
Direct Effects of Leptin on Gonads, Gametes, and Embryos: Is Too Much a Bad Thing?

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Abstract

Swain JE, Smith GD. *Direct Effects of Leptin on Gonads, Gametes, and Embryos: Is Too Much a Bad Thing?* *ARBS Ann Rev Biomed Sci* 2004;6:99-108. Originally discovered as a satiety factor and regulator of metabolism and fat deposition, emerging data suggest a role for leptin in regulation of reproductive function. The vast majority of research in this area focuses on the role of leptin in regulation of neuroendocrine function and the effects of the hormone on the hypothalamo-pituitary-gonadal axis. However, evidence exists indicating a direct role of leptin in regulation of the gonads, gametes and preimplantation embryos. The presence of leptin, its receptor, as well as studies demonstrating effects on function, development and activation of transcriptional and signal transduction pathways, all support the notion that leptin is directly affecting the ovary, testis, oocyte, spermatozoan and preimplantation embryo. These direct effects may be associated with reduced fertility.

Key-words: leptin, oocyte, ovary, sperm, testis, embryo.

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Introduction

Since its discovery, leptin has been the focus of an immense amount of scientific research. A vast majority of this work has focused on leptin's role in regulation of weight gain and fat deposition. A 16-kDa protein product of the obese gene, leptin is a satiety hormone primarily secreted by mature adipocytes and is involved with the regulation of appetite and metabolism through interactions within the hypothalamus (Zhang *et al.*, 1994; Pelleymounter *et al.*, 1995). However, recent research indicates a role for leptin in regulation of reproductive function.

Due to the long-known relationship between nutritional status and fertility, there has been a search to identify a signal linking adipose tissue stores and the reproductive system. Too little body fat has been shown to be associated with reproductive abnormalities (Frisch, 1990), while obesity is also correlated with reduced fertility. Interestingly, there is a positive correlation between leptin levels and percentage of body fat in humans (Dagogo-Jack *et al.*, 1996; Klein *et al.*, 1996; Maffei *et al.*, 1995; Considine *et al.*, 1996). Therefore, it is suggested leptin may serve as the signal from adipose tissue to the reproductive system, indicating whether adequate energy stores are available for normal reproduction (Tataranni *et al.*, 1997).

The role of leptin in regulation of neuroendocrine function, onset of puberty and control of fertility has been the focus of several reviews (Rene-Gonzalez *et al.*, 2000b; Spicer, 2001; Smith *et al.*, 2001; Moschos *et al.*, 2002). However, there is an increasing body of literature suggesting direct effects of leptin on gonadal, gamete and embryo development and function. The objective of this review is to summarize the literature surrounding leptin's mode of action and its impact on development and function of male and female gonads and gametes, as well as its effects on the mammalian preimplantation embryo.

Leptin and the Ovary

Several lines of evidence have emerged indicating a direct role for leptin in regulation of the mammalian ovary. Firstly, leptin receptor has been identified through the use of RT-PCR in human theca, granulosa (Karlsson *et al.*, 1997; Agarwal *et al.*, 1999; Loffler *et al.*, 2001) and cumulus cells (Cioffi *et al.*, 1997). Similarly, reverse transcription experiments have identified leptin receptor mRNA in porcine ovary, corpus luteum, theca and granulosa cells. (Lin *et al.*, 2000; Ruiz-Cortez *et al.*, 2000), as well as in rat ovarian tissue (Zamorano *et al.*, 1997). Immunohistochemistry has also identified the active form of the leptin receptor in mouse theca and granulosa cells, as well as ovarian stroma and corpora lutea (Ryan *et al.*, 2002).

Secondly, the ovary has been proposed to be a possible site of leptin synthesis. Indeed, leptin and its mRNA have been identified in the human ovary (Cioffi *et al.*, 1997; Loffler *et al.*, 2001). Also, follicular fluid has been found to contain leptin (Karlsson *et al.*, 1997; Barroso *et al.*, 1999; Butzow *et al.*, 1999), suggesting its availability to activate the leptin receptor within the follicle.

Thirdly, *in vitro* studies have indicated functional roles for leptin in the ovary of several species. High doses of leptin cause a dose dependent decrease in progesterone and androstenedione production by bovine theca cells (Spicer & Francisco, 1998) and inhibits insulin-like growth factor I (IGF-1)-augmented human thecal cell steroidogenesis (Agarwal *et al.*, 1999). Several studies have also examined effects of leptin on granulosa cell function. Leptin inhibits IGF-1-induced estradiol production by cultured rat (Zachow & Magoffin 1997) and human granulosa cells (Agarwal *et al.*, 1999) and insulin-induced estradiol production by bovine granulosa cells (Spicer & Francisco, 1997). Similarly, leptin also inhibits LH-induced estradiol production by human granulosa cells (Karlsson *et al.*, 1997) and impairs the synergistic effect of TGF- α and FSH on estradiol synthesis in rat granulosa cells (Zachow *et al.*, 1999). Furthermore, luteinized human granulosa cells show decreased hCG-induced progesterone production in response to increasing doses of leptin

(Brannian *et al.*, 1999). These data indicate high levels of leptin may be inhibitory to ovarian function as they seem to attenuate stimulated, but not basal ovarian steroidogenesis. However, it has been reported high doses of leptin can inhibit unstimulated estrogen, but not progesterone production by human granulosa-lutein cells (Ghizzoni *et al.*, 2001). Additionally, physiological levels of leptin are able to inhibit glucocorticoid-induced production of pregnenolone, progesterone, and 20 α -hydroxy-4-pregnen-3-one in rat granulosa cell lines was increased in the presence of leptin (Barkan *et al.*, 1999). Conversely, it has been found leptin is able to stimulate aromatase activity and estrogen production in luteinized human granulosa cells (Kitawaki *et al.*, 1999). Physiological doses of leptin injected into rats increase adrenodoxin and StAR expression, as well as progesterone secretion, suggesting that the steroidogenic capacity of the rat ovary is elevated, compared to control treatments (Almog *et al.*, 2001). Also, a biphasic effect of leptin was observed in pig granulosa cells, where physiological levels of leptin were stimulatory to steroidogenesis, while only higher levels resulted in inhibition of estrogen production (Ruiz-Cortez *et al.*, 2003).

The majority of studies examining leptin's role in regulation of ovarian steroidogenesis utilize cell culture systems. Unfortunately, these systems don't account for various autocrine/paracrine effects present in the ovary and follicle, which may affect function. Thus, an increasing number of studies have begun to examine the effects of leptin on follicular development and function. It has been reported leptin inhibits FSH-stimulated follicular growth in a dose dependent manner, as well as cAMP and forskolin-induced estrogen production (Kikuchi *et al.*, 2001). In agreement with these findings, increasing doses of leptin during individual follicle culture was also found to inhibit follicle growth (Swain *et al.*, unpublished data.). During follicular culture, it was also found leptin inhibits mouse granulosa cell proliferation (Kikuchi *et al.*, 2001). Conversely, hypertrophy of granulosa cells was observed in rats following leptin injection (Almog *et al.*, 2001) and this injection reduces follicular apoptosis (Almog *et al.*, 2001), suggesting a possible stimulatory effect on growth.

Follicle culture studies have also been utilized to examine ovarian function. Administration of increasing amounts of recombinant mouse leptin to follicular culture increases estrogen, testosterone and progesterone production (Swain *et al.*, unpublished data). Supporting these findings, injection of rats with physiological doses of leptin increases testosterone and progesterone production in the ovary (Cannady *et al.*, 2000). Furthermore, high leptin reduces the number of ovulated oocytes in rats (Duggal *et al.*, 2000). However, it has also been reported physiological doses of leptin injected into rats resulted in higher number of ovulations (Almog *et al.*, 2001). Therefore, it is extremely evident many conflicting reports exist concerning the effects of varying concentrations of leptin on follicular development and function.

Leptin may be responsible for regulatory actions within the ovary, in addition to follicle growth and steroidogenesis. It has been hypothesized leptin may be responsible for regulation of processes involved in luteinization of granulosa cells (Ruiz-Cortez *et al.*, 2003). This is supported by studies showing leptin receptor abundance in porcine granulosa cells increases with luteinization *in vitro* and *in vivo* (Ruiz-Cortez *et al.*, 2000).

Further evidence for a direct effect of leptin on the ovary can be seen in alterations in transcriptional activity and signal transduction pathways. Leptin induces c-Jun expression and attenuates the transcriptional activity of the glucocorticoid receptor in granulosa cells, possibly leading to this decreased steroidogenesis (Barkan *et al.*, 1999). Physiological doses of leptin stimulate the JAK-STAT pathway, resulting in increased STAT3 phosphorylation as well as an increase in the concentration of the active form of SREBP1 and StAR promoter activity in porcine granulosa cells (Ruiz-Cortez *et al.*, 2003). However, leptin had no effect on StAR mRNA levels from unstimulated human granulosa-lutein cells (Ghizzoni *et al.*, 2001).

Leptin and the Testes

A possible role for direct effects of leptin in rodent and human testis has been suggested. Leptin receptor has been identified through *in situ* hybridization in Leydig and Sertoli cells of adult rats (Tena-Sempere *et al.*, 2001^b). Reverse transcription-PCR studies have also identified leptin receptor mRNA in rat Leydig cells (Caprio *et al.*, 1999; Caprio *et al.*, 2002). Additional studies indicate leptin receptor mRNA is present in testes from pubertal rats, but declines in adulthood (Tena-Sempere *et al.*, 2001^{a,b}). Leptin receptor has also been identified in human testis (Cioffi *et al.*, 1996). Furthermore, leptin is able to cross the blood-testis barrier (Banks *et al.*, 1999) and is present in human seminal plasma and within the tubuli seminiferi (Camina *et al.*, 2002; Glander *et al.*, 2002), suggesting its availability to interact with its receptor in these tissues.

Functional studies have also revealed a possible direct role of leptin in testes. Rat Leydig cells incubated with increasing levels of leptin led to an inhibition of hCG-stimulated testosterone production (Caprio *et al.*, 1999). Leptin also inhibits hCG-stimulated testosterone production in slices of rat testes (Tena-Sempere *et al.*, 1999, 2001a). This inhibition appears to be dependent on the stage of sexual maturation, as inhibition was observed in adult, but not prepubertal rats (Tena-Sempere *et al.*, 1999; Caprio *et al.*, 2002).

Leptin appears to also affect transcription and signal transduction pathways in the male gonad. Leptin decreases mRNA expression of SF-1, StAR and P450 in rat testes (Tena-Sempere *et al.*, 2001^b). Additionally, leptin induces STAT3 phosphorylation in mouse seminiferous tubules, as well as phosphorylation of ERK1 and ERK2 in isolated interstitial cells (El-Hefnawy *et al.*, 2000). Finally, addition of leptin to rat testes *in vitro* results in decreased levels of leptin receptor mRNA (Tena-Sempere *et al.*, 2001^a).

Indirect evidence for a role of leptin on function and development of testes is apparent from studies utilizing the ob/ob adult male mouse. This mouse is sterile and has abnormal testes, as evidenced by multinucleated spermatids, few spermatozoa and decreased amounts of interstitial Leydig tissue. However, treatment with leptin restores fertility by normalizing testicular weight, spermatogenesis and Leydig cell morphology (Mounzih *et al.*, 1997). Low doses of leptin were also able to restore fertility in young ob/ob mice (Cleary *et al.*, 2001). Interestingly, high serum leptin concentrations were found in azoospermic compared to normozoospermic men (Steinman *et al.*, 2001), suggesting elevated leptin might be compromising normal sperm production.

Leptin and the Oocyte

Due to leptin's presence and action in the ovary, research has also focused on a direct role of leptin in regulation of mammalian oocyte function. Supporting the idea of a regulatory role, leptin has been identified through immunocytochemistry in mature pre-ovulatory human oocytes (Cioffi *et al.*, 1997) and via immunohistochemistry in pig oocytes (Ryan *et al.*, 2002). Western blot analysis has also identified the leptin protein in mouse GV-intact and MII oocytes (Antczak & Van Blerkom, 1997a). Due to the absence of leptin mRNA in these oocytes, it has been suggested the hormone may be maternally-derived and enter the oocyte via a receptor-mediated process, by endocytosis, or by some other unidentified mechanism (Antczak & Van Blerkom, 1997b; Matsuoka *et al.*, 1999).

Further supporting a key action for leptin in the oocyte, its receptor has been identified in mouse oocytes through immunocytochemical analysis and RT-PCR (Antczak & Van Blerkom, 1997a; Matsuoka *et al.*, 1999). Mouse oocytes actually contain splice variant forms of the receptor, both OB-Ra and OB-Rb (Kawamura *et al.*, 2002). Pig oocytes also contain leptin receptor (Ryan *et al.*, 2002). Fluorescence-label intensity for leptin receptor in mouse oocytes was more pronounced in MII oocytes, suggesting a possible stage-dependent regulatory role for leptin in oocyte meiosis (Matsuoka *et al.*, 1999). This hypothesis is further supported by the fact that treatment of MII oocytes with physiological levels of leptin results in tyrosine phosphorylation of STAT3 (Matsuoka *et al.*, 1999). Thus, leptin may alter gene transcription during oocyte development. Leptin is asymmetrically localized in the fully-grown GV-intact and MII mouse and human oocytes.

This localization is coincident with STAT3 distribution and may delineate the future animal pole (Antczak & Van Blerkom, 1997a). Thus, these proteins may act as regulatory molecules and be involved in subsequent development of the resulting embryo.

Functional studies have also indicated a role for leptin in oocyte development. The addition of leptin during *in vitro* oocyte maturation advances the onset of germinal vesicle breakdown (GVBD) and reduces cumulus cell coupling in pig oocytes (Galeati *et al.*, 2000). While leptin had no effect on resumption of spontaneous maturation in denuded or cumulus enclosed pig oocytes, it did result in increased rates of GVBD in oocytes cultured within preovulatory follicles (Ryan *et al.*, 2002). Maturation of pig oocytes in the presence of leptin results in altered oocyte metabolism compared to controls (Swain *et al.*, 2001). Subsequent culture of embryos in leptin derived from these leptin-matured oocytes also results in decreased blastocyst development (Swain *et al.*, unpublished data). It is known oocyte metabolism is predictive of subsequent embryo developmental competence and thus may indicate the quality of the maturing oocyte (Krisher & Bavister, 1999; Spindler *et al.*, 2000). Thus, high doses of leptin may be inhibitory to oocyte developmental competence by causing premature meiotic resumption and/or altering metabolic activity.

Although leptin and its receptor are found within the oocyte, it has been suggested the protein hormone is not required for normal oocyte development. Oocytes from *ob/ob* mice are able to undergo normal fertilization and embryo development, resulting in pregnancy and live birth, following transplant to a normal surrogate (Runner & Gates, 1954). Similarly, transplant of ovaries from obese mothers to normal surrogates result in offspring (Hummel, 1957). Finally, treatment of *ob/ob* females with exogenous gonadotropins also allows for successful ovulation, fertilization and embryo development (Smithberg & Runner, 1957), suggesting the sterility defect in these obese mice is not at the level of the oocyte. Supporting this notion, *in vitro* maturation of cumulus enclosed mouse oocytes in the presence of leptin had no effect on GVBD or MII development (Swain *et al.*, unpublished data). Similarly, oocytes isolated from mouse follicles cultured for 9d in the presence of varying doses of leptin had no differences in GVBD or MII following *in vitro* maturation (Swain *et al.*, unpublished data). A similar study in rats showed no effect on meiotic resumption in oocytes isolated from gonadotropin-stimulated follicles (Duggal *et al.*, 2002). Thus, it remains to be determined whether leptin is required for normal oocyte development and function.

Leptin and Sperm

Functional leptin receptor has also been identified in male mouse germ cells (El-Hefnawy *et al.*, 2000). Immunohistochemistry revealed age and stage dependent distribution of leptin receptor throughout the testis, showing expression in spermatogonia of young mice and localization to spermatocytes in adults (El-Hefnawy *et al.*, 2000). It is suggested leptin may be responsible for regulation of growth and differentiation of these developing germ cells.

Leptin may also be involved in regulating sperm function. Leptin levels show a negative correlation with percentage of motile spermatozoa (Glander *et al.*, 2002). However, it has been reported there is no correlation between leptin levels and sperm concentration, motility, vitality or morphology (Camina *et al.*, 2002). Thus, additional research is required to elucidate leptin's role in regulation of sperm development and function.

Leptin and the Preimplantation Embryo

There is a growing amount of literature indicating a direct role for leptin in regulation of preimplantation embryo development. It has been shown leptin is differentially distributed and coincident with STAT3 between daughter blastomeres in cleavage stage embryos, the inner and outer cells of the morula, and the inner cell mass and trophectoderm of the blastocyst. (Antczak & Van Blerkom, 1999). The authors suggest that this distribution may be of developmental significance, possibly

involved in regulation of gene transcription, aiding in the understanding of fragmentation of the embryo, or establishment of the inner cell mass and trophectoderm in the blastocyst. Also, leptin is present in the oocyte and therefore may play a role in early stages of preimplantation embryo development. Leptin mRNA is present in the mouse blastocysts and hatched blastocysts (Kawamura *et al.*, 2002), indicating that, at these stages, mouse embryos are actively making the protein hormone. Indeed, it has been reported human blastocysts secrete leptin, and that levels are higher than arrested embryos (Gonzalez *et al.*, 2000).

Leptin receptor has also been identified in the mammalian preimplantation embryo. Leptin OB-R receptor has been identified in mouse embryos using immunofluorescence, showing localization to the nuclear region with diffuse cytoplasmic staining (Antczak & Van Blerkom, 1999). Both OB-Ra and OB-Rb mRNAs were detected in oocytes, decreased at the 2-cell stage, and increased in morula stage embryos (Kawamura *et al.*, 2002), indicating a switch of leptin responsiveness from maternal to embryonic control.

Several *in vitro* studies have shown a direct functional effect of leptin on embryo development. Culture of 2-cell or 8-cell embryos in leptin at 10ng/ml is able to increase development to the blastocyst stage, while 100ng/ml of leptin at the 2-cell stage is inhibitory (Herrid & McFarlane, 2001), indicating a possible dose and stage dependent response. Also, maturation of porcine oocytes and culture of subsequent embryos in the presence of increasing doses of leptin reduces blastocyst development (Swain *et al.*, 2001). However, addition of leptin to 1-cell mouse embryos at varying doses had no effect on subsequent cleavage or degeneration at 24 hours, or to blastocyst or hatched blastocyst by 96 hours (Swain *et al.*, unpublished data). Similarly, addition of leptin during *in vitro* culture of porcine embryos had no effect on blastocyst development (Swain *et al.*, 2001). Contradicting these findings are reports that increasing doses of leptin during mouse embryo culture increase development to blastocyst, expanded blastocyst and hatched blastocyst stages (Kawamura *et al.*, 2002). These data were supported by the observed inhibitory response to rate of blastocyst formation when mouse embryos were cultured in the presence of leptin and anti-OB-R antibody (Kawamura *et al.*, 2002). Furthermore, leptin increases total blastocyst cell number by increasing proliferation of trophectoderm and inner cell mass (Kawamura *et al.*, 2002).

Indirect evidence for a regulatory role on leptin on the developing preimplantation embryo also exists. Leptin levels have been negatively correlated with human follicular oxygen content and may be a marker of follicular hypoxia (Barroso *et al.*, 1999), which may result in compromised embryo development. Also, patients who successfully became pregnant from IVF had lower follicular fluid (Mantzoros *et al.*, 2000) and serum levels of leptin than those who did not (Brannian *et al.*, 2001). Lower leptin levels in these patients resulted in a greater proportion of high quality embryos, yet had no effect on number of mature oocytes retrieved or fertilized, suggesting leptin may be affecting post-fertilization events, such as embryo development or implantation (Brannian *et al.*, 2001). Additionally, leptin mRNA was detected in mouse oviduct and uterus, while immunohistochemical staining indicated leptin was more abundant in these tissues of pregnant mice, compared to virgins (Kawamura *et al.*, 2002). Uterine fluid contains detectable levels of leptin, while oviductal fluid does not (Kawamura *et al.*, 2002), suggesting a possibly direct role of leptin in normal blastocyst function. Human endometrium contains mRNA for both leptin and the long form of the leptin receptor (Rene-Gonzalez *et al.*, 2000a). Therefore, leptin may serve as a signal for implantation between the blastocyst and human endometrium. These data suggest leptin may exert some sort of stage regulatory action on the developing preimplantation embryo as it migrates through the reproductive tract.

Concluding Remarks

Based on a review of the literature, it is apparent there is an abundance of contradictory information regarding the direct effects of leptin on the mammalian gonads, gametes and preimplantation embryo. However, considering the present data, it appears as if leptin is directly regulating gonadal, gamete and preimplantation development and function. These direct effects may be contributing to altered fertility in overweight patients with elevated levels of leptin.

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