypothalamus to regulate GnRH neuronal activity and secretion, as well as acting directly on gonadotropes.

Role of leptin in ovary function

Not only does leptin participate in the control of gonadotropin secretion via its hypothalamic/pituitary actions, but circulating or locally produced leptin may also provide direct modulation of ovarian function. Leptin protein has been found in follicular fluid, with concentrations corresponding to those reported in serum [55]. Leptin plays a role in both follicular development, where leptin transcript has been detected at early follicular stages, whereas leptin protein appears only in mature follicles [56], and subsequent luteal function. Moreover, LEPRs have been identified in granulosa, theca and interstitial cells of the human ovary [55, 57]. In these, several in vitro studies have demonstrated that treatment with medium-high physiologic doses (beginning from 10 ng/mL) of leptin-inhibited steroidogenesis in human granulosa and theca cells [58, 59] and lead to a marked decline in the number of ovulated oocytes [60]. Thus, high leptin concentrations in the ovary may suppress estradiol production and interfere with the development of dominant follicles and oocyte maturation, predisposing to anovulation. Therefore, conditions with excess energy stores or metabolic disturbances, such as obesity and polycystic ovarian syndrome, leptin have an inhibitory effect on the gonads. However, in suboptimal nutritional status, such as eating disorders, exercise-induced amenorrhea, and functional hypothalamic amenorrhea, leptin deficiency results in HPG dysfunction [52], raising the possibility that relative leptin resistance or deficiency may be at least partly responsible for the reproductive abnormalities that occur in these pathophysiological conditions.

Role of leptin in preinplantational embryo

Relatively little research has focused on receptor-mediated events in maturing oocytes and pre-implantation embryos. However a significant role of leptin in implantation has been proposed. While leptin correlates with progesterone concentrations throughout the cycle [61], LEPRs in oocytes might also influence oocyte maturation and development [62]. In fact, in mouse oocytes [63–65] leptin

induces tyrosine phosphorylation of STAT3, a major intracellular leptin signal transduction protein in mouse metaphase II stage oocytes [64]. Moreover, LEPR and leptin mRNA is specifically expressed at the blastocyst stage, suggesting a function in the blastocyst-endometrial dialog [8]. In a human in vitro model, it was observed that leptin was present in conditioned media from human blastocysts whether or not they were cocultured with endometrial epithelial cells [66]. Thus, in embryo culture media, leptin promoted the development of embryos from the two-cell stage to blastocysts, fully expanded blastocysts, and hatched blastocysts [63]. The higher leptin secretion found in competent human blastocyst cultures, compared with arrested blastocysts, suggests that this molecule may be a marker of cell viability. In line with this, leptin has concentration and stage-dependent effects on embryonic development in vitro. Differences between arrested and competent blastocysts suggest autocrine/paracrine regulation of leptin between endometrial epithelial cells and preimplantation embryos [67]. However, exposure of porcine or ovine oocytes to leptin during in vitro maturation and subsequent embryo culture after IVF resulted in the formation of fewer blastocysts relative to controls [68]. Since the endocrinology of pregnancy, in general, and leptin, in particular, are not well conserved between species, the extrapolation of data from rodent to human physiology is not feasible [69].

Role of leptin in implantation

Embryo implantation represents the most critical step of the reproductive process, involving a complex sequence of signaling events that are crucial to the establishment of pregnancy. A large number of identified molecular mediators have been postulated to be involved in this early feto-maternal interaction, including hormones, adhesion molecules, cytokines, growth factors, lipids and others [70]. In this sense, it has been reported that both leptin and LEPR are expressed in the glandular and luminal tissues of the endometrium throughout the menstrual cycle [8, 62, 71]. More specifically, low LEPR levels observed during the early proliferative phase are followed by a gradual increase and peak in the early secretory phase of the menstrual cycle, suggesting that LEPRs may be regulated by ovarian steroids, and that leptin might have a physiological role in the implantation of a fertilized egg [66].

The obligatory nature of leptin signaling in mammalian implantation was illustrated by experiments in the mouse demonstrating that endometrial LEPR expression was pregnancy-dependent and that intrauterine injection of a leptin peptide antagonist or a leptin antibody impaired implantation, suggesting that secretory endometrium is also a target tissue for leptin action. In fact, the blastocyst becomes intimately connected to the maternal endometrial surface to form the placenta [72] and oocytes and preimplantation embryos also express LEPR mRNA, as mentioned above, indicating that leptin may be necessary for embryonic development. In line with this, a deficiency in functional LEPR expression in the endometrium has been found in patients with subfertility who had evidence of an endometrial maturation defect [73]. Further evidence for the importance of leptin in implantation is the fact that in cytotrophoblasts, leptin increases the expression of matrix metalloproteinases (MMP-2 and MMP-9), which have been implicated in trophoblast invasion [74–76].

Role of leptin in placentation

The placenta is a complex organ that enables the mammalian embryo to survive within the intrauterine environment. The diversity of functions performed by the placenta is impressive, ranging from anchoring the embryo and preventing its rejection by the maternal immune system to enabling the transport of nutrients and waste between mother and the embryo [77]. Similarly to adipose tissue, placenta is a potent endocrine organ capable of expressing and secreting leptin. In fact, human placental leptin is identical to that derived from adipose tissue in terms of size, charge, and immunoreactivity [78], but it has a specific upstream enhancer, known as the placental leptin enhancer region [79], implying that leptin gene expression is regulated differently in placenta than in adipose tissue. In this regard, it has been reported that gestational hormones, such as b-hCG, estrogen progesterone and human placental lactogen (hPL) as well as hypoxia, insulin, glucocorticoids, several interleukins (IL-1a, IL-1b, IL-6), interferon-γ and cAMP, regulate placental leptin expression [74, 76, 80–86]. In humans, with the progression of placentation, two pathways of cytotrophoblast differentiation lead to the formation of two distinct phenotypes. In the villus, cytotrophoblast cells undergo cellular fusion and differentiation to form syncytiotrophoblast, while the extravillous trophoblast proliferates and migrates into the decidua, remodeling the pregnant endometrium [87]. In this sense, leptin, as well as LEPRs have been shown to be localized to the syncytiotrophoblast of the placenta facing maternal circulation [16] suggesting that leptin may act

through a paracrine or autocrine mechanism on placental function. Besides, previous studies have demonstrated the interactions between leptin and some placental hormones, implicating leptin also as a modulator of placental endocrine function [88].

Short and long LEPR isoforms as well as the soluble receptor have been characterized in human placenta [16]. Moreover, it has been reported that multiple signal transduction pathways are activated in response to leptin both in JEG-3 cell culture and in human term placenta [89]. Leptin is able to stimulate Janus kinase (JAK)-signal transducers and activators of transcription (STAT) pathway by promoting JAK-2 and STAT-3 tyrosine phosphorylation, which have been correlated with trophoblast invasiveness [90]. The signal transduction pathways involving mitogen-activated protein kinase (MAPK), which mediates a proliferative response, as well as PI3K, which regulates the invasive differentiation of human trophoblast, have also been found to be signaling pathways activated in response to leptin [89, 91]. Therefore, as trophoblast cells produce leptin locally, once bound to placental receptors, leptin triggers local and peripheral effects. In this way, it has been reported that placental leptin induces hCG production in trophoblast cells and increases the synthesis of extracellular matrix proteins and metalloproteinases (MMP-2 and MMP-9) that are involved in extracellular matrix remodeling [76]. In addition, leptin is a trophic and mitogenic factor for trophoblastic cell line by virtue of inhibiting apoptosis and promoting proliferation [92]. In this context, it has reported that leptin promotes growth, proliferation and cell survival of trophoblastic cells [89] by activating JAK-STAT, MAPK, and PI3K signaling pathways [93–96]. More specifically, leptin enhances cell proliferation in a dose- and time-dependent fashion, displaced the cells towards a G2/M phase as well as upregulated cyclin D1 expression, one of the key cell cycle-signaling proteins [92]. In fact, it was demonstrated that the MAPK pathway is the major signaling pathway to mediate the antiapoptotic effect of leptin in placenta [89] while that PI3K activation may mediate other functions of leptin in placenta. In this sense, both PI3K and MAPK pathways were reported to mediate the protein synthesis effect of leptin in placenta, via activation of the translational machinery (phosphorylation state of EIF4EBP1 and EIF4E) [94, 95]. This may be relevant both physiologically and pathophysiologically since a decrease in EIF4EBP1 phosphorylation has been recently found in fetuses with intrauterine growth restriction resulting from impaired placental development [94]. Recently, we have described the participation of the RNA binding protein Sam68 in leptin signaling in human trophoblastic cells, mediating the growth promoting effect of leptin [97, 98]. Concentrations of leptin in human cord blood correlate with placental size. This is in agreement with the role of leptin in regulating placental growth, which potentially leads to placental hypertrophy under conditions of leptin overproduction, such as the placenta of women with gestational diabetes (GDM) [99, 100]. Increased leptin and LEPR expression in placenta from GDM patients have been well described [86, 101]. Besides, it has been also reported an increased phosphorylation state of different proteins implicated on the initiation stage of translation, and as a result, an increased protein synthesis rate in placentas from GDM, suggesting a molecular mechanism for the observed increase in the placenta weight in GDM [86, 102]. In fact, the placental dysfunction in women with GDM is also associated with increased amino acid transport [103].

More physiological effects of placenta-derived leptin include angiogenesis and immunomodulation. Leptin has been shown to be a regulator of angiogenesis by enhancing expression of vascular endothelial growth factor (VEGF) and its receptor VEGF-R2 and by inducing neovascularization [104, 105]. Data suggest that alterations in leptin levels and the soluble LEPRs may disrupt normal angiogenic events and remodeling events during placental development and could lead to hyperactivation of the angiogenic pathways thus resulting in endothelial dysfunction. Moreover, leptin might modulate the activation of natural killer (NK) cells (70% of the decidual leukocyte population), which produce an array of angiogenic growth factors including angiopoietin-1 (Ang-1), Ang-2, and VEGF-C and have been implicated in decidual vascular remodeling [106, 107].

Leptin is a key modulator of the inflammatory and immune responses, preventing the embryo rejection by the maternal immune system [77] and regulating generation of arachidonic acid products, nitric oxide induction, and T cell cytokines [78], which play an important role in a number of normal and abnormal inflammatory processes, including the initiation and progression of human labor and delivery [108, 109]. Thus, placental leptin may have a local autocrine immunomodulatory or anti-inflammatory role [110]. However, deeper understanding of the immunology of the maternal-fetal interface promises to yield significant insight into the pathogenesis of many human pregnancy complications, including preeclampsia, intrauterine growth restriction, spontaneous abortion, preterm birth, and congenital infection.

In summary, the localization of leptin and its receptor in human placental indicates that leptin may have both autocrine and paracrine activities as a local immunomodulatory signal [111]. Deregulation of the autocrine/

paracrine function of leptin at the feto-placentomaternal interface may be implicated in the pathogenesis of GDM, preeclampsia, and intrauterine fetal growth restriction [112, 113], as described in the next section.

Role of leptin in pregnancy

Pregnancy with its associated hormonal changes (especially insulin, glucocorticoids, estrogens, and PRL) appears to be a state of physiologic hyperleptinemia and leptin resistance, with uncoupling of eating behavior and metabolic activity [114]. It is well known that serum leptin levels are higher in pregnant as compared to non-pregnant women [115–117]. Moreover, serum leptinemia correlates with maternal body weight [118]. However, this elevation does not appear to be mediated by increased body weight and adiposity, since circulating leptin concentrations increase dramatically well before the occurrence of increased body weight [116]. Current data demonstrate that placenta is capable of contributing significantly to the higher levels of leptin seen in maternal circulation during healthy pregnancy [61, 114, 119, 120]. In fact, placental leptin expression patterns coincide with maternal serum leptin levels. Maternal leptin serum levels steadily increase during the first and second trimesters and peak in late second or early third trimester [61, 114, 121]. These high levels are maintained throughout the remainder of gestation and decline drastically postpartum. Moreover, in pregnancy, increases in LEPRe are also observed. Hence, leptin resistance may result from an inability of leptin to disassociate from leptin-LEPRe complex leading to decreased free leptin and decreased binding to membrane bound receptors or by the inability of leptin-LEPRe complex to cross the blood-brain barrier and reach its target tissue. This central leptin resistance may act as a compensatory mechanism to meet the developing fetal energy needs akin to the maternal insulin resistance that occurs in later gestation. Interestingly, normal weight pregnant women and obese non-pregnant individuals seem to have similar increases in circulating leptin levels compared with their counterpart non-pregnant or healthy controls. They also exhibit alterations in signaling of the appetite center of the brain, (i.e., both exhibiting a form of leptin resistance).

The current view is that the maternal metabolic environment may generate stimuli within the placenta resulting in the increased production of leptin and inflammatory cytokines whose expression is minimal under normal pregnancy. In this sense, there are several pregnancy molecules and hormones, commonly increased in the course of pregnancy, involved in leptin up-regulation in the placenta. Leptin expression has been shown to be up-regulated by different pregnancy hormones, such as chorionic gonadotrophin, and 17beta-estradiol, and by second messengers, such as cyclic adenosine 5′-monophosphate, mediated through MAPK and PI3K signaling pathways [83, 122–126]. Hyperinsulinemia in the pregnancy, probably also may regulate placental leptin production, perhaps acting as a circulating signal to control fetal homeostasis [102]. In fact, insulin is an inducer of leptin production in human placenta as shown in vivo [84], enhancing the activity of leptin promoter region [85]. This may be relevant in gestational diabetes [6, 86, 112, 127], a state of greater insulin resistance and hyperinsulinemia than normal pregnancies. In this line, numerous studies have reported that circulating leptin levels, as well as placental leptin and LEPR expression are significantly higher in pregnant women with GDM compared to healthy control women with uncomplicated pregnancy [128, 129], providing, at least, a molecular mechanism for the placenta overgrowth previously observed in GDM [99, 100]. Others common complications of pregnancy, such as type 1 diabetes mellitus (T1DM) and preeclampsia are also associated with an increase in the concentration of leptin in the maternal blood as well as an increase in placental leptin gene expression [130, 131]. In addition, it has been observed an increase in the LEPRe expression in the cytotrophoblast layer of the placenta in preeclampsia compared to healthy pregnancy [16].

Intriguingly, elevated leptin levels in venous cord blood correlate significantly with the development of preeclampsia in human females [132]. This increase in leptin levels occurs before clinical symptoms of preeclampsia are present, which usually are observed in the third trimester of pregnancy, a time at which maternal serum leptin levels typically decline. However, a causative role for leptin in the development of preeclampsia has not been established yet.

Finally, it has been hypothesized that leptin, in concert with other hormones upregulated during pregnancy (i.e., estrogens), may be a local growth factor acting as a functional link between adipocytes and epithelial cells of the mammary gland, providing information on the adequacy of energy stored in adipose tissue. Intriguingly, the highest level of LEPR expression occurred during mid-pregnancy when active growth of the mammary gland is initiated, indicating that the LEPR may be important in regulating mammary gland growth and development during pregnancy and lactation [133]. Thus, absence of leptin may result in failure of mammary gland growth and

subsequent failure of lactation, as evidenced by the complete failure of lactation in *ob/ob* female mice after an otherwise normal delivery [134]. These results argue against an important role of leptin on pregnancy, however, leptin has also been detected in colostrum and breast milk and results from regional production by mammary epithelial cells and diffusion from the maternal circulation [135]. In this sense, leptin may play an important regulatory role in suckling offspring, possibly affecting growth and/or food intake [135, 136].

The argument against a relevant role of leptin in pregnancy has also been raised in humans. Thus, it has recently been reported a clinical case that describes a spontaneously conceived pregnancy in a woman with a LEPR mutation and the child's growth and development have been normal, an observation that calls into question the belief that leptin is necessary for normal reproductive function [137]. In any case, since placenta trophoblast is developed from the embryo, the placenta from this woman with a LEPR mutation may express LEPR, and therefore, leptin may be important for the placenta growth and function.

In summary, leptin may have regulatory roles in pregnancy, and the leptin resistance in healthy pregnancy seems to be central and beneficial for mobilizing energy stores to support adequate fetal growth.

Role of leptin in fetal development

Several studies have shown that leptin also regulates fetal growth and development [7, 82, 138], however, whether the placenta contributes to circulating fetal leptin is still under debate. Leptin are localized in villous vascular endothelial cells in direct contact with maternal and fetal blood, respectively [139]. Even though leptin is secreted by the placenta into the fetal circulation, the rate of this secretion is minimal (98.4% released into the maternal and 1.6% into the fetal circulation) [140] and increases during late pregnancy in parallel with an upregulation of expression of the shorter isoforms of the LEPR in the placenta [141, 142]. To date leptin levels in fetal blood are still believed to be mostly independent from placental and/or maternal contributions and correlate more with fetal fat mass, as it does in the adult, reinforcing this notion [120, 143]. In accordance with this, an increase in circulating leptin levels in macrosomic fetuses and decreased leptin levels in growth restricted fetuses have been reported [128, 144].

However, it has been demonstrated that augmentation of placental leptin expression may have a contribution to fetal growth, independently of maternal glucose control [145]. In addition, fetal concentrations of leptin and insulin are increased in venous cord blood without modification of maternal circulating leptin levels [30], suggesting that placental leptin release is more important for fetal than for maternal leptin levels. It is interesting to note that elevated venous cord leptin levels have also been shown to correlate significantly with increased birth weight [146], suggesting that increased levels of leptin in venous cord blood could possibly be a causative factor for the higher birth weights typically observed in infants born from diabetic women. In this sense, insulin has been involved in the regulation of placental leptin [85, 86] and recent data have provided new molecular mechanisms that might underlie the increased growth of placenta and fetal overgrowth observed in GDM [85, 86]. Briefly, an increased leptin production stimulated by insulin might act as a fetal growth factor and as result, giving rise to large-for-gestational-age infants. In fact, cord blood leptin levels are elevated in infants of diabetic mothers and in large-for-gestational-age newborns [130]. However, whether increased leptin production is due to increased fetal fat mass [147, 148] or others factors could affect adipose tissue in the fetus remain unclear, and further investigations regarding the feto-maternal leptinemia are necessary to clarity this point [149, 150].

Thus, it is possible that fetal plasma leptin could be derived from the placenta (leptin mRNA is detected from early gestation, i.e., weeks 7–14, up to term [151]) and from fetal adipose tissue, which appears and develops progressively from 14 weeks of gestation to term [152]. Anyway, there is evidence that leptin may have a range of neuroendocrine and endocrine actions in the fetus. Leptin has been shown to bind to LEPRs in fetal organs, suggesting that leptin may be able to influence fetal growth and development [111]. The high level of expression of leptin (and its receptor) [140] in fetal bone suggests a role for leptin in bone or cartilage development, as well as in the development of ossification. In fact, leptin plays an important role in the regulation of fetal skeletal development, acting on both chondrocyte and osteoblast differentiation and proliferation [138]. More specifically, fetal serum leptin levels are negatively correlated with serum markers of bone resorption [153], suggesting a possible effect of leptin on the overall increase of bone mass by decreasing bone loss. Leptin may also be associated with fetal pulmonary system during intra-uterine development [82]. In addition, leptin has a role stimulating myelopoiesis, erythropoiesis, lymphopoiesis, and thus, it may also promote maturation of the fetal immune system [140, 154]. The presence of mature leptin protein in several tissues of

the fetus contrasts with the absence of leptin in the corresponding adult tissues [140, 155]. This suggests that leptin is a growth factor in fetal development, rather than acting as a signal of fetal energy stores to the fetal CNS, as it does in adults. Thus, leptin may be considered an important factor during fetal organogenesis.

Finally, a role for leptin in the early programming of later obesity has also been suggested. A programmed alteration in the synthesis, secretion or actions of leptin may play a role in the early origins of later obesity following exposure to either relative over- or undernutrition in early life [156].

In summary, these data suggest that leptin has a role in intrauterine and neonatal development and that the placenta provides a source of leptin for the growing fetus.

Conclusions

As discussed in this review, leptin control of reproduction has been intensely studied over the past several years. Clearly, neuroendocrine actions of leptin and signaling mechanisms have received most of the attention and are the best understood at this time. We can conclude that leptin controls reproduction depending on the energy state of the body, and sufficient levels of leptin are a prerequisite for the maintenance of reproductive capacity. Leptin plays an integral role in the normal physiology of the reproductive system with complex interactions at all levels of the HPG axis. At the central level, leptin has a stimulatory effect in the regulation of gonadotropin secretion. Whether this is a direct or indirect effect of leptin's action on kisspeptin neurons is yet to be fully resolved. At the peripheral level, in the ovary, leptin antagonizes the effect of growth factors on gonadotropin-stimulated steroidogenesis, to augment reproductive function of females.

Moreover, leptin signaling mainly involves activation of JAK/STAT, MAPK/ERK and PI3K pathways in the cell, however, the LEPR-Tyr1077-Stat5 might serve as an important mechanism by which leptin modulates endocrine function, linking body adiposity and the reproductive axis. Future studies have to resolve the question of how leptin Tyr1077-Stat5 signaling controls reproduction.

Leptin and LEPRs are expressed in other sites, such as mammary epithelial cells, blastocyst, endometrium, placenta, immune system and fetal tissues, leading to the suggestion that leptin may have additional regulatory roles in successful establishment of pregnancy, fetal growth and lactation. However, further work is needed to provide a clearer and precise role of leptin in each of these critical reproductive processes. The role of leptin in mitogenic, antiapoptotic, protein synthesis, angiogenic, immune modulation and placental nutrient transport is a widely accepted fact. Its deregulation in the placenta has been implicated in the pathogenesis of various disorders during pregnancy, such as GDM and preeclampsia. Finally, observational studies have demonstrated that states of leptin excess, deficiency, or resistance can be associated with abnormal reproductive function. Future interventional studies involving leptin administration are expected to further elucidate these complex relationships and potentially provide new and better options in our therapeutic arsenal for the reproductive function in women.

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Antonio Pérez-Pérez obtained his specialty in Clinical Biochemistry and his PhD in 2007 at the Virgen Macarena University Hospital and in the University of Seville, respectively. He investigated leptin and leptin receptor expression as well as the signaling pathways in placenta. He was supervised by Dr Víctor Sánchez-Margalet. Currently, he works in the laboratory of Clinical Biochemistry of the Virgen Macarena University Hospital, Seville, Spain. His research is focused on investigating the molecular mechanism involved in the gestational diabetes and the role of leptin and insulin in this process.

As a biochemist, Flora Sánchez-Jiménez started her specialty in Clinical Biochemistry and her PhD studies in 2006 at the Virgen Macarena University Hospital and University of Seville, respectively. In 2010 she finished her formation period as a collaborator and team member in the group headed by Dr. Víctor Sánchez-Margalet. There, she developed work related to the Sam68 protein and leptin signal transduction. Currently, she works in the Clinical Biochemistry Unit at the Virgen Macarena University Hospital. Her current research is focused on investigating the role of the Sam68 protein in insulin and leptin related-cancer.

Julieta L. Maymó graduated in Molecular Biology from Universidad de Buenos Aires (UBA), Buenos Aires, Argentina. She obtained her PhD in the Department of Biological Chemistry at the UBA. She investigated the regulation of leptin expression and its function in placenta. Currently, she works as investigator, in the laboratory of Placental Molecular Physiology (UBA). She is supervised by Dr Cecilia Varone. Her research is focused on investigating the amniotic placental stem cells (hAECS) and their regenerative properties. Specifically her studies are centered on examining the hepatic diffentiation of the hAECs and the cellular and molecular process involved.

José L Dueñas obtained his MD in 1979 and the PhD in 1982 at the University of Valladolid, Spain. He was a postdoctoral fellow in the University Pierre and Marie Curie and the Northwestern University of Chicago. He is now Professor of Obstetrics and Gynecology in the Medical School of Seville, and Chief of the Ob-Gyn Service in the Virgen Macarena University Hospital, in Seville, Spain. His research interests include anticonception, fertility, and pathology of pregnancy.

Cecilia Laura Varone received her PhD in Biology from the School of Sciences of the University of Buenos Aires. She joined the National Council for Scientific Research of Argentina in 2002, where she is currently an independent researcher. She is the Director of the Laboratory of Placental Molecular Physiology, Department of Biological Chemistry, School of Natural and Exact Sciences, University of Buenos Aires. She also is a Teaching Professor of Molecular Biology. Her research is focused on investigating the regulation of leptin expression and function in placenta.

Víctor Sánchez-Margalet obtained his MD in 1988, and PhD in 1991 at the Medical School, University of Seville. He obtained the specialty of Clinical Biochemistry in 1993 at the Virgen Macarena University Hospital. He was a postdoctoral research fellow in the University of San Francisco (UCSF) for 2 years, investigating molecular mechanisms of insulin action and resistance. He has been Clinical Chemist at the Virgen Macarena University Hospital, Seville Spain since October 1994, and he is full Professor of Biochemistry and Molecular Biology, and Clinical Biochemistry since 2004, at the Medical School, University of Seville, Spain. He is a member of the Royal National Academy of Medicine, Spain, as specialist in Clinical Biochemistry. His research interests include obesity, insulin resistance, diabetes and the role of leptin in immune cells and the placenta.