0013-7227/07/$15.00/0
Printed in U.S.A.

Endocrinology 148(4):1784–1796

doi: 10.1210/en.2006-1487

Development of *l*-CDB-4022 as a Nonsteroidal Male Oral Contraceptive: Induction and Recovery from Severe Oligospermia in the Adult Male Cynomolgus Monkey (*Macaca fascicularis*)

Sheri Ann Hild, Gary R. Marshall, Barbara J. Attardi, Rex A. Hess, Stefan Schlatt, David R. Simorangkir, Suresh Ramaswamy, Sailaja Koduri, Jerry R. Reel, and Tony M. Plant

Division of Reproductive Endocrinology and Toxicology (S.A.H., B.J.A., S.K., J.R.R.), BIOQUAL, Inc., Rockville, Maryland 20850; Department of Cell Biology and Physiology (G.R.M., S.S., D.R.S., S.R., T.M.P.), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15260; and Department of Veterinary Biosciences (R.A.H.), University of Illinois, Urbana, Illinois 61802

The present study was undertaken to examine the antispermatogenic effect of *l*-CDB-4022 in the adult male cynomolgus monkey. Monkeys (four per group) were dosed via nasogastric tube for 7 d with *l*-CDB-4022 at 12.5 mg/kg·d or vehicle (d 0 = first day of dosing). Plasma levels of *l*-CDB-4022 and its deesterified metabolite were nondetectable prior to treatment and in all vehicle-treated monkeys. Peak levels of *l*-CDB-4022 and its metabolite were observed at 4 h after dosing with steady-state levels apparent around d 4. Sperm concentration and total sperm per ejaculate were decreased to levels below 1 × 10^6 sperm/ml or sperm/ejaculate in *l*-CDB-4022-treated monkeys by d 17 and remained suppressed through wk 6. Sperm motility also declined to 0% for 6 wk. Testicular volume was reduced in *l*-CDB-4022-treated monkeys through d 21. The left testis and epididymis were removed from all monkeys on d 24. At this time, the most mature germ cells in the seminiferous tubules of testes from *l*-CDB-4022-treated monkeys were either spermatocytes or round spermatids. Immature germ cells, but not mature sperm, were found in the efferent ducts and collapsed epididymal lumen of *l*-CDB-4022-treated monkeys. A steady recovery in sperm motility, concentration, and total sperm per ejaculate was observed in *l*-CDB-4022-treated monkeys such that these parameters were not different from those of vehicle-treated monkeys by wk 16. Volume of the remaining testis increased in vehicle- and *l*-CDB-4022-treated monkeys after hemicastration; however, the increase in *l*-CDB-4022-treated monkeys was delayed compared with that observed in the vehicle-treated monkeys. The morphology of the remaining testis and epididymis, which were removed on wk 17, was normal. Serum inhibin B levels were increased in *l*-CDB-4022-treated monkeys during the dosing interval; thereafter serum inhibin B levels declined such that there was no difference between the groups by wk 3. *l*-CDB-4022 treatment did not affect circulating levels of testosterone, LH, FSH, or estradiol. In conclusion, these data indicate that in the cynomolgus monkey, a representative higher primate, *l*-CDB-4022 exerts a selective antispermatogenic action, which was reversible under the conditions of this study and thus has potential as a nonhormonal oral male contraceptive. (*Endocrinology* 148: 1784–1796, 2007)

INDENOPYRIDINES, INCLUDING CDB-4022, have been shown to possess antispermatogenic activity in rats, mice, and dogs (1–9). The indenopyridine, CDB-4022, induces infertility in adult male rats; however, this antispermatogenic effect appears to be irreversible unless endogenous testosterone production is suppressed by GnRH agonist or antagonist treatments before administration of CDB-4022 (7, 9). In contrast, other indenopyridine analogs have been shown to induce reversible infertility in mice, rats, and dogs (1, 2, 4, 6). Differences in reversible *vs.* irreversible infertility may be related to differences in the potency of indenopyridine analogs, the dosing regimen used, the dose itself, and/or the species used for testing. Infertility was reversible in the majority of male mice treated with a single oral dose of 10 mg/kg of the indenopyridine Sandoz 20–438; however, higher doses of 30 or 90 mg/kg resulted in reduced recovery (2). Thus, indenopyridine analogs may induce reversible infertility at low doses and permanent testicular damage and sterility at high doses.

A series of *in vivo* experiments in our laboratory indicated that the Sertoli cell was the principal target of CDB-4022 action in the rat (8), as CDB-4022 demonstrated acute and specific adverse effects on Sertoli cells which included decreased seminiferous tubule fluid secretion, decreased production of the Sertoli cell-specific proteins, inhibin B and androgen binding protein, and vacuolization of Sertoli cell cytoplasm observed at the light microscopic level (8). Apoptotic germ cells were also observed in acute response to CDB-4022 treatment (8); however, the depopulation of the germinal epithelium may have occurred as a secondary response to the effects on Sertoli cells (10). The finding that the rat Sertoli cell is a primary target of CDB-4022 action may explain our observation of irreversible infertility in this species since the majority of Sertoli cell toxicants cause irreversible infertility (10). In particular, the degree of Sertoli cell

First Published Online January 11, 2007

Abbreviations: ANOVA-RM, ANOVA for repeated measures; DMSO, dimethylsulfoxide; LC-MS-MS, liquid chromatography and tandem mass spectrometry.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

vacuolization and disorganization of the seminiferous epithelium observed in the testes of CDB-4022-treated rats suggest severe structural damage to the Sertoli cells. In contrast, CDB-4022 did not affect Leydig cell morphology or function. Serum testosterone levels were not altered by CDB-4022 treatment, there were no detectable effects on the androgen-dependent accessory sex organs, and animals exhibited normal libidos (1, 2, 4, 6, 7, 9). These observations, in combination with the lack of genetic or overt toxicity, and other adverse side effects of indenopyridine analogs (1, 2, 11), indicate that these antispermatogenic compounds may have utility as male contraceptives. However, demonstration of a reversible antispermatogenic effect of CDB-4022 in primates was critical for its continued development as a male contraceptive. A preliminary study performed in adult cynomolgus monkeys (Hild, S. A., B. J. Attardi, and J. R. Reel, unpublished observation) suggested that the purified *l*-enantiomer of CDB-4022 induces reversible suppression of sperm production after 1 wk of daily oral dosing. Therefore, we undertook the present systematic study to examine the effects of *l*-CDB-4022 on the testes, epididymides, and serum hormone levels of sexually mature cynomolgus monkeys.

Materials and Methods

Animals

Adult sexually mature male cynomolgus monkeys (*Macaca fascicularis*) were purchased from Magee Womens Research Institute (Pittsburgh, PA) or Three Spring Scientific, Inc. (Perkasie, PA). All monkeys were bred in China and imported by Primate Products Inc. (Miami, FL). The monkeys (5–9 yr of age; 6.0–8.5 kg in body weight) were individually housed in stainless steel primate cages under controlled photoperiod (12 h light, 12 h dark) and temperature (20–22 C) at the University of Pittsburgh Primate Facility. The animals were fed a high-protein monkey chow (Lab Diet 5045; PMI Nutrition International, Brentwood, CA), which was supplemented with fresh fruits and/or vegetables daily. Drinking water was provided *ad libitum*. The study protocol, including dispensation for social grouping, was approved by the University of Pittsburgh's Institutional Animal Care and Use Committee, and animals were maintained according to the National Research Council Guide for the Care and Use of Laboratory Animals (12).

Chemicals

l-CDB-4022, [4aS,5R,9bS]2-ethyl-2,3,4,4a,5,9b-hexahydro-8-iodo-7-methyl-5-[4-carbomethoxyphenyl]1H-indeno[1,2-c]pyridine-hydrochloride (also known as RTI-4587–073), was synthesized by Research Triangle Institute (Research Triangle Park, NC) and was considered more than 98% pure based on HPLC analysis (Fig. 1). The CDB-4022 studied previously (7–9) was a racemic mixture of *l*- and *d*-enantiomers, whereas the purified *l*-enantiomer was used in this study because it is the active enantiomer (5, 13). Bouin's solution, modified Davidson's fixative, and other reagent or molecular biology grade chemicals were purchased from Sigma-Aldrich Inc. (St. Louis, MO).

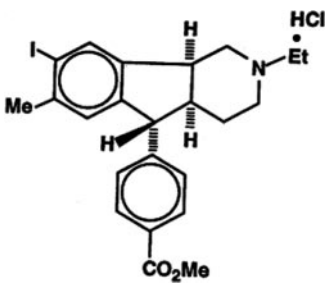

FIG. 1. Chemical structure of *l*-CDB-4022.

Treatment

The study design is depicted in Fig. 2. Male monkeys (four per group) were dosed using a nasogastric tube with either vehicle or *l*-CDB-4022 at 12.5 mg/kg·d on d 0–6 of the study (wk 0; d 0 = first day of dosing). *l*-CDB-4022 was formulated in 50% ethanol/bacteriostatic water at a concentration of 12.5 mg/ml and dosed at 1 ml/kg body weight. Monkeys were sedated with ketamine hydrochloride (100 mg per monkey im; Ketaject; Phoenix Scientific Inc., St. Joseph, MO) for insertion of the nasogastric tube. Before dosing on d 1–6, monkeys were fed a portion of banana, and the nasogastric tube was flushed with water instead of vehicle to prevent emesis.

Collection and analysis of plasma for l-CDB-4022 and its de-esterified metabolite

Blood samples (3 ml each) were obtained from all monkeys on d 0, before treatment, and at 4 and 8 h after the initial dose and then daily on d 1–7 and 15 to determine plasma levels of *l*-CDB-4022 and its deesterified metabolite. Plasma was harvested and stored at −20 C until analyzed by liquid chromatography and tandem mass spectrometry (LC-MS-MS). The deesterified metabolite is the proposed active form because of its rapid production from *l*-CDB-4022, and the authentic carboxylic acid of *l*-CDB-4022 exhibited potent antispermatogenic activity in the mouse (5). LC-MS-MS analyses were performed under the direction of Dr. Arijan Grootenhuis (N.V. Organon, Oss, The Netherlands).

All solvents were of HPLC grade and were obtained from JT Baker (Philipsburg, NJ). Demineralized water was prepared using a Milli-Q system (Millipore, Molsheim, France), and analyses were performed on a API 4000 mass spectrometer (Applied Biosystems, Palo Alto, CA), operated in positive APCI mode. Processed samples were injected on a C18 HD cartridge of a Symbiosis system (Spark Holland, Emmen, The Netherlands) in XLC mode (on-line SPE). The column was washed with demineralized water containing 0.1% formic acid, followed by 20% methanol and 0.1% formic acid in water. The sample was eluted from the cartridge in focusing mode using a solution of 95% methanol, 4.9% water, and 0.1% formic acid. After elution from the SPE cartridge, the sample was focused on the analytical column (Polaris C18A, 50 × 3 mm, 5 μm) and eluted using a 1.5-min gradient from 22 to 95% methanol in demineralized water containing 0.1% formic acid at a flow rate of 1 ml/min. Quantitative analyses were performed with the multiple reaction monitoring mode: *m/z* 476→245 for *l*-CDB-4022 and *m/z* 462→262 for its metabolite. 3D-testosterone (CAS no. 77546–39-5; CDN-Isotopes, Québec, Canada) was used as an internal standard to control for system performance (*m/z* 292→97).

Stock solutions of *l*-CDB-4022 and the carboxylic acid of *l*-CDB-4022 were prepared in dimethylsulfoxide (DMSO) at 100 μM which was diluted further to prepare standards of 1 nM to 20 μM in DMSO. Calibrators were prepared by adding one part of blank plasma, one part of DMSO standard, and four parts of acetonitrile containing 3D-testosterone, the internal standard. All these procedures were automated using a Tecan automated liquid handler (Genesis 150; Tecan, Seattle, WA). Quality controls were prepared by diluting the 100 μM standard in DMSO with blank plasma to create controls of 1, 10, 100, 1,000, and 10,000 nM. The precipitated standards, quality controls, and samples were vortexed for 15 min and centrifuged for 20 min at 10,000 rpm in 96 deep-well plates. After centrifugation, the plate was transferred to the autosampler, which left the protein pellets at the bottom of the wells. The limit of detection was 1 nM for both *l*-CDB-4022 and the carboxylic acid of *l*-CDB-4022. Assay variations for *l*-CDB-4022 and the carboxylic acid of *l*-CDB-4022 were 7.3 and 5.1%, respectively, for between assays and 11.6 and 3.6%, respectively, for within assays.

Semen collection and analysis

Semen samples were collected from each monkey by electroejaculation, using a rectal probe (14) weekly for 3 wk before treatment to establish baseline sperm parameters. Monkeys were sedated as described above. Semen samples were analyzed microscopically, and sperm concentration and motility were determined. Total sperm per ejaculate was calculated based on sperm concentration and ejaculate weight (14). Semen volumes in this study were 213 ± 31 μl (mean ± SE, n = 136). Semen samples were collected and analyzed on d 3, 7, 10, 14,

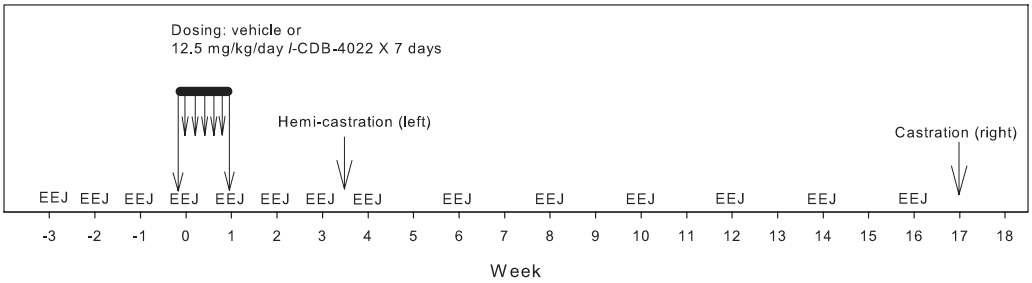

FIG. 2. Adult male cynomolgus monkeys (four per group) were dosed for 7 d with *l*-CDB-4022 at 12.5 mg/kg·d or vehicle (50% ethanol in bacteriostatic water) at 1 ml/kg·d using a nasogastric tube (d 0 = first day of dosing; wk 0 = d 0–6). Blood samples were collected on d 0–7 and weekly thereafter for measurement of serum inhibin B by ELISA and serum testosterone, FSH, LH, and estradiol using specific RIAs. Additional blood samples were obtained at 0, 4, and 8 h and d 1–7 and 15 for detection of plasma levels of *l*-CDB-4022 and its deesterified metabolite by LC-MS-MS. Weekly measurements of testis were obtained to determine testicular volume. Semen samples were collected by electroejaculation (EEJ) and analyzed on d 3, 7, 10, 14, and 17 and wk 4, 6, 8, 10, 12, 14, and 16. Testis and epididymis were surgically obtained on wk 3.5 (d 24, *left*) and wk 17 (*right*), preserved, and processed for morphological evaluation.

17, and 21 and wk 4, 6, 8, 10, 12, 14, and 16. In one instance, a monkey did not respond to electroejaculation, but a second attempt a few days later was successful.

Testicular volume

Testicular dimensions were measured with calipers weekly to determine the length and width of the testis while the monkeys were sedated with ketamine hydrochloride (see above). Testicular volume was calculated from these measurements using the formula for an ellipsoid as described by Marshall *et al.* (14). These measurements were obtained once per week for 3 wk before treatment to establish a baseline testicular size and continued until each testis was surgically removed. The mean of the three baseline measurements for each monkey was used to define pretreatment (assigned 100%), and subsequent measurements were expressed as percent of pretreatment.

Morphological evaluation of testes and epididymides

Surgical removal of the left testis and epididymis of all monkeys was performed on d 24 after weekly measurements of testicular volume and sperm counts observed from d 0–17 indicated maximal disruption of spermatogenesis had been achieved. Animals were sedated with ketamine hydrochloride and anesthetized by isoflurane inhalation (1–2% in oxygen; Abbott Animal House, North Chicago, IL), and all surgical procedures were performed under sterile conditions. On the day of surgery and for 3 d thereafter, the animals received a single im injection of penicillin (Pen-G, 300,000 U; Phoenix Scientific Inc., Fort Dodge, IA). Analgesia was achieved with ketoprofen (2 mg im twice daily for 4 d; Fort Dodge Animal Health, Fort Dodge, IA). The right testis and epididymis of all monkeys were surgically removed on wk 17 after full restoration of spermatogenesis in the remaining testes was apparent from measurements of testicular volume and sperm counts. The freshly excised epididymis was carefully dissected from the testis such that the efferent ducts remained intact. This was accomplished by taking a portion of the rete testis with the epididymis. The entire epididymis was preserved in modified Davidson's fixative, embedded in glycol methacrylate medium, and sections (2 μm) were evaluated for morphological changes (15). Portions of the testis were preserved in Bouin's solution, embedded in paraffin, and 5-μm sections prepared for morphological assessment (16).

Collection and analysis of serum for hormone measurements

Blood samples (3 ml each) were collected weekly from wk −3 through 16 of the study for all monkeys, and on d 0–6, before administration of the daily dose and on d 7, approximately 24 h after the last dose. Serum was harvested and stored at −20 C until shipment to BIOQUAL for hormone assays.

Serum samples were assayed for inhibin B using a specific ELISA (Serotec, Oxford, UK), testosterone and estradiol using specific RIAs (Diagnostic Products Corp., Los Angeles, CA), and FSH and LH by RIA using reagents supplied by Dr. A. F. Parlow (National Hormone and Peptide Program, Torrance, CA). The concentrations of the recombinant cynomolgus monkey LH (rec-moLH-RP-1) and FSH (rec-moFSH-RP-1) standards were expressed as μg/ml when they were reconstituted. The sensitivities of the rec-moFSH and rec-moLH RIAs were 0.065 and 0.572 ng/tube, respectively, and 200-μl aliquots of serum were used in each assay. All samples were analyzed in a single assay, ELISA or RIA. The limits of detection for the volumes assayed and the intraassay variations, respectively, were as follows: 15.6 pg/ml and 3.8% for inhibin B, 0.08 ng/ml and 2.7% for testosterone, 11.01 pg/ml and 6.8% for estradiol, 0.32 ng/ml and 1.3% for moFSH, and 2.86 ng/ml and 3.4% for moLH. Although this LH assay was unusually insensitive, LH levels were detectable in all samples, and, therefore, the insensitivity did not impact the assay results.

Statistical analysis

All statistical analyses were performed using SigmaStat for Windows (version 3.00; SPSS Inc., Chicago, IL). All tests were two tailed with significance set at $\alpha = 0.05$. For all parameters examined, the data failed to meet the basic test assumptions of normal distribution and/or homogeneity of variances for a parametric two-way ANOVA for repeated measures (ANOVA-RM) for the full 16-wk study interval. Therefore, statistically significant effects of treatment, vehicle *vs.* *l*-CDB-4022, on sperm concentration, sperm per ejaculate, and sperm motility were detected by a series of one-way ANOVA-RM tests with comparisons to wk 0 as control. Friedman's ANOVA-RM on ranks followed by Student-Newman-Keuls *post hoc* test for a significant F value was used to detect differences in sperm per ejaculate and sperm concentration, whereas a parametric one-way ANOVA followed by Holm-Sidak *post hoc* test was used to detect differences in sperm motility. Since hemicastration is known to affect testicular function, particularly with regard to testicular size, potential treatment effects on right testicular volume were detected by two-way ANOVA-RM on data for wk 0–3 (hemicastration performed on d 24). Because a significant treatment × time interaction was observed, one-way ANOVA-RM tests were performed on the data for each treatment group (vehicle and *l*-CDB-4022) to clarify time related effects. To evaluate treatment effects on circulating hormones, we focused on the dosing interval. The area under the curve was determined for levels of each hormone for wk 0–1, and a Student's *t* test was used to detect potential differences between the two treatment groups (vehicle *vs.* *l*-CDB-4022). If a significant difference was detected, one-way ANOVA-RM tests were performed to detect significant differences in daily levels, compared with d 0. Differences that were statistically significant based on $P < 0.05$ are noted in the text, whereas nonstatistical changes that may still be biologically relevant are simply mentioned.

Results

Circulating levels of l-CDB-4022 and its metabolite

Plasma levels of *l*-CDB-4022 and its deesterified metabolite were below the limit of detection (1 nM) in monkeys dosed

with the vehicle (not shown) and in *l*-CDB-4022-treated monkeys before treatment (time 0; Fig. 3). Peak levels of *l*-CDB-4022 and its deesterified metabolite were observed in the plasma at 4 h after dosing. Both *l*-CDB-4022 and its deesterified metabolite reached steady-state levels around d 4 of dosing. It was particularly noteworthy that plasma levels of the deesterified metabolite were approximately 10-fold greater than the levels of the parent compound. This observation implies that the parent compound is rapidly converted by esterases to the deesterified metabolite.

l-CDB-4022-induced oligospermia and recovery

Pretreatment evaluation of semen samples indicated that the eight monkeys assigned to this study exhibited normal sperm parameters. Treatment with the vehicle had no effect on sperm concentration, sperm per ejaculate, and sperm motility ($P = 0.331$, 0.188, and 0.919, respectively). Sperm concentration and total sperm per ejaculate were greatly increased on d 3 in *l*-CDB-4022-treated monkeys ($P < 0.05$) but not vehicle-treated monkeys (Fig. 4). Thereafter sperm concentration and total sperm per ejaculate decreased to levels less than 1×10^6 sperm/ml and 1×10^6 sperm/ejaculate by d 17, indicating severe oligospermia (Fig. 4). *l*-CDB-4022's effect on sperm concentration was significant ($P < 0.05$) on wk 4 (0.11×10^6 sperm/ml *vs.* 183.10×10^6 sperm/ml on wk 0). After a significant increase ($P < 0.05$) in sperm motility on d 3, sperm motility dropped to 0% in all four *l*-CDB-4022-treated monkeys by d 17 (Fig. 5), and testicular volume was significantly suppressed ($P < 0.05$) in *l*-CDB-4022-treated monkeys through wk 3 (Fig. 6).

After hemicastration, sperm concentration and total sperm per ejaculate declined slightly, but not significantly, in the vehicle-treated monkeys by wk 6 and remained at this lower level through wk 10 (Fig. 4). By wk 12–14, these parameters were similar to those observed in the monkeys before treatment (253.00×10^6 sperm/ml at wk 14 *vs.* 208.71×10^6 sperm/ml for mean pretreatment value and 30.70×10^6 sperm/ejaculate at wk 14 *vs.* 36.39×10^6 sperm/ejaculate for mean pretreatment value). An increase in right testicular volume was apparent by wk 6 in vehicle-treated monkeys (Fig. 6), and this continued to increase until reaching a plateau at wk 11. Sperm concentration and total sperm per ejaculate remained suppressed to less than 1×10^6 in *l*-CDB-4022-treated monkeys through wk 6 (mean values of 0.11, 0.08, 0.01, and 0.65×10^6 sperm/ejaculate on wk 2.5, 3, 4, and 6 *vs.* pretreatment value of 23.58×10^6 sperm/ejaculate), indicating induction of severe oligospermia. Sperm motility was 0% in all four *l*-CDB-4022-treated monkeys through wk 6 (Fig. 5). Indeed, sperm motility was significantly suppressed ($P < 0.05$) in *l*-CDB-4022-treated monkeys on wk 1.4 (d 10), 2, 2.4 (d 17), 3, 4, 6, 10, and 14. A gradual increase in sperm concentration, total sperm per ejaculate, and sperm motility was observed in the *l*-CDB-4022-treated monkeys through wk 12 (Figs. 5 and 6). However, one *l*-CDB-4022-treated monkey (no. 3178) exhibited a delay in recovery, compared with the other three monkeys in this group. It is interesting to note that monkey 3178 also exhibited the highest plasma levels of *l*-CDB-4022 and its metabolite. By wk 16, all four *l*-CDB-4022-treated monkeys exhibited sperm concentrations and total sperm per ejaculate, which were not different from those observed in vehicle-treated monkeys. As observed in vehicle-treated monkeys, right testicular volume increased in *l*-CDB-4022-treated monkeys after hemicastration but remained less than was observed in vehicle-treated monkeys until wk 10 (Fig. 6). A delayed increase in right testicular volume in monkey 3178 correlated with the time lag in sperm recovery in this monkey.

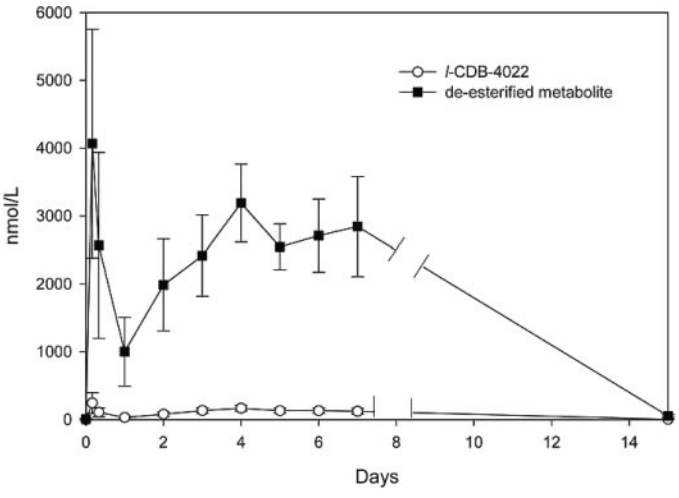

FIG. 3. Plasma levels of *l*-CDB-4022 and its deesterified metabolite in *l*-CDB-4022-treated monkeys. The deesterified metabolite is the proposed active form because it exhibited potent antispermatogenic activity in the mouse (5), and *l*-CDB-4022 is rapidly metabolized in the monkey. The *break in the lines* indicates a gap in blood sample collection. *Symbols* and *brackets* represent mean ± SE, n = 4.

Testicular and epididymal morphology

Normal testicular morphology, namely seminiferous epithelium consisting of Sertoli cells and germ cells through testicular spermatozoa, was observed in the left testes of the four vehicle-treated monkeys (Fig. 7, A and B). In the *l*-CDB-4022-treated monkeys, seminiferous tubules on d 24 were smaller in diameter and exhibited a reduction in the number of spermatocytes and round spermatids, whereas few, if any, elongated spermatids were present (Fig. 7, C–E). The fourth monkey, no. 3178, had seminiferous tubules that were conspicuously lacking both round and elongated spermatids (Fig. 7F). This finding correlates with the delay in recovery of spermatogenesis, as detected by testicular volume and sperm counts, in this monkey. By wk 17, the morphology of the right testes obtained from the *l*-CDB-4022-treated monkeys was similar to that observed in vehicle-treated monkeys, particularly in regard to the presence of testicular spermatozoa (Fig. 8).

Morphological evaluation of the left efferent ducts and epididymides removed on d 24 was consistent with the antispermatogenic effect of *l*-CDB-4022 on the testis (Fig. 9). In the left efferent ducts of *l*-CDB-4022-treated monkeys, no sperm were present, the lumen was collapsed, and the nonciliated cells appeared to be regressed (Fig. 9, B and C), compared with vehicle-treated monkeys (Fig. 9A). There were no or few mature sperm in the lumen of the entire epididymis of the *l*-CDB-4022-treated monkeys, whereas ma-

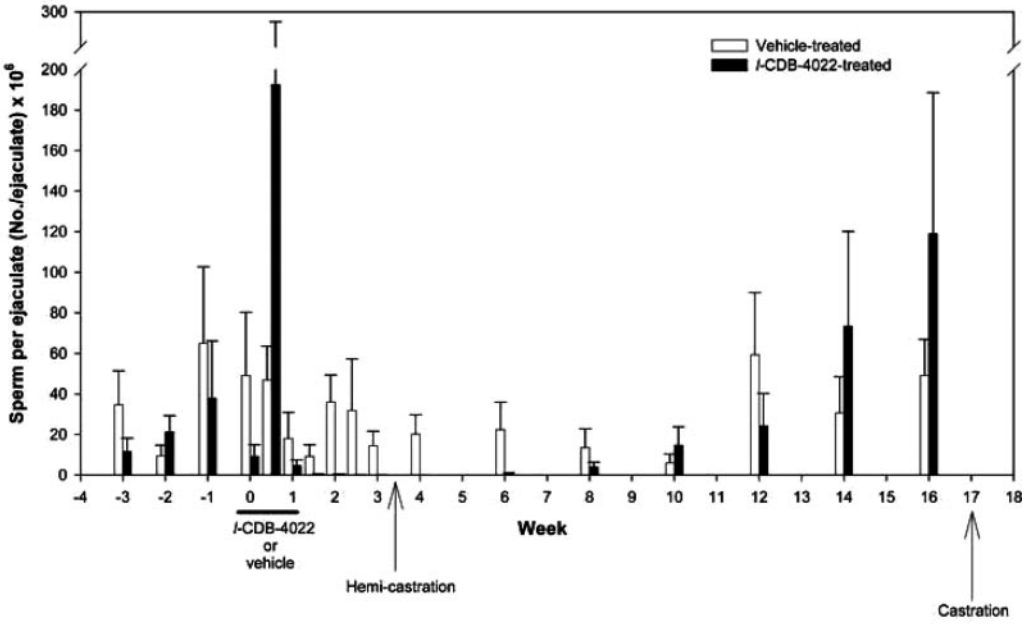

FIG. 4. Total sperm per ejaculate in vehicle- and *l*-CDB-4022-treated monkeys. Sperm concentration exhibited a similar trend (data not shown). Sperm were detected in the semen of *l*-CDB-4022-treated monkeys on wk 2.5, 3, and 4, but numbers were well below 1×10^6 sperm/ejaculate, indicating severe oligospermia (means of 0.11, 0.08, and 0.01×10^6 sperm/ejaculate, respectively; *bars* are not visible on graph). *Bars* and *brackets* represent mean ± SE, n = 4.

ture sperm were present in the epididymis of the vehicle-treated monkeys (Fig. 9, D and F). The lumen was collapsed throughout the three regions of the epididymis, and periodic acid-Schiff-positive material was observed in the lumen of the cauda epididymis. Round spermatids, pachytene spermatocytes, and cytoplasmic bodies were also observed in the epididymal lumen of *l*-CDB-4022-treated monkeys (Fig. 9G). For the most part, the epididymal epithelium appeared normal in *l*-CDB-4022-treated monkeys. However, abnormal basal cells, which were round and eosinophilic with pleomorphic nuclei, were present in the caput and corpus epididymis (Fig. 9E). These abnormal cells were more prominent in the caput epididymis and occasionally were seen migrating toward the lumen. Epithelial cysts were also observed in the cauda epididymis of *l*-CDB-4022-treated monkeys (Fig. 9H). All of these morphological effects in the efferent ducts and epididymis appeared to be completely reversible, as the right efferent ducts and epididymis from *l*-CDB-4022-treated monkeys obtained at wk 17 appeared normal (Fig. 10).

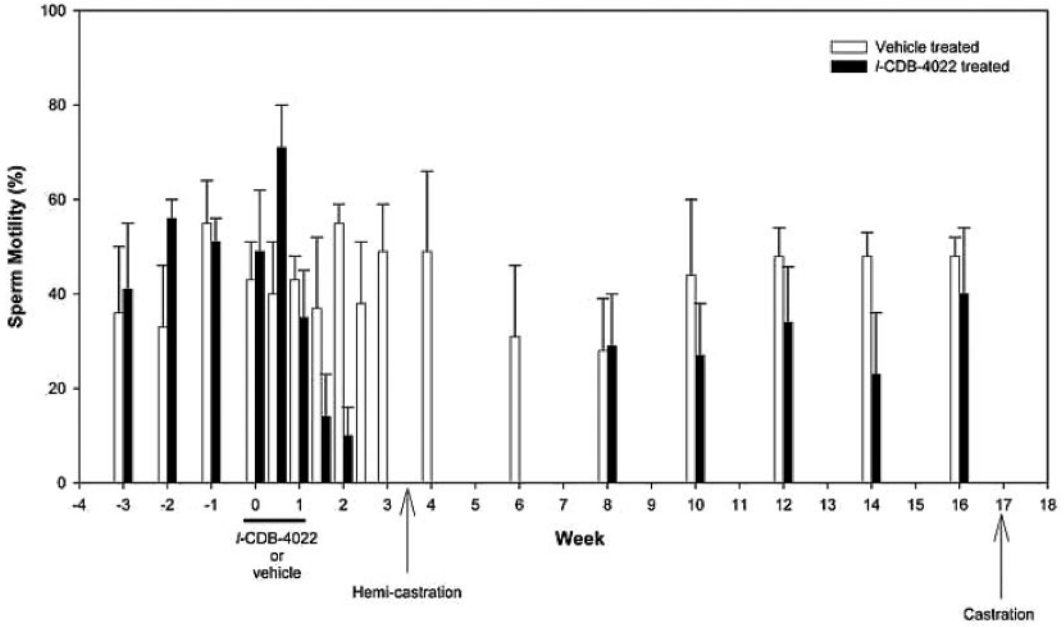

FIG. 5. Sperm motility in vehicle- and *l*-CDB-4022-treated monkeys. *Bars* and *brackets* represent mean ± SE, n = 4.

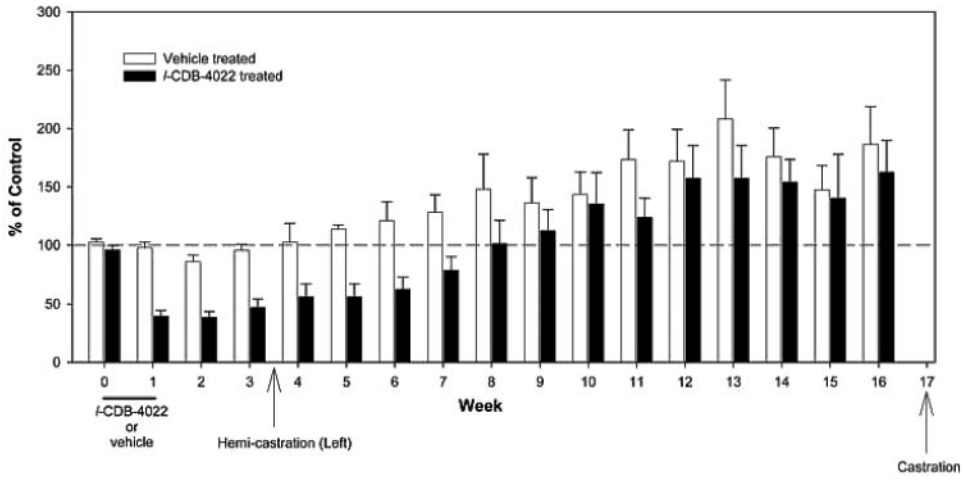

FIG. 6. Right testicular volume in vehicle- and *l*-CDB-4022-treated monkeys. The mean value of the three baseline measurements for each monkey was used to define pretreatment (assigned 100%; *dashed line*), and subsequent measurements were expressed as percent of control. *Bars* and *brackets* represent mean ± SE, n = 4.

Treatment effects on circulating hormones

Serum levels of testosterone tended to range between 2 and 4 ng/ml throughout the study in both vehicle- and *l*-CDB-4022-treated monkeys (Fig. 11A). There were no differences between the two treatment groups ($P = 0.737$). Likewise, serum LH levels averaged around 4 ng/ml in both vehicle- and CDB-4022D-treated monkeys and were not different ($P = 0.817$) between the two groups (Fig. 11B). Hemicastration also did not appear to affect serum testosterone or LH levels measured in weekly blood samples.

Serum inhibin B levels exhibited a slight, but not significant, decline after the first dose, followed by a significant increase ($P < 0.05$) for the duration of the 7-d *l*-CDB-4022 dosing interval (Fig. 12A). Thereafter serum inhibin B levels declined such that there was no difference between the two treatment groups by wk 3. After hemicastration, serum inhibin B levels were decreased by 50% and remained at this new set point for the duration of the study. Although inhibin B levels were increased during *l*-CDB-4022 treatment, serum FSH levels were not suppressed during this interval (Fig. 12B). There was no significant difference ($P = 0.102$) in circulating FSH levels between vehicle- and *l*-CDB-4022-treated monkeys over the treatment interval. Hemicastration resulted in a sustained increase in serum FSH levels in both vehicle and *l*-CDB-4022-treated monkeys. There appeared to be a slightly greater increase in serum FSH levels in *l*-CDB-4022-treated monkeys, compared with that in vehicle-treated monkeys after hemicastration. However, serum levels of FSH were equivalent in both treatment groups by wk 12.

As observed for testosterone, there were no effects of treatment on the low, but detectable, circulating levels of estradiol in these adult monkeys ($P = 0.748$, not shown). Likewise, hemicastration had no discernible effect on mean serum estradiol levels, which ranged from 14 to 35 pg/ml.

Discussion

The results of this study indicated that *l*-CDB-4022 consistently induced severe, but reversible oligospermia (less than 1×10^6 sperm/ejaculate compared with 23.58×10^6 sperm/ejaculate before treatment) when administered orally as seven daily doses to adult cynomolgus monkeys, a representative higher primate. The onset of oligospermia after seven daily doses occurred within 2 wk of initiating treatment and lasted approximately 6 wk with complete recovery occurring in all males by wk 16. An initial increase in sperm in the ejaculates of *l*-CDB-4022-treated monkeys was observed on d 3. This presumably reflects the depopulation of the seminiferous tubules in response to *l*-CDB-4022 treatment and correlates with the decrease in testicular volume observed by wk 1. Morphological evaluation of the testes at d 24 was consistent with *l*-CDB-4022-induced loss of spermatocytes and spermatids. Likewise, mature sperm were absent from the lumen of the epididymides of *l*-CDB-4022-treated monkeys at d 24. These findings are consistent with the loss of immature germ cells from the testis. Recovery from *l*-CDB-4022 treatment was complete based on sperm number in the ejaculates, which were equivalent to pretreatment values, and on testicular and epididymal morphology at wk 17. Unlike our previous results in the rat (7, 9), the antispermatogenic effects of *l*-CDB-4022 on the primate testis were reversible.

The testis appeared to be the primary target of *l*-CDB-4022 action, whereas the effects on the efferent ducts and epididymis were likely to be secondary. The epithelium of the efferent ducts is dependent on stimulation by androgen and estrogen from the luminal fluids, and in the absence of fluid, the nonciliated cells typically regress (17, 18). In the present study, *l*-CDB-4022 resulted in regression of nonciliated cells, whereas ciliated cells of the efferent ducts appeared normal. These results suggest a loss of testicular fluid secretions, which normally contain estrogen (19), entering the efferent ducts in *l*-CDB-4022-treated monkeys. Alternatively, *l*-CDB-4022 may be having a direct effect on the nonciliated cells, possibly decreasing the expression of estrogen receptor α, which could result in the observed effects, as seen in other animal models (15, 18). We were unable to detect any effect of *l*-CDB-4022 treatment on serum levels of estradiol; however, the proposed effect at the level of the efferent ducts and epididymides would not necessarily be detected in the circulation (19). The principal cells appeared fairly normal, suggesting that androgen stimulation was adequate to maintain their structure (20). Morphological effects noted in the basal cells were unusual, and this cell type appeared to be more susceptible to the effects of *l*-CDB-4022 than principal

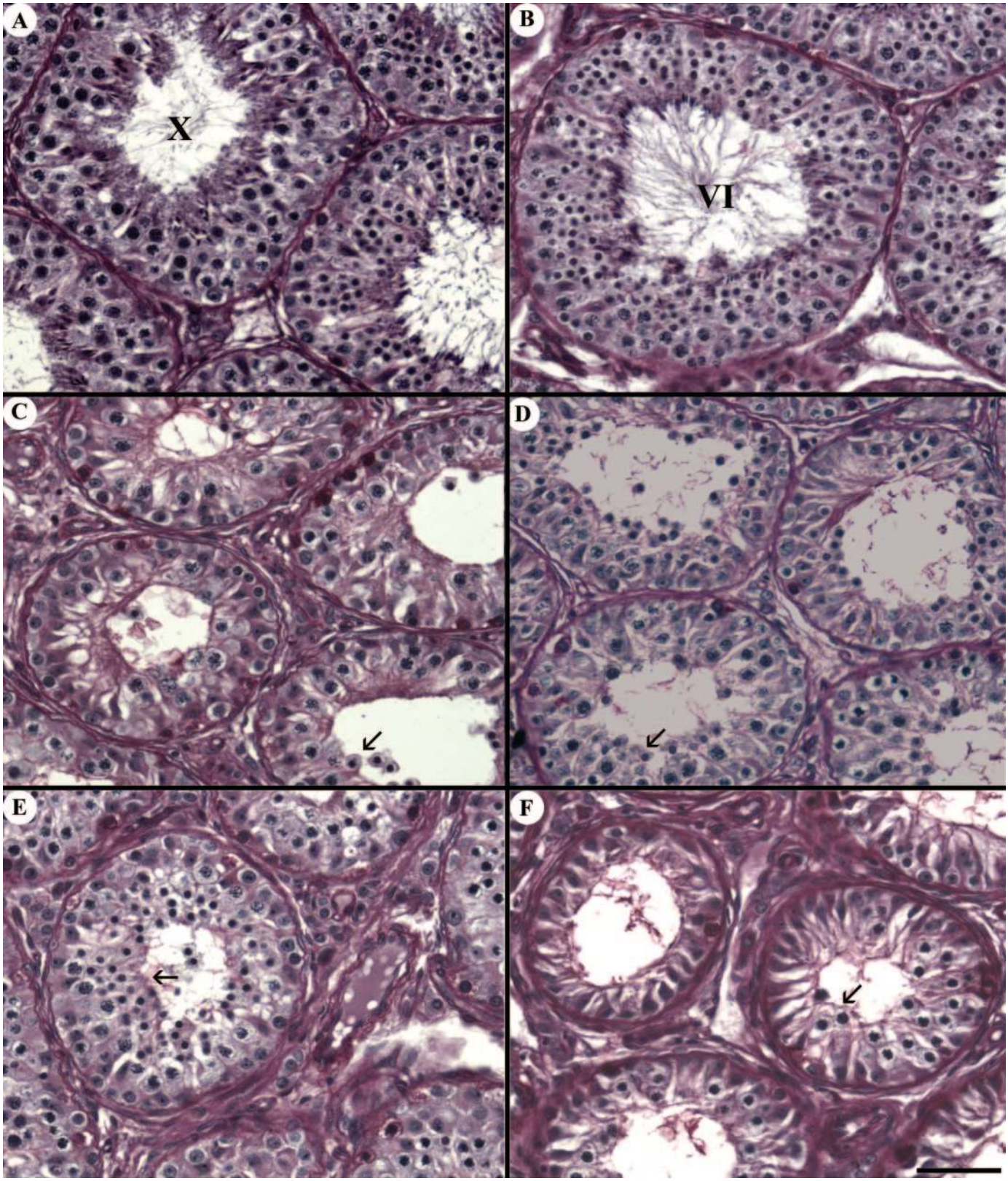

FIG. 7. Morphology of the left testis obtained at wk 3.5 from vehicle- and *l*-CDB-4022-treated monkeys. A and B, Left testis of two representative vehicle-treated monkeys. *Roman numerals in the center of the tubules* indicate the stage of the cycle of the seminiferous epithelium. C–F, Left testis from each of the four *l*-CDB-4022-treated monkeys. These tubules could not be staged due to incomplete spermatogenesis. The most mature germ cells present in each panel are as follows: C, step 9 spermatid (*arrow*); D, step 14 spermatid (also called testicular spermatozoa, *arrow*); E, step 7 to 8 spermatids (*arrow*); F, pachytene spermatocytes (*arrow*); the remaining spermatocytes in this panel are leptotene-zygotene. *Bar*, 50 μm.

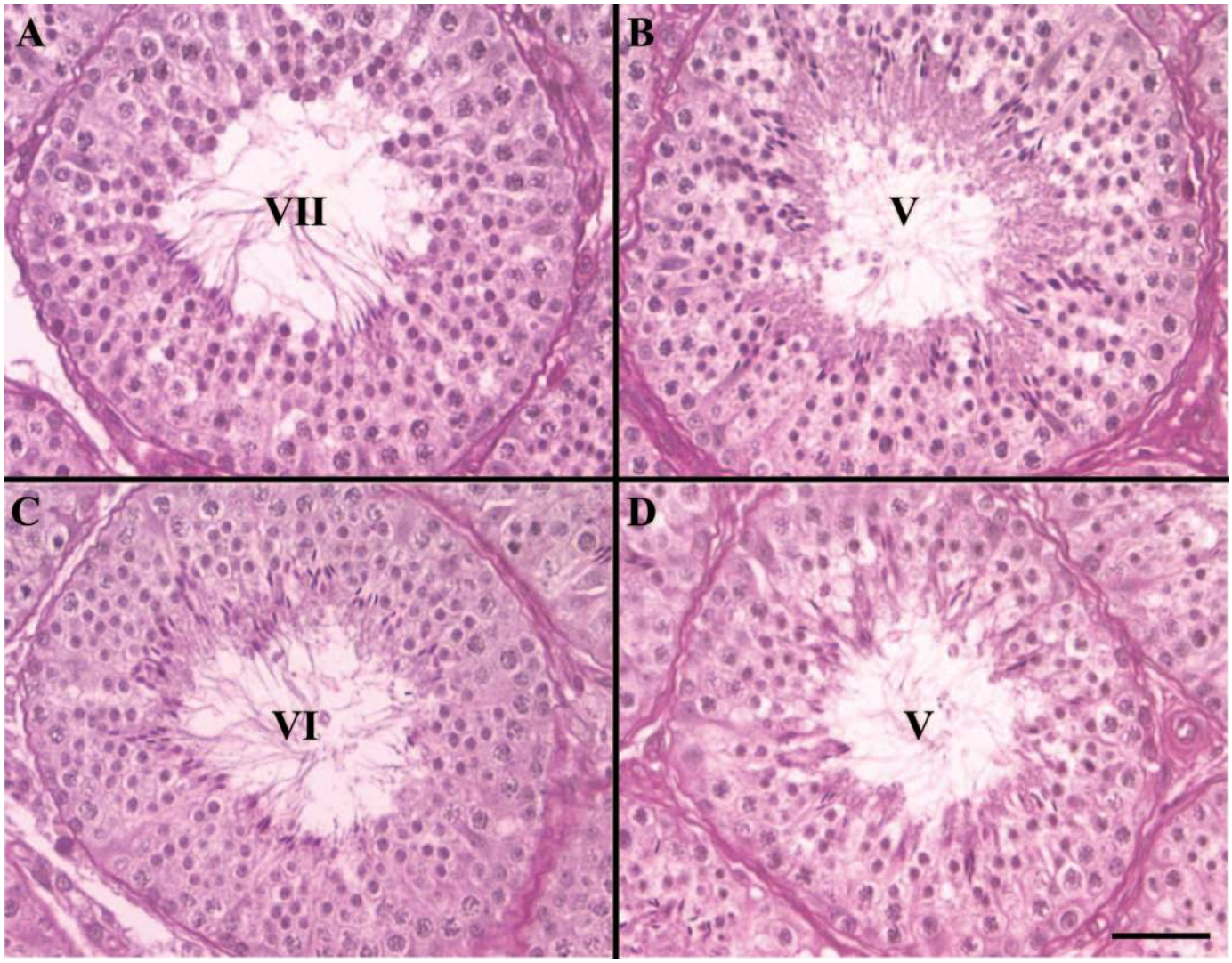

FIG. 8. Morphology of the right testis obtained at wk 17 from each of the four *l*-CDB-4022-treated monkeys. All four monkeys exhibited normal spermatogenesis with formation of testicular spermatozoa. *Roman numerals in the center of the tubules* indicate the stage of the cycle of the seminiferous epithelium. *Bar*, 50 μm.

cells. This could be due to a difference in the origin of these cells. Basal cells are hypothesized to be derived from immune cells or immune-associated cells (21), whereas the other cells of the epididymides arise from the Wolffian duct (22). This difference in cell origin may be responsible for their differential response to *l*-CDB-4022. The basal cells also appear to be more sensitive to loss of luminal contents because they lose their expression of aquaporin 3 after efferent ductule ligation (23). These effects of *l*-CDB-4022 were completely reversible after cessation of treatment. Normal concentrations of sperm were present in the epididymal lumen, and the epithelium of both the efferent ducts and epididymides appeared normal at wk 17.

l-CDB-4022 appeared to have no major effect on the endocrine system, except for the paradoxical changes in inhibin B release from the Sertoli cell of the testis during the treatment interval. Serum inhibin B levels were initially decreased slightly after the first dose of *l*-CDB-4022 followed by a significant increase for the duration of the dosing interval. Inhibin B is the major testicular component of the negative feedback loop regulating FSH secretion in the closely related rhesus monkey (24), and therefore, the lack of suppression in circulating FSH levels during the dosing interval when inhibin B was elevated is surprising. The reason for this is unknown but may be related to concomitant increases in circulating or local levels of activin or other members of the TGFβ superfamily (such as bone morphogenetic proteins) with opposing actions. Thus, inhibin, acting through its coreceptor, betaglycan, can compete with activin for binding to type II activin receptors to block activin signaling, a consequence of which is to suppress synthesis and secretion of FSH at the pituitary level (25, 26). However, parallel increases in circulating or local levels of activin or other TGFβ family members could override inhibin's binding to activin receptors and, thus its negative feedback effect on FSH. On the other hand, the lack of an effect on the LH/testosterone feedback axis indicates that, like in the rat, *l*-CDB-4022 does not affect Leydig cell function in higher primates. This find-

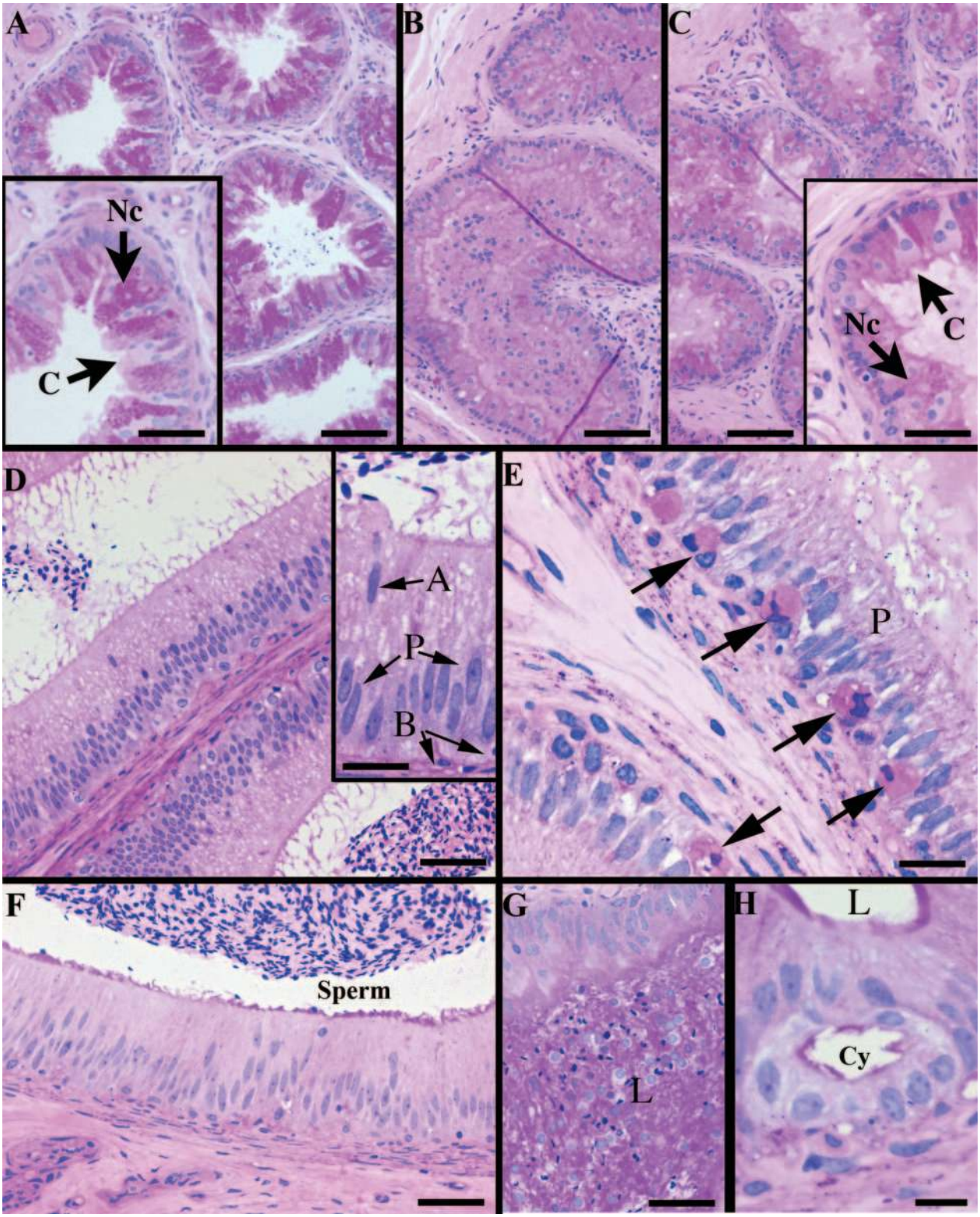

FIG. 9. Morphology of the left efferent ducts and epididymis obtained at wk 3.5 from vehicle- and *l*-CDB-4022-treated monkeys. A, Efferent ducts from a vehicle-treated monkey showing normal ciliated (C) and nonciliated (Nc) cells. B and C, Efferent ducts of *l*-CDB-4022-treated monkeys demonstrating the presence of immature germ cells and absence of mature sperm in the lumen and apparent regression of nonciliated cells (Nc). D and F, Caput and corpus epididymis, respectively, from a vehicle-treated monkey. Note the normal appearance of apical (A), principal (P), and basal (B) cells and the presence of mature sperm in the lumen of the epididymis from the vehicle-treated monkeys. E, Caput epididymis of *l*-CDB-4022-treated monkey; *arrows* point to abnormal basal cells, whereas principal (P) cells appeared normal. The *dark structures* in the basal cells are pleomorphic nuclei. G and H, Cauda epididymis of *l*-CDB-4022-treated monkey showing immature germ cells in the lumen (L) of panel G, and an epithelial cyst (Cy) in panel H (L, lumen). *Bar*, A–C, 100 μm; A and C, *insets*, and D, F, and G, 50 μm; D, *inset*, and E, 25 μm; H, 20 μm.

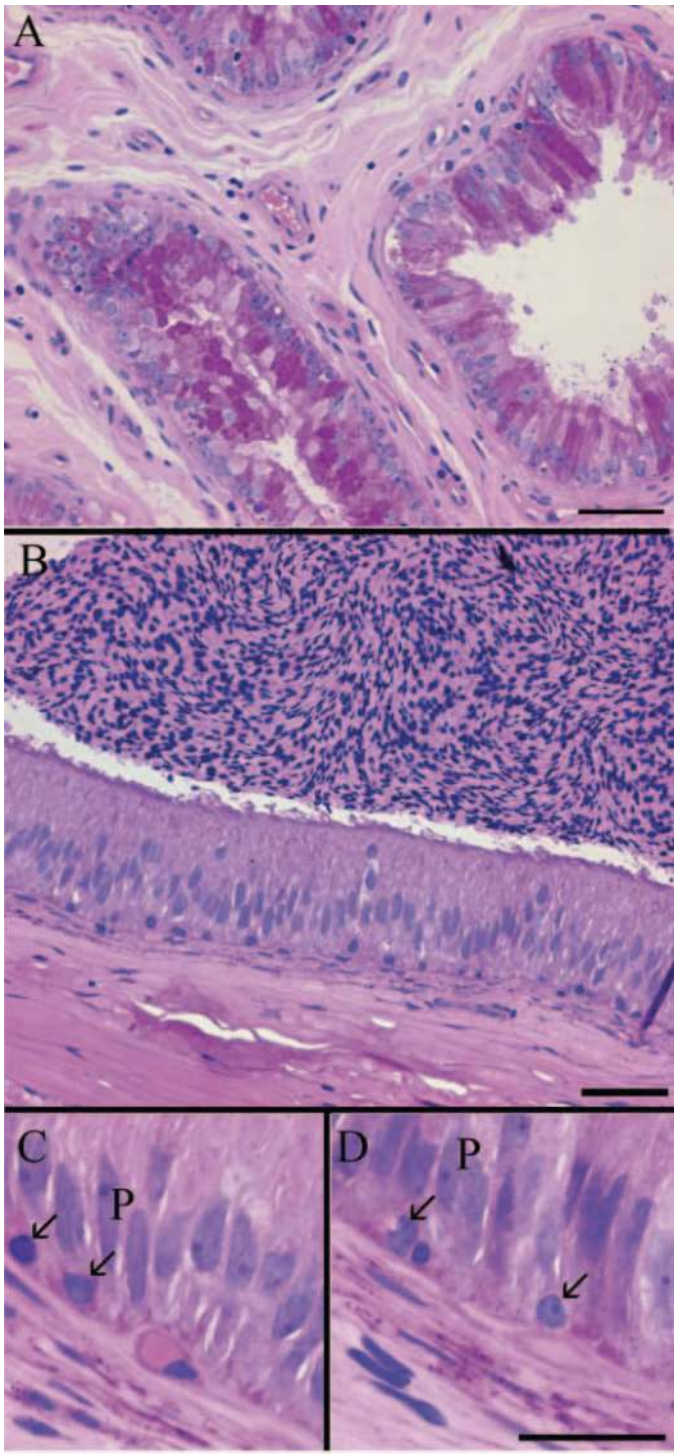

FIG. 10. Morphology of the right efferent ducts and epididymis obtained at wk 17 from representative *l*-CDB-4022-treated monkeys. A, Efferent ducts exhibiting normal morphology, particularly of nonciliated cells. B-D, Corpus epididymis; note the normal appearance of the epididymis with mature sperm present in the lumen (B) and normal morphology of principal (P) and basal (*arrows*) cells (C and D). *Bar*, A and B, 50 μm; C and D, 25 μm.

ing bodes well for the continued development of *l*-CDB-4022 as a potential contraceptive for men.

The changes in serum inhibin B levels in *l*-CDB-4022-treated monkeys suggest that *l*-CDB-4022 acts, at least in part, by affecting Sertoli cell function. In the rat, the Sertoli cell appears to be the primary target cell in the testis, with serum inhibin B levels dropping to nondetectable levels in rats rendered irreversibly infertile (8, 9). In contrast, the antispermatogenic effect in monkeys was spontaneously reversible, and serum inhibin B levels were increased after a slight decline on d 1. An increase in serum inhibin B levels was also observed in response to CDB-4022 treatment in rats that had received acyline, a GnRH antagonist, for 4 wk before the single oral dose of CDB-4022 (9). These acyline-pretreated rats exhibited reversible infertility after treatment with CDB-4022. Thus, increases in serum inhibin B levels were associated with reversibility of CDB-4022-induced antispermatogenic effects in both species. Additional experiments using a nonhuman primate model, such as the monkey, are required to elucidate the testicular target cell and mechanism of action of *l*-CDB-4022. Differences in reversibility, the onset of the antispermatogenic effect (within 2 wk in the monkey *vs.* 4 wk in the rat), and the effects on inhibin B secretion from the Sertoli cell suggest that the mechanism of action of *l*-CDB-4022 in primates differs to some degree from that in the rat.

The results associated with hemicastration of the male monkeys in this investigation are consistent with those from a previous study in rhesus monkeys (27) and confirm that the adult primate testis undergoes compensatory hypertrophy. After hemicastration, right testicular volume increased in both vehicle- and *l*-CDB-4022-treated monkeys. The increase in right testicular volume in *l*-CDB-4022-treated monkeys presumably reflects both recovery from the antispermatogenic effect of *l*-CDB-4022 and compensatory hypertrophy, as testicular volumes increased to levels comparable with those in vehicle-treated monkeys by wk 10. Associated with the increase in testicular size, sperm numbers increased such that by wk 17, the values were similar to those obtained before treatment. These data confirm that the monkey testis does not function at maximal spermatogenic capacity and therefore, unlike the rodent testis, is able to compensate after removal of one testis (27). In addition, hemicastration had no apparent effect on weekly serum levels of LH and testosterone, suggesting that steroidogenic compensation occurred in less than 1 wk. This result concurs with the study in rhesus monkeys in which compensation of the LH/testosterone pathway occurred within 48 h after hemicastration. However, inhibin B levels decreased by 50% after hemicastration, and a sustained increase in serum FSH levels was observed in both vehicle and *l*-CDB-4022-treated monkeys. These data confirm that, in adult macaques, the remaining testis cannot compensate in terms of inhibin B synthesis and secretion, which is presumably limited by the finite number of Sertoli cells in the remaining testis (27). The sustained increase in serum FSH levels was presumably due to reduced circulating inhibin B, the major negative feedback signal regulating FSH in the monkey (24), and suggest that the inhibin-FSH feedback loop was operative again at this point.

At present, there are few new promising candidates for male contraceptives, particularly orally active treatments. The only reliable and Food and Drug Administration-approved methods are condoms or vasectomy, and both have several limitations. Steroid hormonal contraceptives are en-

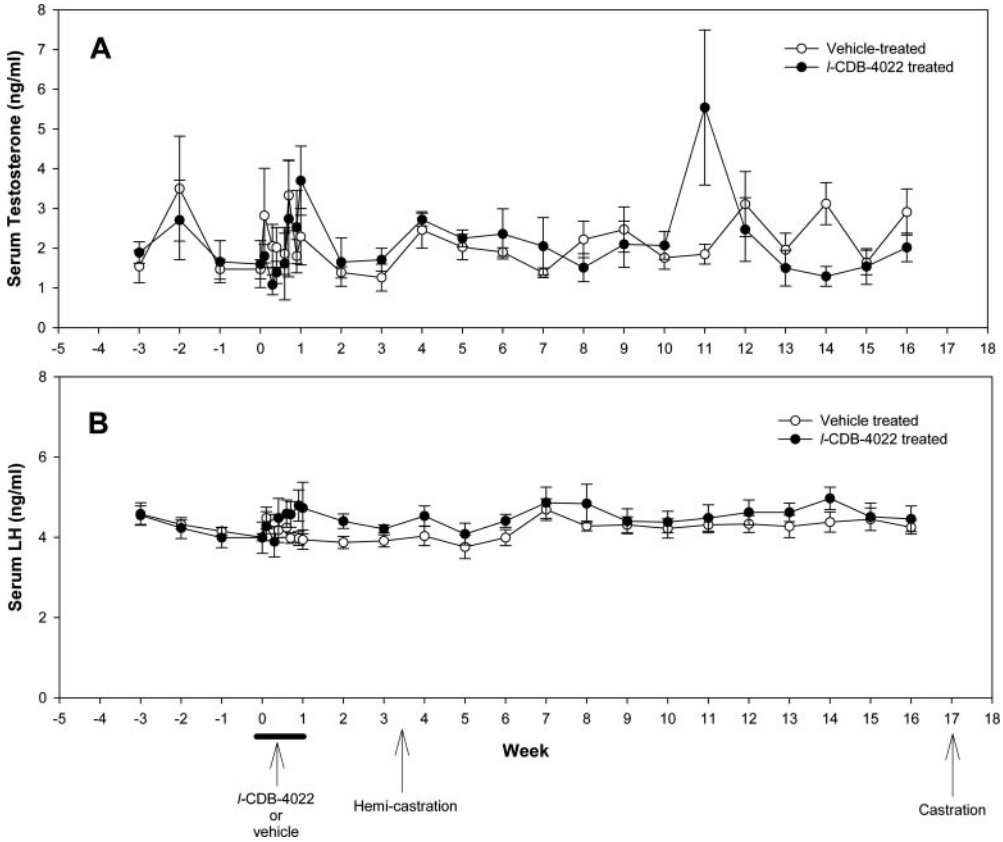

FIG. 11. Serum testosterone (A) and LH (B) levels in vehicle- and *l*-CDB-4022-treated monkeys. *Symbols* and *brackets* represent mean ± SE, n = 4.

tering phase III clinical trials and may be reaching the marketplace within a few years (28–30). However, hormonal contraceptives still have disadvantages including the long delay between initiation of treatment and induction of infertility and the lack of a long-acting injectable or orally active androgen as part of the treatment regimen. Other proposed nonhormonal male contraceptives including gossypol and tripterygium have not moved forward due to low efficacy and/or toxicity issues (31). An alkylated imino sugar, *N*B-DNJ (*N*-butyldeoxynojirimycin), appeared to be a promising candidate in mice; however, a recent study in men indicated a lack of efficacy and undesirable side effects (32). Results from the present study indicate that *l*-CDB-4022 has potential as a nonsteroidal oral contraceptive in men because it induced reversible severe oligospermia in primates after seven daily oral doses. The onset of oligospermia was rapid (within 2 wk of initiation of dosing) and spontaneously reversible after a sustained interval of oligospermia (~6 wk). The lack of genetic or overt toxicity and other adverse side effects of indenopyridines supports the continued development of these compounds, including *l*-CDB-4022, for male contraception (1, 2, 11, 33).

In conclusion, seven daily oral doses of *l*-CDB-4022 induced reversible severe oligospermia in nonhuman primates without affecting circulating hormone levels, except for a transient increase in inhibin B levels. The testis appeared to be the primary target of *l*-CDB-4022 action with secondary effects on the efferent ducts and epididymis due primarily to a decrease in luminal content and loss of testicular factor(s). Further studies are required to elucidate the target cell type(s), Sertoli and/or germ cell, of *l*-CDB-4022 action in the primate testis. The results of this study suggest that the mechanism of action in the primate testis may differ from that in the rat. In particular, the antispermatogenic effect in the monkey was spontaneously reversible under the conditions of this study, whereas a single oral dose of *l*-CDB-4022 induced infertility in rats that was irreversible. Continued development of *l*-CDB-4022 as a male contraceptive is dependent on favorable results in additional long-term dosage efficacy studies in nonhuman primates and minimal toxicity in safety studies.

Acknowledgments

The authors are grateful to Dr. Arijan Grootenhuis and his colleagues at N.V. Organon (Oss, The Netherlands) for the LC-MS-MS analysis of the plasma samples for levels of *l*-CDB-4022 and its deesterified metabolite. The authors express thanks for the technical expertise of the following technicians: Emily Hua, Margaret Krol, Trung Pham, and Bruce Till (BIOQUAL); Michael Cicco, Lisa Nieman Vento, Scott Hergenrother, and Rachel Rosland (Center for Research in Reproductive Physiology, University of Pittsburgh); and Kay Carnes (University of Illinois). We thank Dr. Richard Blye (Contraception and Reproductive Health Branch, National Institute of Child Health and Human Development) for his input into the design of this study and review of this manuscript.

Received November 8, 2006. Accepted December 28, 2006.

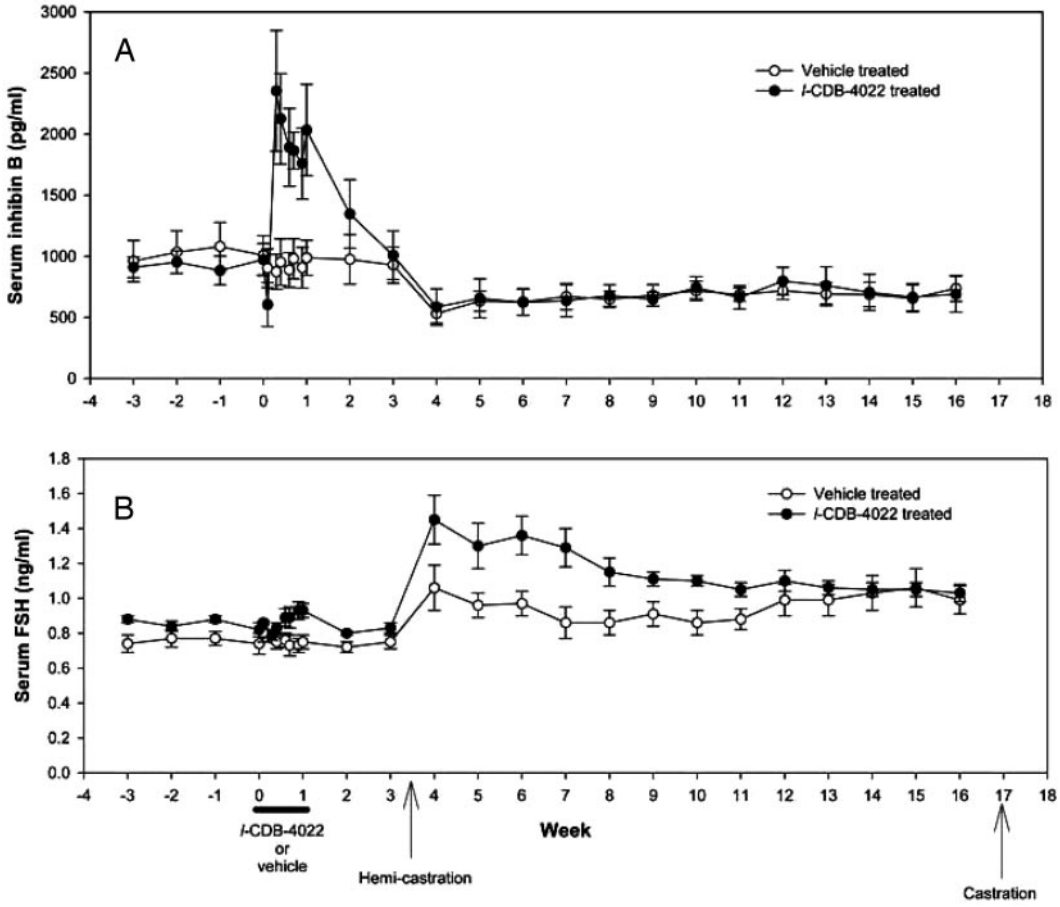

FIG. 12. Serum inhibin B (A) and FSH (B) levels in vehicle- and *l*-CDB-4022-treated monkeys. *Symbols* and *brackets* represent mean ± SE, n = 4.

Address all correspondence and requests for reprints to: Dr. Sheri Ann Hild, BIOQUAL, Inc., 9600 Medical Center Drive, Rockville, Maryland 20850. E-mail: shild@bioqual.com.

This work was supported by National Institute of Child Health and Human Development Contract NO1-HD-2-3338 awarded to BIOQUAL, Inc. and National Institutes of Health Grant U54 HD08160 awarded to the University of Pittsburgh.

This work was presented in part at the 31st Annual Meeting of the American Society of Andrology, Chicago, IL, April 2006.

The authors have nothing to disclose.

References

1. **Hodel C, Suter K** 1978 Reversible inhibition of spermatogenesis with an indenopyridine (20–438). Arch Toxicol Suppl 1:323–326
2. **Matter BE, Jaeger I, Suter W, Tsuchimoto T, Deyssenroth H** 1979 Actions of an antispermatogenic, but not mutagenic, indenopyridine derivative in mice and *Salmonella typhimurium*. Mutat Res 66:113–127
3. **Cook CE, Wani MC, Jump JM, Lee Y-W, Fail PA, Anderson SA, Gu Y-Q, Petrow V** 1995 Structure-activity studies of 2,3,4,4a,5,9b-hexahydroindeno[1,2-c]pyridines as antispermatogenic agents for male contraception. J Med Chem 38:753–763
4. **Fail PA, Anderson SA, Wani MC, Lee YW, Cook CE** 1995 Contraceptive potency of two indenopyridine analogs of Sandoz 20–438 tested in Swiss mice. J Androl 15(Suppl 1):P-48 (abstract 99)
5. **Cook CE, Jump JM, Zhang P, Stephens JR, Lee Y-W, Fail PA, Anderson SA** 1997 Exceptionally potent antispermatogenic compounds from 8-halogenation of (4aRS,5SR,9bRS)-hexahydroindeno-[1,2-c]pyridines. J Med Chem 40:2111–2112
6. **Chang CLT, Fung HP, Lin YF, Kuo CY, Chien CW** 2002 Indenopyridine hydrocloride induced testicular spermatogenesis failure with high seminal alkaline phosphatase levels in male dog. Biol Pharm Bull 25:1097–1100
7. **Hild SA, Meistrich ML, Blye RP, Reel JR** 2001 Lupron Depot prevention of antispermatogenic/antifertility activity of the indenopyridine, CDB-4022, in the rat. Biol Reprod 65:165–172
8. **Hild SA, Reel JR, Larner JM, Blye RP** 2001 Disruption of spermatogenesis and Sertoli cell structure and function by the indenopyridine CDB-4022 in rats. Biol Reprod 65:1771–1779
9. **Hild SA, Attardi BJ, Reel JR** 2004 The ability of a gonadotropin-releasing hormone antagonist, acyline, to prevent irreversible infertility induced by the indenopyridine, CDB-4022, in adult male rats: the role of testosterone. Biol Reprod 71:348–358
10. **Boekelheide K, Johnson KJ, Richburg JH** 2005 Sertoli cell toxicants. In: Skinner MK, Griswold MD, eds. Sertoli cell biology. San Diego: Elsevier Academic Press; 345–382
11. **Fail PA, Anderson SA, Cook CE** 2000 28-Day toxicology test: indenopyridine RTI 4587–056 in male Sprague-Dawley rats. Reprod Toxicol 14:265–274
12. **National Research Council** 1996 Guide for the care and use of laboratory animals. Washington DC: Institute of Laboratory Animal Resources Commission on Life Sciences, National Academy Press
13. **Hild SA, Attardi BJ, Burgenson J, Reel JR** 2003 Antispermatogenic activity of the purified enantiomers of the indenopyridine CDB-4022: potential interactions of the isomers *in vivo* and *in vitro*. Proc 36th Annual Meeting of the Society for the Study of Reproduction, Cincinnati, OH, 2003, p 134 (Abstract 55)
14. **Marshall GR, Wickings EJ, Luedecke DK, Nieschlag E** 1983 Stimulation of spermatogenesis in stalk-sectioned rhesus monkeys by testosterone alone. J Clin Endocrinol Metab 57:152–159
15. **Cho HW, Nie R, Carnes K, Zhou Q, Sharief NA, Hess RA** 2003 The antiestrogen ICI 182,780 induces early effects on the adult male mouse reproductive tract and long-term decreased fertility without testicular atrophy. Reprod Biol Endocrinol 1:57–74
16. **Marshall GR, Plant TM** 1996 Puberty occurring either spontaneously or induced precociously in rhesus monkey (*Macaca mulatta*) is associated with a marked proliferation of Sertoli cells. Biol Reprod 54:1192–1199
17. **Hess RA, Zhou Q, Nie R** 2002 The role of estrogens in the endocrine and paracrine regulation of the efferent ductules, epididymis and vas deferens. In: Robaire B, Hinton B, eds. The epididymis: from molecules to clinical practice. New York: Kluwer Academic/Plenum Publishers; 317–338
18. **Oliveira CA, Mahecha GA, Carnes K, Prins GS, Saunders PT, Franca LR, Hess RA** 2004 Differential hormonal regulation of estrogen receptors ERα and

ERβ and androgen receptor expression in rat efferent ductules. Reproduction 128:73–86
19. **Turner KJ, Morley M, Atanassova N, Swanston ID, Sharpe, RM** 2000 Effect of chronic administration of an aromatase inhibitor to adult male rats on pituitary and testicular function and fertility. J Endocrinol 164:225–238
20. **Smithwick EB, Young LG** 2001 Histological effects of androgen deprivation on the adult chimpanzee epididymis. Tissue Cell 33:450–461
21. **Holschbach C, Cooper TG** 2002 A possible extratubular origin of epididymal basal cells in mice. Reproduction 123:517–525
22. **Robarie B, Hermo L** 1988 Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. In: Knobil E, Neill JD, eds. The physiology of reproduction. New York: Raven Press, Ltd.; 999–1080
23. **Hermo L, Krzeczunowicz D, Ruz R** 2004 Cell specificity of aquaporins 0, 3, and 10 expressed in the testis, efferent ducts, and epididymis of adult rats. J Androl 25:494–505
24. **Majumdar SS, Mikuma N, Ishwad PC, Winters SJ, Attardi BJ, Perera AD, Plant TM** 1995 Replacement with recombinant human inhibin immediately after orchidectomy in the hypophysiotropically clamped male rhesus monkey (*Macaca mulatta*) maintains follicle-stimulating hormone (FSH) secretion and FSHβ messenger ribonucleic acid levels at precastration values. Endocrinology 136:1969–1977
25. **Vale W, Rivier C, Hsueh A, Campen C, Meunier H, Bicsak T, Vaughn J, Corrigan A, Bardin W, Sawchenko P, Petraglia F, Yu J, Plotsky P, Spiess J Rivier J** 1999 Chemical and biological characterization of the inhibin family of protein hormones. Recent Prog Horm Res 44:1–34
26. **Wiater E, Vale W** 2003 Inhibin is an antagonist of bone morphogenetic protein signaling. J Biol Chem 278:7934–7941
27. **Ramaswamy S, Marshall GR, McNeilly AS, Plant TM** 2000 Dynamics of the follicle-stimulating hormone (FSH)-inhibin B feedback loop and its role in regulating spermatogenesis in the adult male rhesus monkey (*Macaca mulatta*) as revealed by unilateral orchidectomy. Endocrinology 141:18–27
28. **Wenk M, Nieschlag E** 2006 Male contraception: a realistic option? Eur J Contracept Reprod Health Care 11:69–80
29. **Amory JK** 2006 Male hormonal contraceptives. Minerva Ginecol 58:215–226
30. **Matthiesson KL, McLachlan RI** 2006 Male hormonal contraception: concept proven, product in sight? Hum Reprod Update 12:463–482
31. **Lopez LM, Grimes DA, Schulz KF** 2005 Nonhormonal drugs for contraception in men: a systematic review. Obstet Gynecol Surv 60:746–752
32. **Amory JK, Muller C, Page ST, Liefke E, Pagel ER, Bhandari A, Subramanyam B, Bone W, Radlmier A, Bremner WJ** Miglustat has no apparent effect on spermatogenesis in normal men. Hum Reprod 22:702–707
33. **Suter KE, Hodel C, Gradient F, Fluckiger E** 1977 Antispermatogenic activity of an indenopyridine derivative. Experientia 33:810

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**