Leptin Impairs the Synergistic Stimulation by Transforming Growth Factor-β of Follicle-Stimulating Hormone-Dependent Aromatase Activity and Messenger Ribonucleic Acid Expression in Rat Ovarian Granulosa Cells

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ABSTRACT

Leptin blocks the insulin-like growth factor-I-induced increase in FSH-dependent estradiol-17β (E₂) production by rat ovarian granulosa cells (GC) in vitro. To determine whether the leptin effect extended to another positive modulator of FSH-dependent E₂ production, the direct ovarian effects of leptin on transforming growth factor β (TGF-β) were investigated. Reverse transcription-polymerase chain reaction demonstrated that theca-interstitial cells (TIC) from hypophysectomized rats expressed only a non-signaling transducing isoform (OB-Ra) of leptin receptor mRNA. Leptin had no effect on TIC androgen production. In contrast, mRNAs for OB-Ra and the signal-transducing (OB-Rb) leptin receptor isoforms were expressed in GC. When GC obtained from 26-day-old rats were cultured (48 h) with FSH and androstenedione, both estrone (E₁) and E₂ levels increased over those in untreated controls. In the presence of FSH (0.1 IU/ml), TGF-β (10 ng/ml) potentiated E₂ and E₁ accumulation by 2.7- and 1.4-fold, respectively. Leptin did not alter basal or FSH-stimulated E₁ and E₂ levels. However, leptin suppressed the effect of TGF-β on FSH-dependent E₂ and E₁ production by 30% and 29%, respectively. Aromatase cytochrome P450 (P450 arom) mRNA expression and P450 arom activity were increased by FSH and further augmented by the addition of TGF-β. Leptin abolished the TGF-β effect on P450 arom mRNA expression, and it decreased P450 arom activity by approximately 27%. These data support the hypothesis that leptin antagonizes the stimulatory effects of TGF-β on FSH-dependent estrogen production by a mechanism involving the leptin-induced attenuation of P450 arom activity and mRNA expression in GC.

INTRODUCTION

Leptin is a 16-kDa protein produced principally by adipocytes [1]. Leptin was initially identified because of its effects on appetite suppression and fat metabolism in rodents [2]. Additional studies demonstrated that exogenous leptin exerts regulatory functions within the rodent reproductive-endocrine axis by restoring fertility in the leptin-deficient (Ob/Ob) mouse [3]. The positive effects on fertility appear to be related to increased secretion of LH and FSH, as a result of an increased production of LHRH [4]. Collectively, these data indicate that leptin promotes fertility at the neuroendocrine level by increasing gonadotropin release. However, recent evidence demonstrates that direct ovarian effects of leptin cannot be ruled out [5–7].

Using mouse brain [8], pancreatic β cells [9], and transfected cell lines [10], it has been demonstrated that leptin binds with high affinity to a cell-surface receptor (OB-R). OB-R is structurally similar to the class I cytokine receptor gp130 subunit [10]. Three major classes of leptin receptor isoforms have been identified in the rat brain: 1) the long, signal-transducing isoform, OB-Rb; 2) truncated isoforms, OB-Ra, OB- Rc, and OB-Rd; and 3) a soluble isoform, OB-Re [11]. The cellular distribution of leptin receptor isoforms within the rodent ovary has not been reported. Upon receptor activation, an intricate signaling cascade that is mediated by one or more Janus kinase isoforms, and signal transducers and activators of transcription (STAT) proteins has been shown to mediate leptin bioactivity [10]. At present, the intracellular mechanisms that regulate leptin bioactivity within the ovary are unknown.

In the ovary, FSH is required for the recruitment of small antral follicles into the growing preovulatory cohort. As a consequence of FSH stimulation, the granulosa cells (GC) differentiate into estradiol-17β (E₂)-producing cells, and E₂ is required for continued follicle viability. It has become apparent that a group of intraovarian growth factors and cytokines modulates the FSH-dependent growth and differentiation of GC [12]. Many of these regulatory factors have redundant actions in GC. For example, both insulin-like growth factor-I (IGF-I) and transforming growth factor β (TGF-β) augment FSH-dependent E₂ production. Hence, TGF-β appears to be a key supportive intraovarian factor because it promotes GC growth [13] and augments FSH-dependent E₂ synthesis [14].

Leptin has been shown to exert direct inhibitory effects on ovarian GC. In cultures of rat [5], bovine [6], and human [7] GC, leptin suppressed the sensitizing effect of insulin and/or IGF-I on FSH-dependent E₂ production. Because E₂ production is essential for ovarian follicle growth and ovulation, and IGF-I appears to be an obligatory mediator of FSH-dependent follicle development [15], it appears that leptin can interfere with an important regulatory mechanism supporting follicle viability and ultimately ovulation. In light of the redundant modulation of FSH action by growth factors and cytokines, it was of interest to determine whether leptin caused a general antagonism of the stimulatory modulators of FSH-dependent E₂ production. This study tested the effects of leptin on TGF-β regulation of E₂ production by GC, and investigated intraovarian mechanisms whereby leptin suppresses E₂ synthesis in GC.

MATERIALS AND METHODS

Reagents and Supplies

Recombinant murine leptin (carrier-free) and recombinant human transforming growth factor β1 (TGF-β; lyophilized with BSA as carrier) were purchased from R&D Sys-
tems (Minneapolis, MN). Human recombinant FSH and ovine LH were supplied by the National Hormone and Pituitary Program of the NIDDK, NICHD, and USDA (Rockville, MD). McCoy’s 5a medium (M5a, serum-free) and Medium 199 were purchased from Gibco-BRL (Grand Island, NY). Culture plates were purchased from Falcon (Lincoln Park, NJ). [1β-3H(N)]Androstenedione (A4; 21.5 Ci/mmol) was obtained from Du Pont NEN (Boston, MA). The E2 RIA kit was obtained from Diagnostic Products Corporation (Los Angeles, CA). The estrone (E1) RIA kit was obtained from Diagnostic Systems Laboratories (Webster, TX). Unless otherwise specified, all assay reagents were purchased from Sigma (St. Louis, MO).

GC Culture

All procedures using live animals were approved by the CSMC Institutional Animal Care and Use Committee. Immature (26-day-old) Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) were killed via CO2 inhalation followed by cervical dislocation. Ovaries were removed and placed in ice-cold Medium 199 supplemented with 0.1% BSA. Ovaries were cleaned of bursa and other extraneous tissues, and GC were collected from the surrounding medium following puncture [16]. GC were centrifuged (250 × g) and resuspended in a known volume of M5a, supplemented with penicillin (100 U/ml), streptomycin sulfate (100 μg/ml), and L-glutamine (2 mM). GC number and viability were determined by trypan blue exclusion using a hemacytometer.

Aliquots containing 50 000–60 000 viable GC were placed in 96-well culture plates. GC were incubated in a final volume of 200 μl M5a/well containing 0.1 μM A4 at 37°C in a humidified atmosphere containing 5% CO2 in air. Control GC were incubated without additional hormones. Designated GC were challenged with FSH (0.001–1.0 IU/ml) with or without TGF-β (10 ng/ml). Separate cultures were treated with FSH (0.001–1.0 IU/ml) plus leptin (10 ng/ml) with and without TGF-β (10 ng/ml). The leptin concentration was chosen on the basis of the reported KD (0.7 nM) for leptin binding [8] and previous studies by our lab [5], as well as serum leptin concentrations in the human [17]. Cultures were terminated at 48 h, and the conditioned media were collected and frozen at −20°C pending RIAs to measure E2 and E1 content. RIAs were conducted according to the manufacturers’ protocols.

Theca-Interstitial Cell (TIC) Culture

To measure the effect of leptin on androgen production, purified populations of TIC were obtained from the enzymatically dispersed ovaries of 26-day-old hypophysectomized rats as previously described [18]. TIC viability was determined using trypan blue exclusion. TIC were incubated in 96-well plates (approximately 4 × 10^5 viable TIC/well) in a final volume of 200 μl. TIC were either cultured in M5a alone (control), leptin (0.1, 1.0, and 100 ng/ml), or LH (0.03–10 ng/ml) to induce steroidogenic differentiation of the cells. Designated TIC were challenged with LH in the presence of TGF-β (10 ng/ml), with and without leptin (10 ng/ml). TIC were incubated for 48 h at 37°C in a humidified atmosphere containing 5% CO2 in air. Cultures were terminated at 48 h, and media were removed and frozen at −20°C until analyzed for androstenedione content by RIA [19]. The cells were frozen at −80°C pending extraction of RNA.

Leptin Receptor (OB-R) Isoform mRNA Expression

GC and TIC were harvested after the 48-h incubation period described above. For all reverse transcription (RT)-polymerase chain reaction (PCR) reactions, total RNA, DNA, and protein were extracted from the cells using the Tri Reagent method, according to the manufacturer’s protocol (Molecular Research Center, Inc., Cincinnati, OH). Four replicate wells were pooled from GC and TIC cultures, and RT was performed as described [20]. Previous studies have shown that the multiple isoforms of the leptin receptor present in rat hypothalamus can be grouped into three classes: short forms with truncated intracellular domains (OB-Ra, OB-Rc, OB-Rd), the full-length signal-transducing isoform (OB-Rb), and a soluble isoform lacking the transmembrane and intracellular domains (OB-Re) [11]. Therefore, as a positive control, RNA was extracted from fresh hypothalamus tissue harvested from intact 26-day-old female Sprague-Dawley rats. All samples were amplified using oligonucleotide primers (synthesized by Gibco-BRL) previously shown to amplify the leptin receptor isoforms OB-Ra, OB-Rb, and OB-Re [11]. After 35 cycles of PCR (94°C, 1 min; 55°C, 1 min; 72°C, 1.5 min), the amplification products were separated on a 2% agarose gel and visualized with ethidium bromide.

Measurement of Aromatase Cytochrome P450 (P450arom) mRNA

In order to determine the effect of leptin on P450arom mRNA expression, RNA was extracted from GC cultures as described above. P450arom mRNA was measured using semiquantitative RT-PCR. Primers (sense: 5’-ACT GTG CCT GTC AGT GCC AT-3’; antisense: 5’-GAC CAG AAT AAG CTT ACC A-5’) were synthesized in our lab (using an Applied Biosystems model 391 DNA synthesizer, Foster City, CA) and were designed to amplify a 426-base pair (bp) segment of the rat P450arom cDNA [21]. To control for variations in individual PCR reactions, a mutant control P450arom cDNA fragment was synthesized by site-directed mutagenesis [22]. In the P450arom cDNA, a C was substituted for a T at base 320 to introduce an MspI restriction site. The resultant mutant cDNA can be amplified by the P450arom primers but can be distinguished from the amplification products by digestion with MspI. The control cDNA (1 pg) was included in each PCR reaction (25 cycles: 94°C, 1 min; 55°C, 2 min; 72°C, 1 min), and all samples from each experiment were amplified at the same time in the presence of [32P]dCTP. The amplification products were separated on a 2% agarose gel and visualized with ethidium bromide. The individual bands were cut from the gel and counted in a β-spectrometer. P450arom mRNA values were normalized to β-actin mRNA levels measured [23] in the same samples to account for procedural variability and differences in cell number.

P450arom Activity

P450arom activity was estimated by measuring the production of [3H]H2O from [1β-3H]-A4 [24]. GC (5 × 10^5 viable GC/well, 1 ml final volume) were incubated in 6-well plates without hormones (control), with FSH alone (0.1 IU/ml), with FSH plus TGF-β (10 ng/ml), and with a combination of FSH, TGF-β, and leptin (10 ng/ml). After 48 h, fresh hormones were added to the appropriate wells and [1β-3H]-A4 (2 × 10^9 cpm, 0.1 μM) was added to all wells. After a 4-h incubation at 37°C, the media were removed and the
FIG. 1. OB-R mRNA expression in GC and TIC. GC and TIC were isolated as described in Materials and Methods. GC (5–6 × 10⁴ viable cells/well) were incubated in the presence and absence (control) of FSH (0.1 IU/ml) with A4 (0.1 μM). TIC (approximately 4 × 10⁴ viable cells/well) were incubated with and without LH (0.3 ng/ml). Freshly removed rat hypothalamus was homogenized and used as a positive control for the presence of OB-R isoform mRNAs. At 48 h, GC and TIC were harvested, and total RNA was extracted from the cells. The presence of OB-R isoform mRNAs was determined by RT-PCR using oligonucleotide primers designed to amplify unique regions of the cDNA sequences for OB-Ra (347 bp), OB-Rb (375 bp), and OB-Re (305 bp). β-Actin mRNA was amplified (517 bp) as a positive control for procedural variables. Amplification products were visualized on a 2% agarose gel stained with ethidium bromide.

FIG. 2. The effect of leptin on androsterone production by TIC. Purified populations of TIC were obtained from the enzymatically dispersed ovaries of immature rats using Percoll density-gradient centrifugation. TIC (approximately 4 × 10⁵ viable cells/well, 200 μl final volume) were incubated with and without LH (0.01–10 ng/ml), leptin (10 ng/ml), TGF-β (10 ng/ml), or the indicated combinations of hormones. Control TIC were incubated in M5a without added hormones. After 48 h in vitro, androsterone levels in TIC-conditioned media were measured using RIA. Values represent the mean ± SEM of two experiments, with four replicates per experiment. *Significantly different from LH alone (P ≤ 0.05).

FIG. 3. The effect of leptin on E₂ production stimulated by FSH or FSH plus TGF-β. GC obtained from the ovaries of immature rats were incubated (5–6 × 10⁴ viable cells/well) in the presence of A₄ (0.1 μM) with FSH (0.001–1.0 IU/ml), or FSH and TGF-β (10 ng/ml). Separate GC cultures were incubated with leptin (10 ng/ml) in the presence and absence of FSH, or leptin with FSH plus TGF-β. Controls were incubated without added hormones. All cultures were terminated at 48 h, and E₂ content in culture-conditioned media was measured by RIA. Data represent the mean ± SEM from three independent experiments, with four replicates per experiment. Within each FSH concentration, symbols with different letters are significantly different (P < 0.05).

amount of ³H₂O produced was measured [24]. Briefly, trichloroacetic acid (TCA) was added, and the precipitated proteins were removed by centrifugation at 1700 × g for 15 min. The supernatants were collected, and 1 ml of H₂O-saturated chloroform was added. The reactions were vigorously shaken for 5 sec; then the aqueous phase was aspirated from each tube and mixed with an ice-cold 5% charcoal, 0.5% Dextran T-70 solution to remove the unreacted substrate. The mixtures were centrifuged (1700 × g, 15 min). The supernatants were collected, scintillation fluid was added, and then supernatants with scintillation fluid added were counted in a β-spectrometer. To control for variations in cell numbers, GC were scraped from the wells and protein levels were measured using the Bradford method [25].

Statistical Analyses

Treatments were administered in quadruplicate, and each experiment was repeated a minimum of three times. Mean values from independent experiments were statistically analyzed by unpaired t-test, and multiple comparisons were performed using one-way ANOVA followed by Tukey’s test. Values were determined to be significant when P ≤ 0.05.

RESULTS

Leptin Receptor Isoform mRNA Expression in Rat GC and TIC

We examined the cell-specific expression of OB-Ra, OB-Rb, and OB-Re in the immature rat ovary. In GC, the mRNA for OB-R isoforms OB-Ra (Fig. 1, lane 1) and OB-Rb (Fig. 1, lane 2) were expressed. In contrast, TIC expressed only the OB-Ra mRNA (Fig. 1, lane 1). OB-Re mRNA was not detected in either GC or TIC (Fig. 1, lane 3).
FIG. 4. The effect of leptin on E1 production stimulated by FSH or FSH and TGF-β. GC obtained from the ovaries of immature rats were incubated (5–6 × 10⁴ viable cells/well) in the presence of A₄ (0.1 μM) with FSH (0.001–1.0 IU/ml), or FSH and TGF-β (10 ng/ml). Separate GC cultures were incubated with leptin (10 ng/ml) in the absence of FSH, or leptin with FSH plus TGF-β. Control GC were incubated without added hormones. All cultures were terminated at 48 h, and E₁ content in culture-conditioned media was measured by RIA. Data represent the mean ± SEM from three independent experiments, with four replicates per experiment. Within each FSH concentration, symbols with different letters are significantly different (P < 0.05).

Effect of Leptin on GC Estrogen Production

FSH stimulated E₂ production in GC (Fig. 3). The maximal stimulatory effect of FSH was detected in the presence of 0.1 and 1.0 IU/ml FSH. In the presence of 0.1 and 1.0 IU/ml FSH, TGF-β augmented FSH-dependent E₂ accumulation by 2.7- and 1.45-fold, respectively (Fig. 3). Leptin did not significantly alter basal or FSH-dependent E₂ production, whereas leptin did impair the synergistic effect of TGF-β on FSH-stimulated E₂ synthesis (Fig. 3).

In order to determine whether there was a selective effect of leptin on the conversion of E₁ to E₂ (17β-hydroxysteroid dehydrogenase activity), E₁ levels in GC-conditioned media were measured. As expected, FSH induced a dose-dependent increase in E₁ accumulation, and leptin did not alter the FSH effect (Fig. 4). In the presence of FSH (0.01, 0.1, and 1.0 IU/ml), TGF-β significantly augmented E₁ accumulation. When leptin was added to FSH- and TGF-β-stimulated cells, E₁ levels were diminished at the 2 highest concentrations of FSH tested (0.1 and 1.0 IU/ml) (Fig. 4).

Effects of Leptin on P450arom mRNA Levels and Activity

In order to understand the mechanism of leptin interference with TGF-β stimulation of estrogen production, the effect of leptin on P450arom mRNA expression was examined. In the absence of FSH, P450arom mRNA expression was tested the direct effect of leptin on androgen production in TIC. As shown in Figure 2, there was no effect of leptin on LH-dependent androgen production in the presence or absence of TGF-β. Leptin (0.1–100 ng/ml) did not alter LH-dependent androsterone levels after 48 h in vitro (data not shown). These results showing a lack of an effect of leptin on TIC androgen production are consistent with the observation that the TIC did not express the signal-transducing isoform of the leptin receptor (OB-Rb).

FIG. 5. The effect of leptin on P450arom mRNA levels. GC (5–6 × 10⁴ viable cells/well, 200 μl final volume) were incubated for 48 h in the presence and absence of A₄ (0.1 μM), FSH (0.1 IU/ml), TGF-β (10 ng/ml), and combinations of hormones. At 48 h, culture-conditioned media were used for RIA to measure E₂ and E₁ levels (Figs. 3 and 4). RNA, DNA, and protein were simultaneously extracted from the GC, and P450arom mRNA was measured using semiquantitative RT-PCR. The PCR products were separated and visualized on a 2% agarose gel stained with ethidium bromide, and the bands were cut from the gel and counted in a β-spectrometer. P450arom mRNA values were normalized to DNA and protein content in untreated (control) GC cultures to account for variations in cell number between treatment groups. Data represent the mean ± SEM of three experiments. Bars with different letters are significantly different (P ≤ 0.05).

FIG. 6. The effect of leptin on P450arom activity. GC (5 × 10⁴ viable GC/well) were incubated in 6-well plates, in the presence and absence of FSH (0.1 IU/ml), or FSH plus TGF-β (10 ng/ml), with and without leptin (10 ng/ml). Controls were incubated without added hormones. At 48 h, fresh hormones were added to the appropriate groups and [1β-3H]-A₄ (2 × 10⁶ cpm, 0.1 μM) was added to all wells, including controls. After a 4-h incubation, the conditioned media were removed and [1H₂O] was extracted and counted as described in Materials and Methods. GC were harvested and protein levels were measured using the Bradford method. Values represent the mean ± SEM of three experiments. Bars with different letters are significantly different (P ≤ 0.05).
was not stimulated above control levels by TGF-β, leptin, or TGF-β plus leptin (Fig. 5). FSH (0.01 and 0.1 IU/ml) induced an increase in P450arom mRNA above control levels that was not significantly altered by leptin. TGF-β augmented the stimulatory effect of FSH (0.1 IU/ml) on P450arom mRNA levels (Fig. 5). When GC were treated with leptin in the presence of FSH plus TGF-β, P450arom mRNA levels were reduced to levels equivalent to FSH-stimulated levels.

We next measured the effect of leptin on P450arom activity. As shown in Figure 6, P450arom activity was increased in FSH-treated GC compared to untreated control cells. In the presence of TGF-β, FSH-stimulated P450arom was augmented 4-fold. Addition of leptin reduced the up-regulation in FSH-dependent P450arom activity by 27% (Fig. 6).

**DISCUSSION**

Several isoforms of the leptin receptor (OB-R) have been identified in rodents [11]. This report demonstrates that the signal-transducing isoform of the leptin receptor (OB-Rb) [26] was expressed in leptin-sensitive rat GC. Interestingly, the pattern of expression of OB-R isoforms was cell-specific within the immature rat ovary. In GC, OB-Rb and OB-Ra mRNAs were detected, whereas, in LH-treated TIC obtained from hypophysectomized rats, only isoform OB-Ra mRNA was expressed. The demonstration that the GC responded to leptin and the TIC did not provides further evidence that leptin bioactivity in the immature rat ovary is mediated by the long OB-Rb isoform and not by the short OB-Ra isoform. These data are consistent with reports that OB-Ra may bind leptin but is not involved in leptin signaling [26]. Whether OB-Ra can bind leptin in the ovary has not been determined. In human theca, the long form of the OB-R is expressed [7, 27], and the theca cells respond to leptin [7]. The difference in OB-R expression between rat and human theca may be due to the fact that the rat theca were obtained from immature hypophysectomized animals. Alternatively, there may be species differences. For example, in the bovine ovary, 125I-labeled leptin binding was detected in theca cells [28], whereas, in the human ovary (GC and theca cells) both long and short isoforms of the leptin receptor may be expressed [7, 27]. The precise intracellular mechanisms of leptin action are unknown, but a point of convergence between the FSH/TGF-β and leptin signaling pathways appears to occur. Further studies will be required in order to determine how leptin affects FSH/TGF-β signaling cascades and how these changes regulate P450arom mRNA expression and P450arom enzyme activity in GC.

Several lines of evidence support the conclusion that TGF-β is an important intraovarian regulator that potentiates FSH action in GC. First, TGF-β mRNA is expressed by GC [29] and TIC [30], and TGF-β is secreted by TIC [31]. Second, in rat GC, TGF-β augments the FSH-stimulated cAMP-dependent second messenger pathway by increasing FSH-induced cAMP levels [32] and synthesis of both cAMP-dependent protein kinase RIβb subunit mRNA and protein [33]. Third, TGF-β stimulates GC growth [13] and steroidogenesis [14] in vitro. The present study expands this body of knowledge by demonstrating an up-regulatory effect of TGF-β on FSH-stimulated P450arom mRNA expression and P450arom activity in rat GC.

In previous reports by our laboratory and others, leptin was shown to reduce the synergistic effect of IGF-I and/or insulin on FSH-dependent E2 synthesis in rat [5], bovine [6], and human [7] GC in vitro. These observations raised the question whether the direct effect of leptin in GC was specific to IGF-I or if leptin exerted a generalized effect on multiple positive modulators of FSH action. This report demonstrated that leptin blocks the positive modulatory effects of TGF-β on FSH-dependent estrogen (E1 and E2) production. Hence, the actions of TGF-β and IGF-I, two well-characterized stimulatory modulators of GC function, are impaired by leptin. These data support the concept that the intracelluar signaling pathways mediating TGF-β and IGF-I enhancement of P450arom gene transcription in GC converge at a common point. Leptin appears to block P450arom gene transcription distal to the point of convergence.

In the present study, leptin blocked the TGF-β-dependent increase in FSH-stimulated estrogen synthesis. It appears that there was a greater effect of leptin on estrogen production than on aromatase activity. Whether or not the apparent difference is important is unclear. The concentrations of estrogens measured in conditioned medium reflect the net metabolism of A4 over a 48-h period of time by a combination of steroidogenic enzymes. E2 synthesis from A4 requires the aromatase and 17β-hydroxysteroid dehydrogenase enzymes and may be influenced by other enzymes such as 5α-reductase that can metabolize the A4 substrate or others that could metabolize the estrogen products. Unlike RIA, the aromatase enzyme assay specifically measures aromatase activity. The results of these experiments indicate that leptin treatment may affect not only aromatase activity but also the activities of other steroidogenic enzymes in granulosa cells.

Leptin mRNA has been detected in human GC [34], and immunoreactive leptin has been found in human follicular fluid [7, 27]. Together the aforementioned show a potential intraovarian leptin system, replete with ligand and one or more OB-R isoforms. Importantly, leptin production by human GC has yet to be demonstrated, and there is no difference between circulating and follicular fluid concentrations of leptin [7, 27], indicating that intraovarian leptin is likely to be of endocrine origin. It appears that the physiologic role of leptin in the ovary may be limited to conditions of obesity. For example, the circulating concentrations of leptin in lean women are too low to alter ovarian function significantly [7]. In contrast, the levels observed in obese women are sufficient to interfere with the sensitizing actions of IGF-I and TGF-β on FSH-dependent E2 production. Such an effect could inhibit fertility because sensitization of small antral follicles to FSH by intraovarian factors (i.e., IGF-I and TGF-β) is thought to be important for selection of dominant follicles [35]. Disruption of E2 production during follicle growth could cause follicle atresia. Thus, by counteracting the effect of TGF-β in GC, leptin may interfere with an essential support mechanism (e.g., augmentation of E2 production) that promotes follicle growth and maturation. Such a mechanism can help to explain how weight loss in obese women can improve their fertility [36].

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