Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1322–1326, February 1995
Medical Sciences

Targeted overexpression of luteinizing hormone in transgenic mice leads to infertility, polycystic ovaries, and ovarian tumors

KIMBERLY A. RISMA*, COLIN M. CLAY†, TERRY M. NETT†, THOMAS WAGNER‡, JUN YUN‡, AND JOHN H. NILSON*§

*Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH 44106; †Animal Reproduction and Biotechnology Laboratory, Department of Physiology, Colorado State University, Fort Collins, CO 80523; and ‡The Edison Biotechnology Center, Ohio University, Athens, OH 45701

Communicated by Anthony P. Mahowald, University of Chicago, Chicago, IL, November 23, 1994

ABSTRACT **Hypersecretion of luteinizing hormone (LH) is implicated in infertility and miscarriages in women. A lack of animal models has limited progress in determining the mechanisms of LH toxicity. We have recently generated transgenic mice expressing a chimeric LH β subunit (LHβ) in gonadotropes. The LHβ chimera contains the C-terminal peptide of the human chorionic gonadotropin β subunit. Addition of this peptide to bovine LHβ resulted in a hormone with a longer half-life. Furthermore, targeted expression of the LHβ chimera led to elevated LH levels and infertility in female transgenics. These mice ovulated infrequently, maintained a prolonged luteal phase, and developed pathologic ovarian changes such as cyst formation, marked enlargement of ovaries, and granulosa cell tumors. Testosterone and estradiol levels were increased compared to nontransgenic littermates. An unusual extragonadal phenotype was also observed: transgenic females developed hydronephropathy and pyelonephritis. The pathology observed demonstrates a direct association between abnormal secretion of LH and infertility and underscores the utility of the transgenic model for studying how excess LH leads to cyst formation, ovarian tumorigenesis, and infertility.**

Maturation of a fertilizable oocyte is a complex process that depends upon the proper development of the oocyte within the highly defined environment of the ovarian follicle. Upon ovulation, the mature follicle ruptures to release the oocyte, and the steroidogenic cells of the ruptured follicle terminally differentiate to form the corpus luteum. The corpus luteum must produce adequate progesterone to maintain pregnancy if fertilization has occurred. The control of these ovarian events is critically regulated by fluctuating levels of pituitary gonadotropins: follicle stimulating hormone (FSH) and luteinizing hormone (LH). Both hormones are heterodimeric glycoproteins containing a common α subunit and a unique β subunit. Heterodimers of LH and FSH are secreted by pituitary gonadotropes and bind to receptors in the gonads to stimulate steroidogenesis and gametogenesis (1).

Several clinical studies have shown a correlation of hypersecretion of LH and polycystic ovarian syndrome (PCOS), infertility, and miscarriage in women (2–4), suggesting that chronically elevated LH impairs fertility. Unfortunately, there are no studies showing a direct relationship between hypersecreted LH and reproductive abnormalities. Since LH is secreted in regulated pulses (5, 6) and its serum $t_{1/2}$ is short [20–30 min (7)], it is difficult to devise protocols for chronic administration of exogenous LH that mimic endogenous pulse patterns of LH. To circumvent this limitation, we report a transgenic mouse model in which elevated hormone levels are maintained chronically, without requiring multiple injections, supraphysiologic dosing, or dampening of the hypothalamic–pituitary–gonadal axis.

We utilized a two-pronged approach to achieve elevated levels of serum LH. (*i*) Increased secretion of hormone from the pituitary was achieved by expression of an LHβ subunit transgene, targeted to the pituitary by a previously characterized bovine α-subunit promoter (8, 9). (*ii*) The LHβ transgene was modified to encode a peptide extension that we proposed would slow the elimination of LH from the serum. This peptide is normally found at the C terminus of the β subunit of human chorionic gonadotropin (hCG) (hCGβ) and is referred to as the C-terminal peptide (CTP) (10). The CTP is thought to be a major determinant of the serum $t_{1/2}$ of hCG and has been shown to increase the $t_{1/2}$ of FSH 2- to 3-fold when fused to its β subunit (11). Accordingly, we constructed a transgene with the coding region of bovine (b) LHβ fused in-frame to the coding region of CTP (bLHβ–CTP).

MATERIALS AND METHODS

Construction of the bLHβ–CTP Transgene. The bLHβ–CTP minigene was engineered by a two-step PCR. (*i*) A short fragment containing the C terminus of bLHβ (30 bp) fused in-frame to the CTP (last 87 bp of the hCGβ gene) was generated. (*ii*) This fragment was lengthened to contain the entire bLHβ cDNA fused in-frame to the CTP. This fusion gene was utilized for transfection experiments but was modified to contain the first intron of bLHβ for the transgene construct. The resulting insert was ligated into a BSK^- plasmid already containing the bovine α-subunit promoter (positions −315 to +45) and the simian virus 40 late polyadenylylation signal. Transgenic mice were generated by microinjecting the bLHβ–CTP insert into fertilized oocytes as described (12). Mice were genotyped by PCR analysis of tail DNA using primers specific to the α-subunit promoter and β-subunit gene.

Analysis of $t_{1/2}$. Recombinant bLH and bLH–CTP heterodimers were generated by stably cotransfecting CHO cells with viral expression vectors containing the bovine α-subunit gene and either the bLHβ or bLHβ–CTP genes as described (13). Serum-free medium was collected and ammonium sulfate-precipitated or concentrated with an Amicon ultrafiltration cell. Female rats were pretreated with 50 μg of antide (Sigma) in 20% (vol/vol) propylene glycol, injected subcutaneously 2 h prior to hormone injections to prevent release of endogenous LH during the course of the experiment. Samples (1 μg) of CHO LH, CHO LH–CTP, and purified hCG (Calbiochem) were dissolved in 1 ml of 0.9% NaCl. All injections and blood sampling were performed by accessing the jugular veins under ether anesthesia. LH (14) and hCG (15) levels were determined by RIA.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LH, luteinizing hormone; FSH, follicle stimulating hormone; hCG, human chorionic gonadotropin; CTP, C-terminal peptide; PCOS, polycystic ovarian syndrome; b, bovine.

*To whom reprint requests should be addressed.

Immunohistochemistry. Male bLHβ–CTP pituitaries were perfusion-fixed in 2% (vol/vol) paraformaldehyde, mounted in paraffin, and sectioned. After deparaffinization, sections were incubated with the following antibodies: (*i*) guinea pig anti-rat LHβ (AFP22238790GPOLHB, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda) for 1 h at 37°C, diluted 1:200; (*ii*) fluorescein isothiocyanate-conjugated anti-guinea pig IgG for 30 min at 37°C, diluted 1:300; (*iii*) rabbit anti-CTP (P79R) for 1 h at 37°C, diluted 1:100; (*iv*) rhodamine-conjugated anti-rabbit IgG for 30 min at 37°C, diluted 1:500. Sections were washed at room temperature with phosphate-buffered saline for 20 min between each incubation. Secondary antibodies were from Organon Teknika–Cappel.

RESULTS AND DISCUSSION

CTP Increases the $t_{1/2}$ of LH. To test directly the effect of the CTP on $t_{1/2}$, we generated recombinant bLH and bLH–CTP heterodimers and measured their elimination from the serum of rats (Fig. 1). The majority of each hormone was eliminated by first-order kinetics. Therefore, $t_{1/2}$ values were estimated for this phase by the amount of time it took to clear from 40 to 20% of hormone. The $t_{1/2}$ of bLH obtained by this analysis was 20–25 min. The addition of CTP to bLHβ dramatically affected clearance of the heterodimer, causing its $t_{1/2}$ to increase 2- to 3-fold. In fact, the elimination curve of bLH–CTP closely approximated the elimination of hCG. These experiments demonstrated that addition of CTP markedly decreased the clearance of bLH–CTP heterodimers in serum and provided impetus for introducing the bLHβ–CTP construct into transgenic mice.

Generation of bLHβ–CTP Transgenic Mice. The bovine α-subunit promoter was used to direct expression of the transgene to gonadotropes (8, 9). Addition of CTP to bLHβ allowed immunologic identification of cells expressing the chimeric subunit. Double immunohistochemical labeling of pituitaries with CTP- and LHβ-specific antibodies demonstrated that all LHβ cells contained the CTP signal (Fig. 2), confirming that expression of the transgene occurred in all gonadotropes. Significantly, no CTP signal was detected in pituitaries from nontransgenic mice (data not shown).

Transgenic Mice Are Infertile. Attempts were made to breed all founder animals. Male mice, however, were subfertile. Although one animal never bred, another male bred after a prolonged (2 month) delay. This permitted establishment of a bLHβ–CTP line. Initial analysis of bLHβ–CTP males indicated that neither LH nor testosterone levels were different in transgenic vs. control males, although testes in transgenic males were significantly smaller than in controls (data not shown).

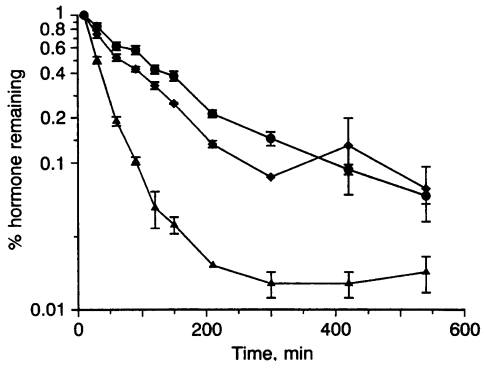

FIG. 1. Analysis of serum $t_{1/2}$ of recombinant bLH–CTP in rats. Recombinant bLH and bLH–CTP heterodimers were purified from CHO cells and injected intravenously into rats to measure elimination *in vivo*. Samples containing 1 μg of purified hCG (●), CHO LH–CTP (◆), or CHO LH (▲) were injected into the jugular vein of female rats (n = 3 or 4 per group) pretreated with antide, a gonadotropin releasing hormone antagonist, and blood was collected as indicated for 540 min. The $t_{1/2}$ was estimated by measuring the time it took to clear from 40 to 20% of hormone from the serum. The addition of CTP to bLH caused its $t_{1/2}$ to increase 2- to 3-fold.

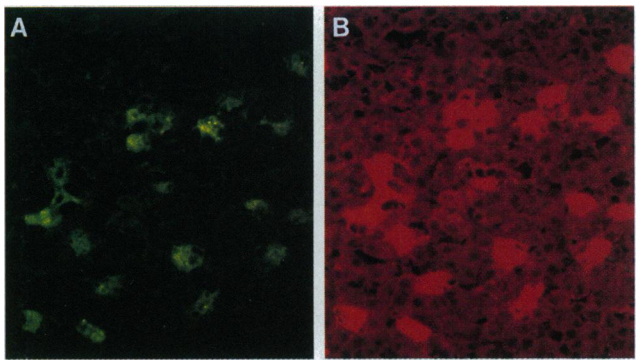

FIG. 2. Immunohistochemical detection of bLHβ–CTP in mouse pituitaries. (×180.) Sections were incubated with primary and secondary antibodies as follows. (*A*) With guinea pig anti-LHβ and fluorescein isothiocyanate-conjugated anti-guinea pig IgG. (*B*) With rabbit anti-CTP and rhodamine-conjugated anti-rabbit IgG. All cells expressing LHβ contained CTP.

Analysis of transgenic females established that expression of bLHβ–CTP had a profound impact upon female reproduction. Two founder females never bred, whereas a third had one litter with one pup that did not survive. Analysis of ovarian cycles by vaginal smear in 12- to 16-week-old F_2 transgenics revealed chronic anovulation. In a subsequent experiment utilizing 8-week-old bLHβ–CTP females from the F_3 generation, 9 of 10 transgenic females mated multiple times, but only 1 generated a litter compared to 5 of 6 littermate controls. Serum LH levels were elevated in 13 transgenic (39.7 ± 8.7 ng/ml; $P < 0.01$) vs. 8 control (2.7 ± 0.48 ng/ml) females (14), demonstrating that hypersecretion of LH had been achieved.

Ovarian Pathology. Morphologic analysis of ovaries from bLHβ–CTP founder and F_1–F_3 generations (age, 4–8 months) revealed three classes of pathologic changes unique to transgenic females: enlarged ovaries packed with corpora lutea, ovaries containing multiple cysts, and ovaries with granulosa and theca-interstitial cell tumors (Fig. 3). The incidence of phenotypic ovarian changes in bLHβ–CTP females is summarized in Table 1. Breeding of four F_1 animals revealed that the infertility and ovarian pathology were transmitted only through two males and not observed in offspring from two F_1 females with normal ovaries. It is not known whether this is due to segregation of multiple integration sites or due to genetic imprinting.

Fig. 3*B* demonstrates a markedly enlarged ovary containing numerous corpora lutea. Mice with this ovarian pattern have vastly elevated progesterone levels (data not shown) and appear to exhibit a prolonged luteal life span. To test whether chronically elevated LH could lengthen the function of corpora lutea, we experimentally induced new corpora lutea and analyzed progesterone production. In rodents, the life span of the corpus luteum during pseudopregnancy (12–14 days) can be measured by mating females with a vasectomized male and determining the length of time to a new estrous cycle. Unfortunately, transgenic females were anovulatory, making this approach problematic. Therefore, we initiated ovulation by hemiovariectomy—a method utilized in rats with pharmacologically induced cystic ovaries (16). Surgeries were performed on both transgenic and nontransgenic littermates and the mice were placed immediately with vasectomized males. Mating was documented by the presence of a semen plug in the vagina. Progesterone levels were analyzed every 3 days and the

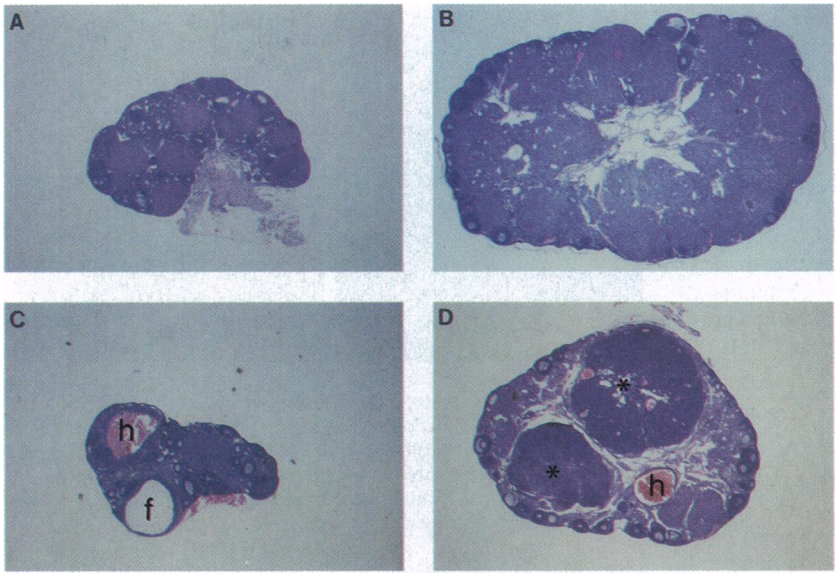

FIG. 3. Ovaries from transgenic females fall into three pathologic phenotypes: enlarged, cystic, and tumorigenic. (×9.) (*A*) A normal ovary is shown containing multiple corpora lutea and follicles. (*B*) Ovaries from multiple bLHβ–CTP females were grossly enlarged and contained multiple corpora lutea (CL), typical of a pseudopregnant ovary. The follicles are pressed to the outside of the ovary and are atretic. (*C*) Other ovaries obtained from bLHβ–CTP females contained numerous cysts—hemorrhagic (h) or fluid-filled (f) and derived from both follicular and luteal tissue. (*D*) Granulosa cell tumors (*) were observed in ovaries from F1 to F3 generation bLHβ–CTP females. Tumors were noted in mice from age 4 to 9 months and contained various amounts of other stromal cells.

termination of the pseudopregnancy was documented by the observation of a new mating or demonstration of a cornified vaginal epithelium by vaginal smear. Both were indicative of ovulation.

Control animals were unaffected by hemiovariectomy and exhibited a normal pseudopregnancy with progesterone levels peaking at day 3 and returning to baseline by day 12, followed by another ovulation (Fig. 4). Two out of four hemiovariectomized transgenics mated and became pseudopregnant. These mice secreted large amounts of progesterone with peak levels occurring at day 12. Remarkably, progesterone remained elevated at least until day 18 and no ovulation was observed. Thus, the progesterone and ovulation data demonstrate that transgene expression prolonged the luteal life span beyond a normal pseudopregnancy.

An increase in luteal life span suggests that chronically elevated LH overcomes the physiologic signals that ordinarily control luteal cell death. The mating stimulus induces pseudopregnancy in rodents by causing twice daily surges of prolactin (17). In turn, prolactin supports progesterone production by maintaining LH receptors on luteal cells (18). Thus, an increase in luteal life span in transgenic mice could reflect a direct action of hypersecreted LH through binding to LH receptors on luteal cells or an indirect effect that sustains the action of prolactin. Although distinguishing between these two mechanisms requires additional experiments, hypersecretion of LH clearly alters the life span of the corpora lutea.

Development of multiple cysts represents another class of phenotypic changes noted in bLHβ–CTP ovaries (Fig. 3*C*). Testosterone in transgenic females was elevated ≈5-fold over control females [transgenic, 1.95 ± 0.61 ng/ml (n = 5); control, 0.38 ± 0.02 ng/ml (n = 5), $P < 0.05$]. Estradiol was also elevated [transgenic, 65.2 ± 12 pg/ml (n = 5); control, 24.8 ± 9 pg/ml (n = 5); $P < 0.05$], although only 2-fold. Thus, these changes result in an elevated androgen/estrogen ratio, analogous to that observed in women with PCOS (19). Although FSH was not measured in these mice, the elevations in estradiol and LH suggest that the LH/FSH ratio may be altered as well. Consequently, these mice represent a model that may be relevant to the study of PCOS, a disease that may affect 20–30% of women (20).

In humans, the etiology of PCOS is multifactorial. Presentation often includes hyperandrogenemia and insulin resistance (2, 21, 22). Hypersecretion of LH is associated with PCOS, but it is unknown whether increased LH alone triggers alteration of the other hormones. Our data indicate that chronically elevated LH is sufficient to cause the PCOS-like syndrome observed in bLHβ–CTP mice. It should be noted, however, that cysts are often more numerous in ovaries from PCOS women than in ovaries from bLHβ–CTP mice. In addition, the thickened fibrous capsule of the ovary typically seen in PCOS (23) is not present in transgenic mouse ovaries, suggesting that other genetic or environmental factors are

Table 1. Summary of ovarian phenotypes in bLHβ–CTP females from founder animals and subsequent generations derived from male founder 94

	No.				
Mice	Normal	Enlarged	Cystic	Tumor	Other*
Founders	2	1	—	—	—
F_1	2	1	2	1	—
F_2†	—	1	4	2	—
F_3†	2	—	2	2	2

*Normal architecture is lacking; remaining stroma is luteinized.
†Includes female offspring from F_1 males only (see text for discussion).

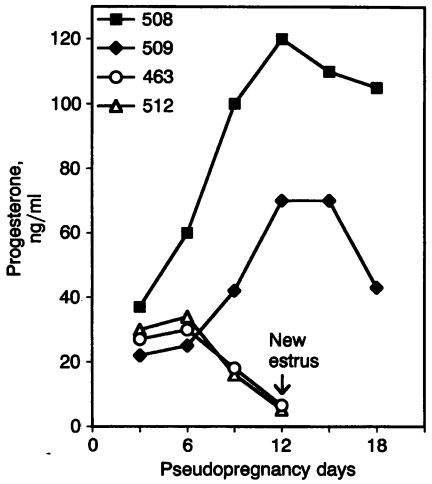

FIG. 4. Demonstration of a prolonged luteal life span in females expressing the bLHβ–CTP transgene. Transgenic females were hemiovariectomized to induce ovulation and then placed immediately with vasectomized males to establish a new pseudopregnancy. Functional luteolysis was analyzed by measuring serum progesterone levels. Progesterone secretion returned to minimal levels by day 12 in control mice (512, ○; 463, △), typical of a normal rodent pseudopregnancy. In contrast, progesterone levels in bLHβ–CTP females were markedly elevated (508, ■; 509, ◆), reaching a peak by day 12 and remaining elevated even until day 18.

necessary to achieve the full spectrum of ovarian pathology. This further underscores the significance of the bLHβ–CTP transgenic mouse model, since additional transgenic alleles encoding PCOS candidate proteins can be bred into the bLHβ–CTP genetic background.

Interestingly, cysts in transgenic ovaries appear to be derived from follicular or luteal tissue and may be present in ovaries that contain corpora lutea (Fig. 3*C*). This suggests that cystic changes may occur during any stage of the estrous cycle, if elevated LH is present. One treatment of PCOS that has been relatively successful in achieving ovulation is wedge resection of the ovary (2), analogous to the hemiovariectomy described above. The two transgenic mice that ovulated after hemiovariectomy had been anovulatory for at least 2 months prior to the surgery, and their ovaries, when inspected upon removal, contained cysts (transgenic mouse 508) or granulosa cell tumors (transgenic mouse 509). Ovaries surgically removed from the two transgenic mice that did not ovulate contained abundant luteal tissue but no cysts or tumors. Thus, it is tempting to speculate that cysts and tumors secrete factors that inhibit ovulation. If so, then a fall in concentration of these factors, which could occur by removal of one ovary, may allow a subsequent ovulation.

Cystic ovaries contained both hemorrhagic and fluid-filled cysts. In addition, hyperemia was found in both cystic and tumor-bearing ovaries. The combination of hyperemia and hemorrhagic cyst formation is reminiscent of ovaries from transgenic mice generated by estrogen receptor gene knockout (24). We suggest that this phenotype is probably due to chronic LH hypersecretion from disruption of the estrogen feedback loop in estrogen receptor knockout mice or from expression of the additional bLHβ allele in our transgenic model.

A subset of bLHβ–CTP females developed ovarian granulosa and theca-interstitial cell tumors by 4–8 months of age (Fig. 3*D*), indicating that stimulation of granulosa and other stromal cells by LH–CTP was tumorigenic. Gonadotropin hyperstimulation has been suggested as a mechanism for tumor formation in ovaries transplanted to the spleen in ovariectomized rats (25), in women undergoing ovarian stimulation for treatment of infertility (26), and in ovaries of inhibin-deficient mice (27). The tumors observed in each of these examples include granulosa cell and other stromal cell tumors. Despite the association of elevated gonadotropins and tumor formation in each of these examples, a direct link between gonadotropin hyperstimulation and tumorigenesis has never been established. Therefore, the finding of granulosa and stromal cell tumors in transgenic mice whose only genetic alteration is the addition of a gene encoding a chimeric gonadotropin strongly suggests that abnormal gonadotropin stimulation is tumorigenic. Interestingly, the recently derived crystal structure of hCG has been used to suggest that glycoprotein hormones can be classified as members of a superfamily of cystine-knot growth factors that includes nerve growth factor, transforming growth factor β, and platelet-derived growth factor β (28). It is feasible that excessive levels of LH in transgenic mice reveal growth factor-like properties of LH not previously realized, resulting in angiogenesis and growth aberrations. Thus, these possibilities illustrate the potential utility of the transgenic model for further study of the molecular mechanisms involved in LH-mediated tumor formation.

Transgenic Females Have Renal Abnormalities. Approximately 25% of infertile females expressing the bLHβ–CTP transgene demonstrated a unique nongonadal phenotype (Fig. 5). These females had enlarged bladders, dilated ureters, and hydronephrosis. In some animals, the hydronephropathy was complicated by acute pyelonephritis, whereas others appeared to have chronic interstitial nephritis. The development of renal pathology was unexpected but may be related to chronically elevated steroids since hydronephrosis has been observed in rats chronically administered estradiol (29). In addition, hydronephrosis is well documented during pregnancy in women and nonhuman primates, although it is disputed whether this results from ureteral obstruction or from physiologic dilatation due to elevated progesterone (30).

CTP Exaggerates the Effect of Hypersecreted LH. We have shown that the presence of CTP on bLHβ greatly reduces the elimination rate of heterodimers from serum. It is probable that the peptide extension contributes to both elevated serum LH levels and the severe gonadal and nongonadal phenotypes. To test this, we generated several additional lines of mice overexpressing the wild-type bLHβ gene by using the same bovine α promoter. All three female founders expressing the

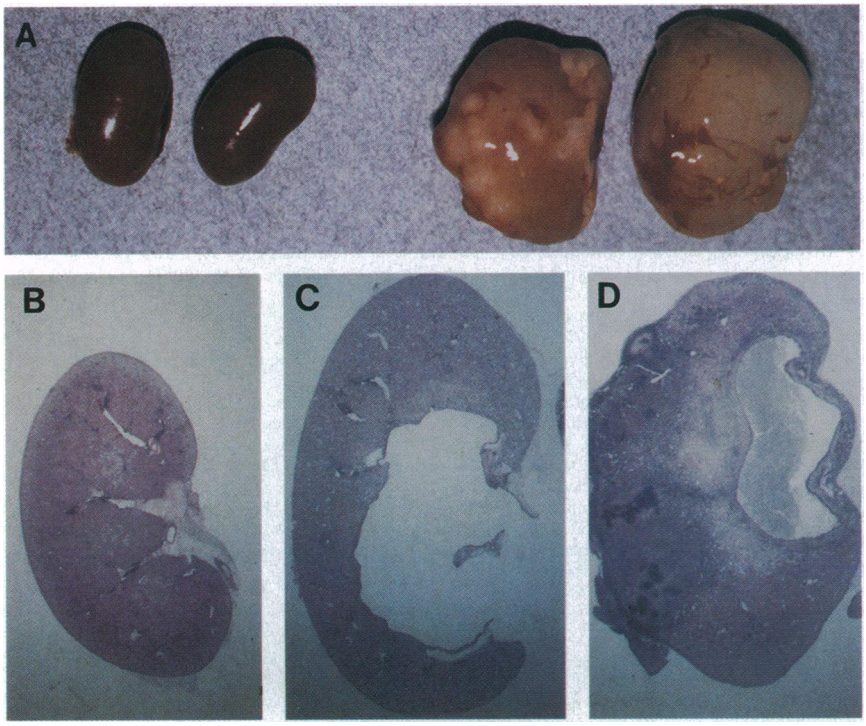

FIG. 5. bLHβ–CTP females developed hydronephrosis and subsequent pyelonephritis. (*A*) Gross morphology of a normal kidney (*Left*) and an infected dilated kidney (*Right*). (*B*) Normal kidney histology with collapsed pelvis and intact cortex and medulla. (*C*) Kidney from female transgenic, with markedly dilated pelvis and collecting ducts, typical of hydronephrosis. (*D*) Histologic section from the infected kidney pictured in *A*. The pelvis and collecting ducts contain bacteria and polynuclear cell infiltrates. There is abscess formation in the cortex and extension into the surrounding capsule (*B–D*, ×5).

bLHβ transgene demonstrated a delay in breeding but, in contrast to bLHβ–CTP females, eventually had litters of normal size. Males expressing bLHβ were fertile, although animals from one line had slightly smaller testes. Females from this particular line were analyzed extensively. Serum LH levels in transgenic females were compared to controls. LH was elevated in 13 transgenic (46.5 ± 13.8 ng/ml; $P < 0.01$) vs. 12 control (3.76 ± 0.62 ng/ml) females (14), demonstrating that hypersecretion of LH led to hormone elevations that were similar to those seen in bLHβ–CTP females. Like bLHβ–CTP mice, many females from the bLHβ line ovulated infrequently and developed cysts. Hemiovariectomy led to ovulation in 3 of 3 mice, and mice induced to pseudopregnancy showed a prolonged luteal life span. Neither tumors nor kidney pathology has been noted in these mice, although fewer animals have been analyzed. Collectively, these data suggest that hypersecretion of wild-type LH can lead to aberrant gonadal phenotypes and that addition of the CTP potentiates the effect of LH.

There are several possibilities for the increased impact of the bLHβ–CTP transgene: (*i*) increased average levels of serum LH, (*ii*) altered pulse pattern of serum LH, or (*iii*) increased activity of bLHβ–CTP heterodimers at the LH receptor. By assuming similar levels of transgene expression, the slower elimination of bLHβ–CTP, compared to bLH, would lead to an overall increase in average LH levels in bLHβ–CTP vs. bLHβ founder animals. In addition, the decreased rate of clearance of bLHβ–CTP may alter the shape of the LH pulse in the serum by decreasing the slope of the elimination curve and elevating trough levels of LH. Finally, we have not yet determined whether addition of the CTP to bLH affects the binding characteristics of the chimera. If the CTP slowed the rate of dissociation of the chimera from the receptor, for example, it may increase the bioactivity of the hormone in the serum. Distinguishing between these three possibilities requires further experimentation.

One additional feature of both bLHβ–CTP and bLHβ transgenes is the bovine α-subunit promoter. We chose this promoter to target transgene expression to gonadotropes because tissue-specific regulatory elements of LHβ promoters are undefined. Nevertheless, our data suggest that the α promoter may be less tightly regulated in females than the LHβ promoter since expression of the transgene led to hypersecretion of LH despite elevated steroid levels. Additionally, this promoter demonstrates sexual dimorphism in our transgenic lines since hypersecretion is not achieved in males. This suggests that the promoter is less efficiently regulated in the females. Finally, in the rat the α-subunit gene is expressed earlier in development than the β gene (31). Although we have not determined the exact day of transgene expression, it is possible that LH may be secreted earlier than usual in bLHβ–CTP and bLHβ mice and contribute to the pathologic phenotype observed. Interestingly, another study (32) showed that expression of a transgenic human FSHβ gene, driven by its own promoter, had little impact upon female or male fertility. Consequently, it should be instructive to test whether linkage of the LHβ promoter to wild-type and chimeric bLHβ subunits leads to the same level of LH overexpression achieved by the α-subunit promoter in our transgenic model.

Summary. We have generated transgenic mice with an allele encoding a chimeric gonadotropin that markedly impairs fertility. In males, expression of the transgene led to reduced fertility and smaller testes, despite normal hormone levels. In females, expression of the transgene led to elevated levels of LH, increased testosterone and estradiol secretion, and extensive pathologic changes in the ovaries. This phenotype was exaggerated by but not solely due to the CTP, since bLHβ females were also affected. Our data also suggests that bLHβ–CTP females have a severely reduced rate of fertility, despite evidence of mating. This may be due to inappropriate luteal support and/or subnormal fertilization. Since excess LH has been implicated in infertility and increased miscarriage rate in women (3, 4), the impact of elevated LH levels on the fertilization of the oocyte and subsequent pregnancy should be studied. The bLHβ–CTP transgenic mouse model will be invaluable for these and other studies as it provides a unique opportunity to study the mechanisms whereby elevated LH leads to prolonged luteal life span, cyst formation, tumorigenesis, and perhaps abnormal fertilization and implantation.

We thank Vernon Stevens for the gift of the CTP antibody and David Schomberg for valuable discussions concerning reproductive physiology.

1. Catt, K. J. & Dufau, M. L. (1991) in *Reproductive Endocrinology*, eds. Yen, S. S. C. & Jaffe, R. B. (Saunders, Philadelphia), 3rd Ed., pp. 105–155.
2. Franks, S. (1989) *Clin. Endocrinol.* **31,** 87–120.
3. Shoham, Z., Jacobs, H. S. & Insler, V. (1993) *Fertil. Steril.* **58,** 1153–1161.
4. Regan, L., Owen, E. J. & Jacobs, H. S. (1990) *Lancet* **336,** 1141–1144.
5. Gibson, M. J., Miller, G. M. & Silverman, A.-J. (1991) *Endocrinology* **128,** 965–971.
6. Karsch, F. J. (1987) *Annu. Rev. Physiol.* **49,** 365–382.
7. Niswender, G. D., Nett, T. M. & Akbar, A. M. (1974) in *Reproduction in Farm Animals*, ed. Hafez, E. S. E. (Lea & Febiger, Philadelphia), 3rd Ed., pp. 57–81.
8. Kendall, S. K., Saunders, T. L., Jin, L., Lloyd, R. V., Glode, L. M., Nett, T. M., Keri, R. A., Nilson, J. H. & Camper, S. A. (1991) *Mol. Endocrinol.* **5,** 2025–2036.
9. Hamernik, D. L., Keri, R. A., Clay, C. M., Clay, J. N., Sherman, G. B., Sawyer, H. R., Nett, T. M. & Nilson, J. H. (1992) *Mol. Endocrinol.* **6,** 1745–1755.
10. Matzuk, M. M., Hsueh, A. J. W., Lapolt, P., Tsafriri, A., Keene, J. L. & Boime, I. (1990) *Endocrinology* **126,** 376–383.
11. Fares, F. A., Suganuma, N., Nishimori, K., LaPolt, P. S., Hsueh, A. J. W. & Boime, I. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4304–4308.
12. Wagner, T. E., Hoppe, P. C. M., Jollick, J. D., Scholl, D. R., Hodinka, R. & Gault, J. G. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6376–6380.
13. Kaetzel, D. M., Browne, J. K., Wondisford, F., Nett, T. M., Thomason, A. R. & Nilson, J. H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7280–7283.
14. Niswender, G. D., Midgley, A. R., Monroe, S. E. & Reichert, L. E. (1968) *Proc. Soc. Exp. Biol. Med.* **128,** 807–811.
15. Vaitukaitis, J., Braunstein, G. D. & Ross, G. T. (1972) *Am. J. Obstet. Gynecol.* **113,** 751–758.
16. Farookhi, R., Hemmings, R. & Brawer, J. R. (1985) *Biol. Reprod.* **32,** 530–540.
17. Terkel, J. (1986) *Ann. N.Y. Acad. Sci.* **474,** 76–93.
18. Niswender, G. D. & Nett, T. M. (1994) in *The Physiology of Reproduction*, eds. Knobil, E. & Neill, J. D. (Raven, New York), 2nd Ed., pp. 781–816.
19. Coney, P. J. (1984) *Fertil. Steril.* **42,** 667–682.
20. Polson, D., Wadsworth, W. J., Adams, J. & Franks, S. (1988) *Lancet* **ii,** 870–872.
21. Dewailly, D. & Cortet-Rudelli, C. (1992) *Horm. Res.* **38,** 41–45.
22. Poretsky, L. (1991) *Endocrinol. Rev.* **12,** 3–13.
23. Stein, I. F. & Leventhal, M. L. (1935) *Am. J. Obstet. Gynecol.* **29,** 181–191.
24. Lubahn, D. B., Moyer, J. S., Golding, T. S., Couse, J. F., Korach, K. S. & Smithies, O. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11162–11166.
25. Biskind, G. R., Bernstein, D. E. & Gospe, S. M. (1952) *Cancer Res.* **13,** 216–220.
26. Matzuk, M. M., Finegold, M. J., Su, J-G. J., Hsueh, A. J. W. & Bradley, A. (1992) *Nature (London)* **360,** 313–319.
27. Willemsen, W., Kruitwagen, R., Bastiaans, B., Hanselaar, T. & Rolland, R. (1993) *Lancet* **341,** 986–988.
28. Lapthorn, A. J., Harris, D. C., Littlejohn, A., Lustbader, J. W., Canfield, R. E., Machin, K. J., Morgan, F. J. & Isaacs, N. W. (1994) *Nature (London)* **369,** 455–461.
29. Andriole, V. T. & Cohn, G. L. (1964) *J. Clin. Invest.* **43,** 1136–1145.
30. Roberts, J. A. (1976) *Urology* **8,** 1–4.
31. Simmons, D. M., Voss, J. W., Ingraham, H. A., Holloway, J. M., Broide, R. S., Rosenfeld, M. G. & Swanson, L. W. (1990) *Genes Dev.* **4,** 695–711.
32. Kumar, T. R., Fairchild-Huntress, V. & Low, M. J. (1992) *Mol. Endocrinol.* **6,** 81–90.