The FASEB Journal express article10.1096/fj.04-2271fje. Published online October 25, 2004.

Leptin induces ovulation in GnRH-deficient mice

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ABSTRACT

Leptin-deficient *ob/ob* mice have reduced gonadotropin-releasing hormone (GnRH) secretion, leading to gonadotropin deficiencies, hypogonadism, and anovulation, which are completely reversed following leptin administration. To determine whether the role of leptin in ovulation is mediated exclusively through GnRH, we studied leptin's action in GnRH-deficient (*hpg*) mice, as well as *ob/ob* mice and normal, prepubertal mice in which the GnRH axis was blocked with antide. Following pretreatment with pregnant mare serum gonadotropin, leptin induced ovulation in all three mouse models. Unlike mature normal mice, these ovulations were not triggered by a luteinizing hormone (LH) surge, as demonstrated by lack of increase in its surrogate marker progesterone. Rather, leptin induced hyperemia and leakage in the follicle, as well as the proteinase ADAMTS-1 (a disintegrin and metalloproteinase with a thrombospondin-like motif), which facilitates extrusion of the follicular content. These data show that on top of its role as an inducer of GnRH secretion, leptin may elicit an LH-independent ovulation.

Key words: gonadotropin-releasing hormone • progesterone • luteinizing hormone • *ob/ob* mice • *hpg* mice

In mammals, the early phase of follicular development consists of basal (slow) follicular growth, associated with proliferation of granulosa cells. This phase does not depend on gonadotropins and is mainly under the control of growth factors of paracrine origin. The second phase, termed terminal follicular growth, is manifested by rapid enlargement of the antrum. This phase depends on the pituitary gonadotropin follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH stimulates granulosa cell proliferation and estradiol production and induces the expression of LH receptors. LH triggers the resumption of meiosis, which is followed by ovulation of mature oocytes, luteinization of granulosa and theca cells and formation of the corpus luteum (CL) (1). Oocyte maturation is initiated by the disintegration of the nuclear membrane, known as the germinal vesicle breakdown and is completed by the formation of the first polar body. Induction of follicle rupture is accompanied by a sharp increase in production of progesterone to prepare the uterus for possible embryo implantation. Exogenous gonadotropin administration is routinely practiced for treatment of infertile women who do not

exhibit the appropriate profile of FSH and LH secretion. In such cases, repeated injections of FSH are followed by administration of human chorionic gonadotropin (hCG, an LH of chorionic origin) to induce ovulation (2).

Leptin, the product of the *ob* gene, primarily regulates food intake and energy homeostasis (3). In addition, leptin plays a critical role in reproduction and the obese, leptin-deficient *ob/ob* mice serve as a rodent model for hypogonadism and sterility. Their infertility is due to hypothalamic-pituitary hormone insufficiency, leading to anovulation, which may be reversed by administration of recombinant leptin (4). Furthermore, administration of leptin to normal prepubertal mice or rats was reported to accelerate puberty and to enhance ovulation (5). Congenital leptin deficiency in humans was also reported and is associated with hypogonadism (6).

The effect of leptin on the reproductive system is primarily mediated by its hypothalamic receptor, eliciting the release of GnRH, which subsequently induces the synthesis and release of pituitary FSH and LH (7, 8). It has also been reported that serum leptin rises during the follicular phase, reaching a peak at the luteal phase of the spontaneous reproductive cycle, a finding that strongly links leptin with ovulation (9, 10). We have previously shown that leptin attenuates follicular apoptosis and accelerates the onset of puberty in immature rats (11). Several studies suggested a possible direct action of leptin on the ovary. Thus, the functional long form of the leptin receptor (OB-Rb) was found to be expressed in ovarian follicles, and leptin was shown to inhibit progesterone synthesis in ovarian granulosa cells in vitro (12)

To further investigate the possible existence of additional leptin-mediated pathways that affect follicular growth and ovulation, we studied the activity of leptin in GnRH-deficient female mice (13) and in mice whose GnRH axis was blocked by the GnRH antagonist antide (14). In such mice, leptin induced ovulation without an increase in serum progesterone. We therefore suggest that leptin induces ovulation independently of LH.

MATERIALS AND METHODS

Reagents

Murine leptin was obtained from R&D Systems (Minneapolis, MN). Pregnant mare serum gonadotropin (PMSG) and hCG were from Sanofi (Ubourne, France). Antide was purchased from Sigma (Rehovot, Israel) and L-15 medium from Gibco-BRL (Bethesda, MD).

Mice and treatments

Homozygous female C57BL/6– *ob/ob* mice and *hpg* mice aged 8–10 wk were purchased from Jackson Laboratories (Bar Harbor, ME). Prepubertal C57BL/6 female mice (21-days-old) were from Harlan (Rehovot, Israel). Prepubertal and C57BL/6– *ob/ob* mice were injected s.c. at time 0 with the GnRH antagonist, antide (1.25 μg/g body weight). The mice were also injected at the indicated times with PMSG (3–5 IU/mouse, s.c.), murine leptin (i.p., 2×5 μg/g body weight at 8 h intervals), hCG (5 IU/animal, i.p.), or their indicated combinations. Humane animal care was overseen by an institutional review board according to guidelines set by Israeli laws.

Histological examination

Ovaries and ampoulae of the oviducts were excised at the indicated times and fixed with 4% paraformaldehyde, and paraffin sections were prepared. Samples were step sectioned with 10-μm gaps and stained with hematoxylin-eosin for histological observation. Photomicrographs were acquired with a Nikon Eclipse E800M microscope and a DXM 1200 digital camera using the Nikon ACT-1 software version 2.62.

Oocyte collection

Oocytes were collected from the ampoulae of oviducts at 72 h, placed in PBS, and counted under a stereomicroscope. Cumulus cells surrounding the oocytes of C57BL/6 mice were removed by incubating with hyaluronidase (1 mg/ml) for 3 min. Oocytes were examined microscopically for maturation as indicated by absence of the germinal vesicle (germinal vesicle breakdown).

Radioimmunoassay (RIA)

Progesterone was determined in mouse sera by RIA (15). LH was determined in mouse sera by a specific RIA, obtained through the National Hormone and Pituitary Program, Harbor-UCLA Medical Center (Torrance, CA).

Quantitative RT-PCR

Preovulatory follicles were obtained from 21-day-old C57BL/6 mice treated with PMSG (5 IU/animal) for 48 h. The follicles were cultured for 17 h in L15 medium containing 10% FCS in the absence or presence of either leptin (250 ng/ml) or hCG (1 IU). Total RNA was isolated from mouse follicles and reverse-transcribed using RNase H^- reverse transcriptase (SuperScript II, Gibco-BRL) with 1 g $(N)_6$ random primer mixture (New England Biolabs (Beverly, MA). Aliquots (2 l) of the reverse transcription products were used for semiquantitative (n=3) and also for quantitative PCR in the LightCycler PCR and Detection System (Roche Molecular Biochemicals, Mannheim, Germany), using the FastStart DNA Master SYBR Green I kit (Roche) according to the manufacturer's instructions. Data are the average of two independent experiments, each done in triplicate. The following PCR primers were used: For ADAMTS-1 the forward primer was 5′ CAGTACCAGACCTTGTGCAGACCTT-3′ and the reverse primer was 5′ CACACCTCACTGCTTACTGGTTTGA-3′. For cathepsin L the forward primer was 5′ TGACACAGGGTTCGTGGATA-3′ and the reverse primer was 5′ ACCGCTACCCATCAATTCAC-3′. The expression levels of ADAMTS-1 and cathepsin L were normalized to expression of ribosomal protein L19 (forward primer 5′ CTGAAGGTCAAAGGGAATGTG-3′ and reverse primer 5′ GGACAGAGTCTTGATGATCTC-3′). The identity of the PCR product was confirmed by DNA sequencing.

Statistical analysis

All experiments were repeated at least three times. Values are the mean ± SE of these experiments where noted. Significance between experimental values was determined by unpaired Student's t test and are significant if $P < 0.05$ when data from all experiments were considered.

RESULTS

Leptin induces follicular development in *ob/ob* mice

Follicular development and ovulation require action of FSH followed by LH. The possible role of leptin in follicular development and ovulation was studied in three different models of hypogonadism: *ob/ob* mice, the GnRH-deficient *hpg* mice (16), and prepubertal C57BL/6 mice. To eliminate the known role of leptin in inducing GnRH release, we treated all *ob/ob* mice and prepubertal mice with the GnRH antagonist antide. Ovarian sections of control hypogonadal *ob/ob* mice show only early stages of follicular growth and small antral follicles (Fig. 1*A* and ref 4). Treatment of female *ob/ob* mice with leptin induced rapid follicular growth, manifested by large antral follicles, which could be observed as early as 9 h and enhanced 72 h after initiation of leptin treatment (Fig. 1*B*). This follicular development was similar to the one observed following treatment of the *ob/ob* mice with PMSG (Fig. 1*C*). When *ob/ob* mice were concomitantly treated with PMSG and leptin, an interstitial cell growth and further ovarian growth were observed at 72 h (Fig. 1*D*). However, leptin alone didn't induce follicular development in either *hpg* mice or antide-treated prepubertal mice (data not shown).

Leptin induces the formation of corpora lutea in PMSG-treated hypogonadal mice

We tested the ability of leptin to replace LH as an inducer of luteinization in the three models of hypogonadal mice following treatment with PMSG. Treatment of *ob/ob* mice with PMSG (3 IU/mouse) alone did not elicit the formation of any corpora lutea (CL, Fig. 1*C*). In contrast, treatment of such mice with PMSG at time 0 followed by leptin at 48 h led to development of CL at 72 h (Fig. 2*A*, see arrow). However, in two out of six ovaries, some luteinized follicles (1–2 per ovary) had an entrapped oocyte (Fig. 2*A*). In a control study, treatment of *ob/ob* mice with PMSG at time 0 followed by hCG at 48 h led to complete follicular development and appearance of CL at 72 h (Fig. 2*B*).

Induction of follicular development in *hpg* mice required a more extensive treatment with PMSG (5 IU/mouse, daily for 4 days). Under these conditions, formation of CL was obtained in four out of seven mice. However, these CL exhibited abnormal morphology, and entrapped oocytes were seen in about half of the CL (Fig. 2*C*). When *hpg* mice were treated with PMSG for 4 days followed by leptin on the 5th day, a large number of CL were seen in all mice (Fig. 2*D*). In a control experiment, PMSG treatment for 4 days followed by hCG (5 IU) resulted in formation of CL in all mice (Fig. 2*E*). In all cases, a significant number of entrapped oocytes was observed.

To check whether these effects of leptin could be obtained in normal mice, we repeated the study in antide-treated prepubertal C57BL/6 mice. Treatment of these mice with PMSG alone (3 IU per mouse) resulted in CL in only 4 out of 12 mice (Fig. 2*F*). Treatment of such mice with PMSG at time 0 and leptin at 48 h gave rise to mature follicles followed by CL at 72 h in all of the mice (Fig. 2*G*). In a control experiment, prepubertal mice were treated with PMSG at time 0 and hCG at 48 h, resulting in mature follicles followed by CL at 72 h in all of the mice (Fig. 2*H*). CL with entrapped oocytes were detected in mice treated with either PMSG alone or PMSG and leptin. These entrapped oocytes probably resulted from inefficient ovulatory response in the absence of LH surge.

Leptin induces ovulation in hypogonadal mice

The development of CL in ovaries of the three GnRH-deficient mouse models following treatment with PMSG and leptin led us to test the possibility that leptin could replace hCG as an inducer of ovulation. To this end, we treated GnRH-deficient female *hpg* mice with PMSG (5 IU per mouse, daily for 4 days), followed on the 5th day by either saline or murine leptin (twice at a 8 h interval, i.p., 5 μg/g body weight). Microscopic examination of serial sections of oviducts isolated on the 6th day revealed that 4 out of 10 mice ovulated with 1–5 oocytes per mouse, indicating a complete leptin-mediated process of ovulation (Fig. 3*A* and Table 1). No ovulation was detected upon treatment of the mice with PMSG alone. In a control study, treatment of *hpg* mice with PMSG followed by hCG led to ovulation in all mice (Fig. 3*B* and Table 1). Although leptin was less efficient than hCG as an inducer of ovulation, the number of oocytes seen in the oviducts was similar in the leptin and hCG groups. However, unlike the hCG group, 44% of the oocytes in the leptin group appeared to be fragmented (Fig. 3*A*).

Similar results were obtained with normal, antide-treated prepubertal C57BL/6 mice. Following treatment with PMSG and either leptin or hCG, mature oocytes were obtained in the oviducts (Fig. 3*C* and 3*D*, respectively). A single mature oocyte was even detected in the ovary of a leptin-treated prepubertal mouse (Fig. 3*E*). No ovulation was detected upon treatment of the mice with PMSG alone. Similar results were obtained with antide-treated *ob/ob* mice following PMSG and either leptin or hCG (data not shown).

We then determined the relative efficacy of leptin and hCG as inducers of ovulation by counting the number of oocytes obtained in the various mouse strains. Oocytes were counted in serial sections of the *hpg* mice, whereas in the cases of prepubertal mice and *ob/ob* mice, ampoulae were carefully separated and oocytes were recovered, microscopically examined, and counted. We found that leptin induced ovulation less frequently than hCG in the three models of hypogonadal mice. Furthermore, the number of oocytes per mouse was significantly lower in the leptin-treated prepubertal mice as compared with the hCG group. In contrast, a similar number of oocytes was counted in ovulating *hpg* and *ob/ob* mice that were treated with either leptin or hCG (Table 1). Although part of the leptin-induced oocytes were immature as indicated by the presence of germinal vesicles, they could undergo spontaneous maturation in vitro, as demonstrated previously with oocytes obtained directly from ovaries (17). In our study, 12 out of 15 oocytes from ovaries of PMSG-treated *ob/ob* mice underwent spontaneous maturation in vitro. Similar results were obtained with oocytes of the prepubertal C57BL/6 mice. These observations indicated that leptin can replace hCG as an inducer of ovulation in mice, albeit with a much lower efficiency.

Serum progesterone is not elevated upon leptin-induced ovulation

We determined serum LH in antide-treated *ob/ob* mice to test whether the leptin-induced follicular development and ovulation is LH-dependent. Serum LH was <0.2 ng/ml, and no LH surge was encountered at different times and combinations of PMSG and leptin (data not shown). Although serum LH remained low, the precise timing of the LH surge is not known and may be easily missed. Therefore, we determined serum progesterone, which serves as a robust marker of the LH surge (18). No significant surge in serum progesterone was seen at 72 h in the PMSG plus leptin groups of *ob/ob* and prepubertal mice, including in those mice that ovulated

(Fig. 4*A*). In a control experiment, prepubertal C57BL/6 mice treated with antide and PMSG at time 0 followed by hCG at 48 h gave the expected surge of serum progesterone, as measured at 72 h (3.4±0.5 ng/ml vs. 0.18±0.06 ng/ml at time 0, $P<0.05$, $n=3$, Fig. 4*A*). In fact, serum progesterone was even higher at 4 h following hCG (7.6±2.4 ng/ml as compared with 0.3±0.09 ng/ml in prepubertal mice treated with antide and PMSG alone, $P<0.05$, $n=3$). In a similar study, no serum progesterone surge was seen in leptin-treated *ob/ob* mice and in PMSG-primed *hpg* mice (Fig. 4*A*). The same results were obtained at 120 h, with *hpg* mice receiving daily PMSG and leptin at the 5th day (Fig. 4*B*). However, the daily injections of PMSG, which may contain trace amounts of LH, gave rise to transient progesterone surges, but not during ovulation at 120 h. These results suggest a mechanism of ovulation independent of LH surge.

The difference between hCG and leptin treatment was also noticed in the appearance of the mouse uterus. During the pro-estrous and estrus days of the cycle the uterus exhibits maximal distension due to high serum estradiol. This appearance was clearly observed in PMSG-treated mice (Fig. 4*C*, panel 1). However, the distension and hyperemia subside following the LH surge (19), as well as following administration of hCG, reflecting the decrease in serum estradiol and the concomitant increase in serum progesterone (Fig. 4*C*, panel 2). In contrast, the uterus of *ob/ob* mice that ovulated upon leptin administration remained hyperemic, exhibiting maximal distension (Fig. 4*C*, panel 3). In fact, treatment with leptin rendered the uterus hyperemic even in the absence of PMSG (Fig. 4*C*, panel 4). The same uterine features were also observed in antide-treated C57BL/6 mice and *hpg* mice (data not shown). These differences in the uterine physiology and appearance further support the notion that the leptin-induced ovulation is independent of LH activity.

Leptin induces the protease ADAMTS-1 in vitro

The preovulatory surge of LH induces ovulation by several mechanisms, including activation of enzymes that weaken the follicular wall to facilitate the extrusion of the follicular content. These enzymes include a disintegrin and metalloproteinase with a thrombospondin-like motif (ADAMTS-1) and cathepsin L (20). To test whether leptin can induce the expression of ADAMTS-1 and cathepsin L by acting directly on the ovary, we isolated pre-ovulatory follicles from C57BL/6 mice and incubated them with leptin in vitro. Leptin (250 ng/ml) induced the expression of ADAMTS-1, as determined by qualitative and quantitative RT-PCR. Significant induction of ADAMTS-1 was evident 17 h after initiation of leptin treatment (Fig. 5). Marginal induction of cathepsin L was noticed, but it was not statistically significant (not shown). These results indicate that leptin can induce the expression of at least one protease that plays a fundamental role in the rupture of the follicular wall.

DISCUSSION

Leptin has a critical role in reproduction, acting as an obligatory inducer of GnRH release. Recently, it was demonstrated that leptin induces the release of GnRH through inhibition of hypothalamic neuropeptide Y (NP-Y) production. Indeed, the fertility of leptin-deficient female *ob/ob* mice could be rescued by deletion of the NP-Y4 receptor (21). However, about half of the double deficient female mice remained sterile, suggesting that leptin might also trigger additional mechanisms needed for efficient ovulation. In the present study, we examined the possible existence of such additional leptin-triggered mechanisms that are independent of GnRH. To

address this question, we used three mouse models: the GnRH-deficient (*hpg*) mice, antide-treated *ob/ob* mice lacking leptin, and antide-treated normal prepubertal C57BL/6 mice. Leptin is not an inducer of FSH (22), and our findings suggest that leptin can mimic FSH in supporting follicular growth and development in *ob/ob* mice. No such effect of exogenous leptin was noticed in the other two mouse models of hypogonadism; however, this lack of effect could not be properly interpreted due to the presence of endogenous leptin in these two models. Furthermore, the leptin-deficient *ob/ob* mice express a very high level of leptin receptors, making them extremely sensitive to exogenous leptin.

The ability of leptin to mimic LH in inducing follicular rupture and ovulation in the three models of hypogonadism is quite intriguing. The lack of concomitant induction of progesterone strongly indicates that the leptin-induced ovulation was indeed LH-independent. Another unexpected result is the rapidity of follicular development (within 3 days) of *ob/ob* mice following leptin or hCG administration, as it was previously reported to take up to 30 days of continuous leptin administration (4). A similarly rapid follicular development was seen in *hpg* mice upon treatment with PMSG, enabling subsequent ovulation by leptin. The appearance of fragmented oocytes upon leptin treatment of *hpg* mice can result either from apoptosis (23) or a disruption of actin filaments (24), or an as yet unknown mechanism (25) .

Therefore, it appears that on top of its role in inducing GnRH, leptin can also mimic LH in its actions on the reproductive system, albeit at a lower efficacy. However, induction of ovulation by hCG (following PMSG) in *ob/ob* mice suggests that leptin is not essential for ovulation.

Our in vivo results are supported by a previous study showing that leptin triggered maturation of mouse oocytes in vitro (26). Yet, exogenous leptin was reported to inhibit ovulation in prepubertal, gonadotropin-primed rats (27). However, timing is most critical in ovulation, and in those studies, hCG was administered following leptin treatment, whereas in our experimental model, leptin was administered instead of hCG. Indeed, it was recently shown that hCG induces a dramatic but transient increase of leptin and its functional receptor in the ovary, and this increase occurs immediately before ovulation (28). It remains to be established whether leptin induced maturation and rupture of the follicle by signaling through the JAK/STAT pathway and/or through the MAPK pathway. Previously, leptin was shown to activate STAT3 during oocyte maturation, and recently, leptin was shown to enhance oocyte nuclear and cytoplasmic maturation by signaling through the MAPK pathway (29, 30).

Although progesterone has a central role in the development and function of the reproductive system (31), induction of ovulation independent of LH and progesterone was previously demonstrated in hypophysectomized rats that were treated with GnRH (32, 33). More recently, it was shown that treatment of Cdk4-deficient mice with hCG resulted in formation of CL despite low progesterone levels (34). Similarly, mice deficient in tissue inhibitor of metalloproteinase 1 (TIMP-1) ovulate normally despite reduced serum progesterone (35). Our results demonstrate that leptin-induced ovulation could occur despite complete absence of progesterone surge.

We have also attempted to address the mechanism of leptin-induced follicular rupture. The cascade of follicular rupture involves the breakdown of the ECM and increase in vascular permeability and blood flow. These changes result in increased intra-follicular pressure and lower tensile strength of the follicular wall. The ability of leptin to induce the expression of

ADAMTS-1 in isolated pre-ovulatory follicles provides a possible mechanism of the leptin-induced breakdown of the ECM, eventually leading to rupture. The rather low efficiency of leptin as inducer of ADAMTS-1 as compared with LH and progesterone (20) correlates well with the relative efficacies of leptin and LH as inducers of rupture. The leptin-induced hyperemia in the ovaries suggests that it may induce the secretion of prostaglandins, as these were implicated in vasodilatation (36). Leptin was reported previously to induce vasodilatation in other tissues (37). The recent finding that LH action in the ovulatory follicle is mediated by induction of EGF-related growth factors may provide an alternative mechanism of leptin-induced ovulation through yet unknown leptin-induced mediators (38).

The present study demonstrated for the first time a cytokine that triggers ovulation independently of GnRH and LH. The mechanism of such ovulation may either involve a local effect of leptin on the ovary, such as induction of ADAMTS-1, or an as yet unknown hypothalamic pathway. In addition, our findings suggest a possible use of leptin in the treatment of female infertility, particularly in subjects who do not respond properly to LH. Furthermore, leptin may also be considered a means to reduce the amount of FSH and LH given to induce ovulation.

ACKNOWLEDGMENTS

We thank Sara Barak for excellent technical assistance. This work was supported by the Serono Group of companies. M.R. is the Edna and Maurice Weiss Professor of Cytokine Research. N.D. is the Phillip M. Klutznick Professor of Developmental Biology. A.H. is the Joyce and Ben B. Eisberg Professor of Molecular Endocrinology and Cancer Research.

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Received May 28, 2004; accepted September 13, 2004.

Table 1

Oocyte count in ampoulae of ovulating hypogonadal mice[a]

Treatment	Oocyte count		
	***hpg* mice**	**C57BL/6 mice**	***ob/ob* mice**
Control	0 (*n*=3)	0 (*n*=5)	N.D.
PMSG at time 0	0 (*n*=7)	0 (*n*=6)	0 (*n*=3)
PMSG at time 0 and leptin at 96 h (*hpg* mice) or leptin at 48 h (all other mice)	2.0 ± 1.7 (range 1–5; 4 out of 10 mice ovulated)	2.0 ± 1.4 (range 1–5; 22 out of 40 mice ovulated)	2.8 ± 2.0 (range 1–6; 10 out of 20 mice ovulated)
PMSG at time 0 and hCG at 96 h (*hpg* mice) or hCG at 48 h (all other mice)	3.5 ± 2.5 (range 1–6; 2 out of 2 mice ovulated)	20 ± 5 (range 12–28; 6 out of 6 mice ovulated)	2.8 ± 2.4 (range 1–6; 4 out of 4 mice ovulated)

[a]*ob/ob* and prepubertal C57BL/6 mice were treated with antide at time 0. Oocytes were counted in oviductal sections of *hpg* mice at 120 h. Oocytes were collected from the ampoulae of C57BL/6 and *ob/ob* mice at 72 h and counted.

Fig. 1

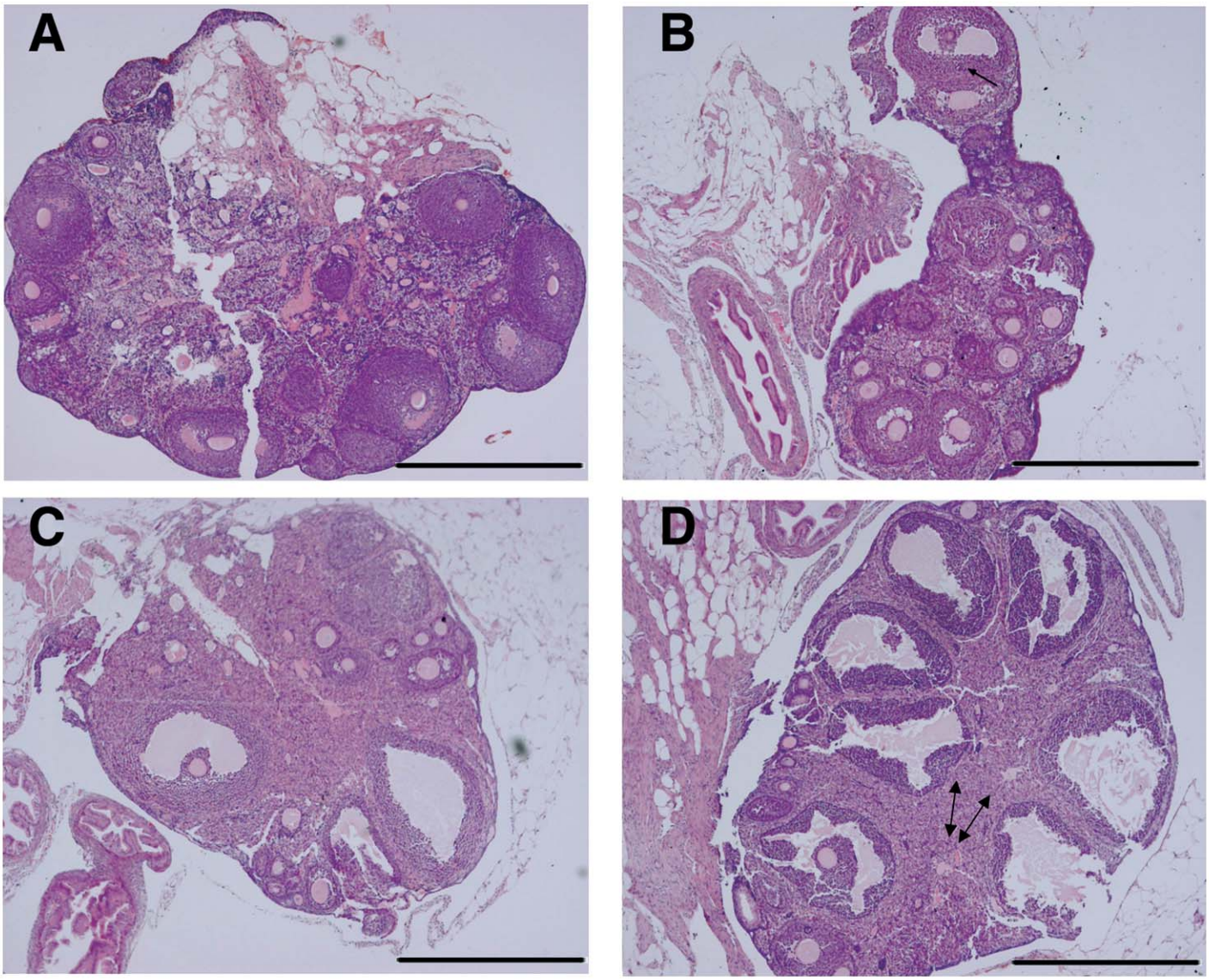

Figure 1. Follicular and ovarian development following hormonal treatment of *ob/ob* mice. Histological examination of ovaries from *ob/ob* mice was performed 72 h following treatment with antide and the indicated reagents. Sections shown are representative examples of the indicated number of ovaries. ***A)*** Control ovaries of *ob/ob* mice (*n*=2). ***B)*** Ovaries of *ob/ob* mice (*n*=4) treated with leptin. A large antral follicle is indicated by an arrow. ***C)*** Ovaries of *ob/ob* mice (*n*=3) treated with PMSG. ***D)*** Ovaries of *ob/ob* mice (*n*=3) concomitantly treated with PMSG and leptin. The arrows indicate growth of interstitial cells. Bar size is 0.4 mm.

Fig. 2

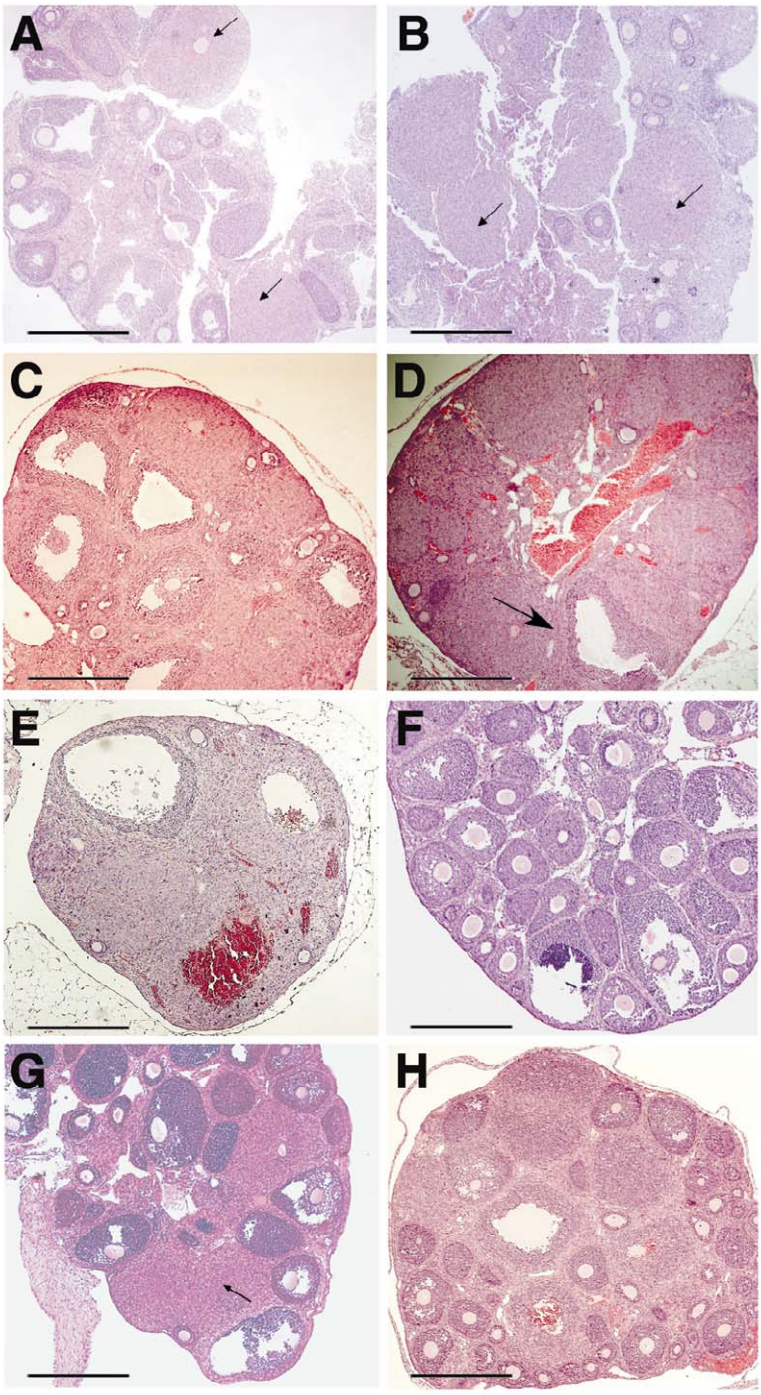

Figure 2. Presence of CL in hypogonadal mice following hormonal treatment. Histological examination of ovaries from *ob/ob* and prepubertal mice was performed 72 h following treatment with antide and the indicated reagents. Sections shown are representative examples of the indicated number of ovaries. ***A)*** Ovaries of *ob/ob* mice (*n*=6) treated with PMSG at time 0 followed by leptin at 48 h. ***B)*** Ovaries of *ob/ob* mice (*n*=3) treated with PMSG at time 0 followed by hCG at 48 h. CL are indicated by arrows. ***C)*** Ovaries of *hpg* mice (*n*=14) treated with PMSG daily for 4 days. ***D)*** Ovaries of *hpg* mice (*n*=20) treated with PMSG daily for 4 days followed by leptin at day 5. The arrow indicates a ruptured follicle. ***E)*** Ovaries of *hpg* mice (*n*=4) treated with PMSG daily for 4 days followed by hCG at day 5. ***F)*** Ovaries of prepubertal mice (*n*=24) treated with PMSG at time 0. ***G)*** Ovaries of prepubertal C57BL/6 mice (*n*=6) treated with PMSG at time 0 followed by leptin at 48 h. ***H)*** Ovaries of prepubertal C57BL/6 mice (*n*=6) treated with PMSG at time 0 and hCG at 48 h. Histological examination of ovaries from *hpg* mice was performed at day 5 following treatment with the indicated reagents. Histological examination of ovaries from all other mouse models was performed at day 3 following treatment with the indicated reagents. Bar size is 0.4 mm.

Fig. 3

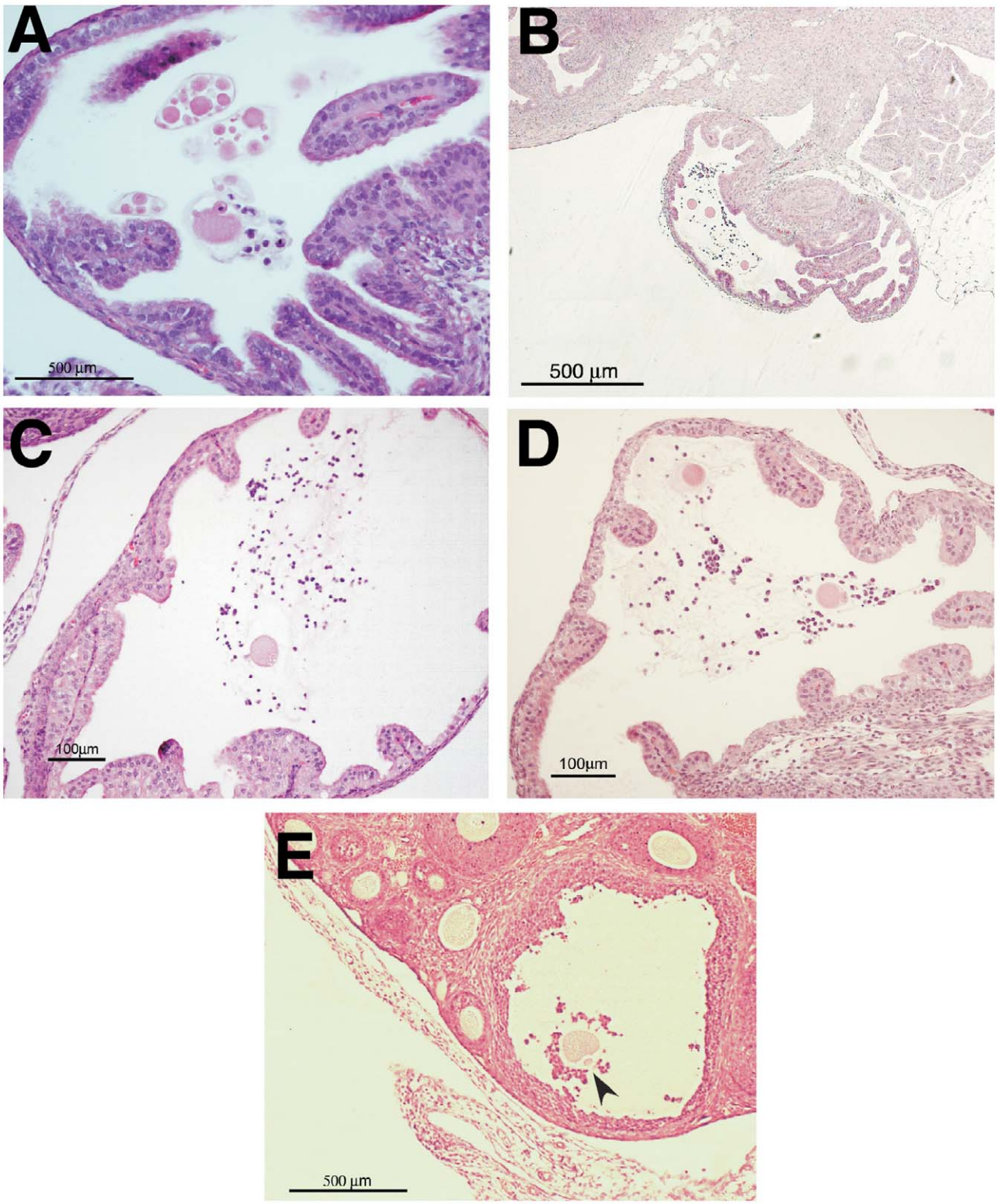

Figure 3. Induction of ovulation by leptin. ***A)*** An oocyte in the ampoula of an *hpg* mouse treated with PMSG daily for 4 days followed by leptin at day 5. ***B)*** Oocytes in the ampoula of an *hpg* mouse treated with PMSG daily for 4 days followed by hCG at day 5. ***C)*** Oocytes in the ampoula of an antide-treated prepubertal C57BL/6 mouse treated with PMSG at time 0 and leptin at 48 h. ***D)*** Oocytes in the ampoula of an antide-treated prepubertal C57BL/6 mouse receiving PMSG at time 0 and hCG at 48 h. ***E)*** A mature oocyte in the ovary of an antide-treated prepubertal C57BL/6 mouse receiving PMSG at time 0 and leptin at 48 h. Notice the polar body (arrow) and cumulus cells.

Fig. 4

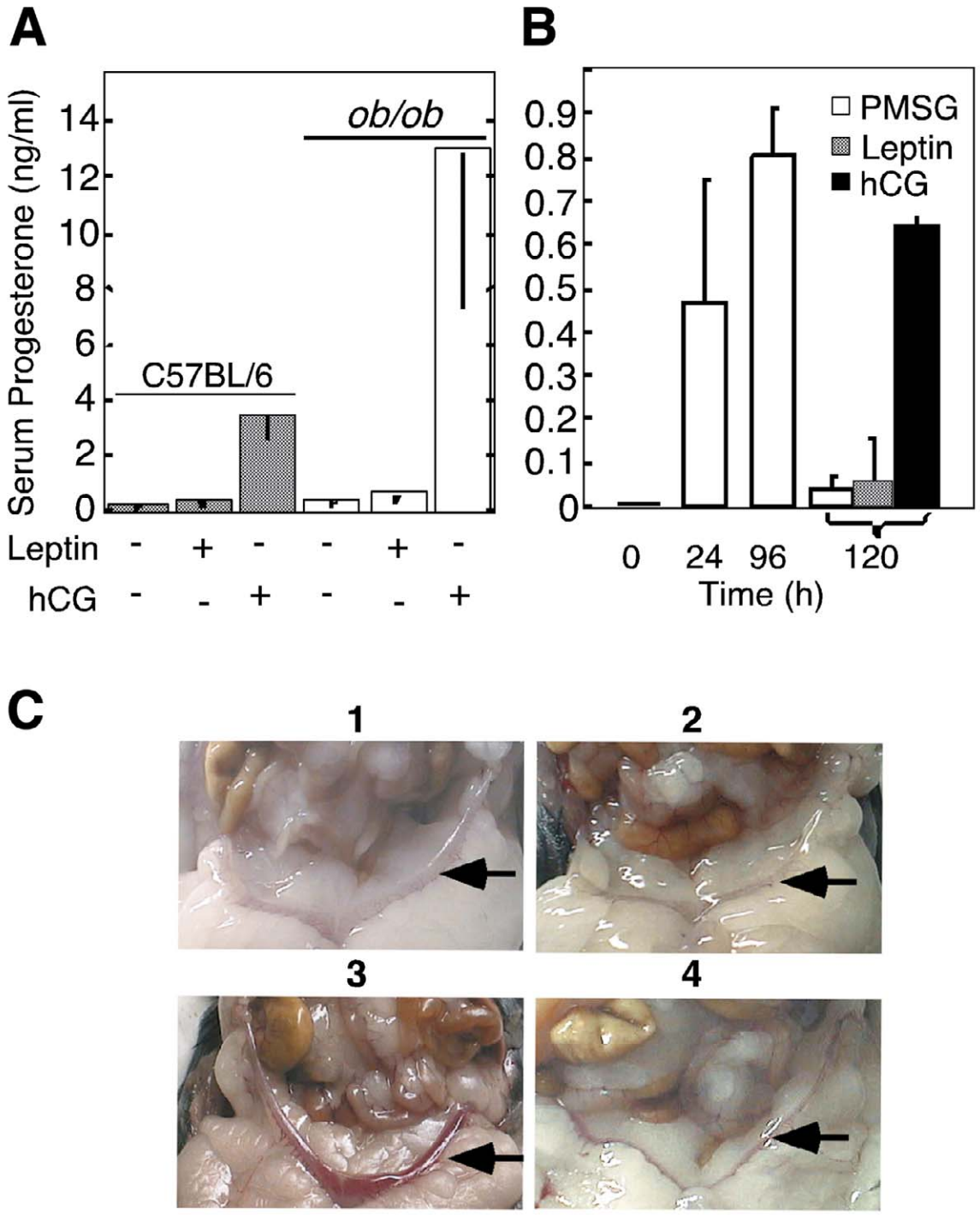

Figure 4. Serum progesterone of *ob/ob*, C57BL/6, and *hpg* mice and the uterine appearance of *ob/ob* mice. ***A)*** Prepubertal C57BL/6 mice (gray bars) and *ob/ob* mice (open bars) were treated with antide and PMSG at time 0 and either leptin or hCG at 48 h. Serum progesterone was determined at 72 h. ***B)*** *hpg* mice were treated with PMSG daily for 4 days followed by either leptin or hCG at day 5 (108 h). Serum progesterone was determined before PMSG (time 0) and at the indicated times following PMSG administration, as well as 8 h after administration of either hCG or leptin (time 120 h). hCG significantly induced progesterone to 645 ± 20 pg/ml (n=2) as compared with PMSG alone at 120 h (13±23 pg/ml; SD; n=3; P<0.02), whereas leptin did not significantly induce progesterone (55±96 pg/ml; SD; n=3) as compared with PMSG alone (P>0.5). ***C)*** Photographs of uteri (arrows) of *ob/ob* mice 72 h after initiation of the following treatments: 1) PMSG at time 0, 2) PMSG at time 0 and hCG at 48 h, 3) PMSG at time 0 and leptin at 48 h, and 4) leptin at time 0.

Fig. 5

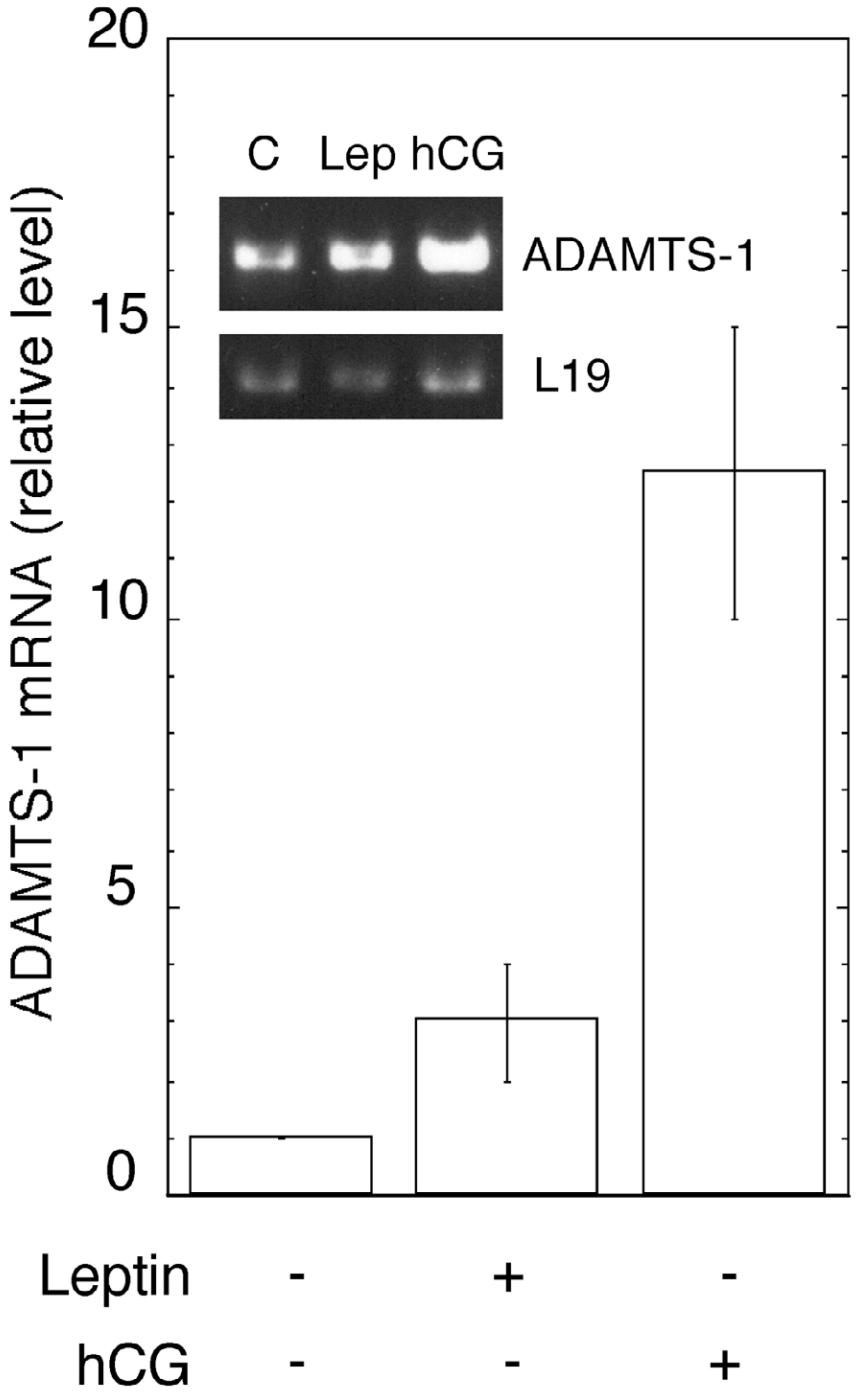

Figure 5. Leptin induces the expression of ADAMTS-1 in mouse preovulatory follicles. Follicles of prepubertal, PMSG-treated C57BL/6 mice were collected and cultured in vitro with either leptin or hCG. Total RNA was isolated from the follicles, and ADAMTS-1 mRNA was measured by quantitative RT-PCR with specific primers. The relative mRNA levels were normalized to those of ribosomal protein L19 mRNA and are presented as fold induction over basal ADAMTS-1 mRNA levels. Inset: Agarose gel electrophoresis of the semiquantitative PCR products at cycle 30 (n=3). ADAMTS-1 mRNA (upper panel) and L19 mRNA (lower panel). The induction of ADAMTS-1 mRNA by leptin was 3.0 ± 0.9-fold (P<0.05, n=3), whereas the induction by hCG was 12.5 ± 2.5-fold (P<0.01, n=3).