

# Gonadotropin-Releasing Hormone Antagonist plus Testosterone: A Potential Male Contraceptive\*

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**ABSTRACT.** No effective hormonal contraceptive has yet been devised for men. Through their suppressive effect on gonadotropin secretion, GnRH antagonists inhibit both testosterone (T) production and spermatogenesis in animals. Long term administration of an antagonist alone would result in androgen deficiency; this would cause unacceptable physiological and behavioral sequelae in men. Therefore, androgen replacement must be included in any GnRH antagonist regimen used in human male contraception. We tested the hypothesis that the combination of a GnRH antagonist plus T would suppress spermatogenesis in the male primate to azoospermic levels while maintaining normal serum T levels. We examined the effects of the GnRH antagonist Deterelix [*N*-Ac-D<sup>1</sup>Nal(2)-D<sup>2</sup>pCl-Phe<sup>2</sup>-D<sup>3</sup>Trp<sup>3</sup>-DhArg(Et2)<sup>6</sup>-DAla<sup>10</sup>-GnRH], alone and with simultaneous T replacement, on sperm production and serum T levels in adult male monkeys (n = 22). After 12 weeks of daily sc antagonist injection, all animals that received antagonist alone (n =

5) and those that 750 µg/kg·day antagonist plus T (n = 5) were azoospermic. After 16 weeks, four of five animals that received 250 µg/kg·day antagonist plus T became azoospermic. Control animals (n = 7) received daily injections of vehicle; sperm counts increased somewhat during the study period in that group. Castrate range T levels were achieved in animals receiving antagonist alone. T levels in the groups that received T supplementation and in the control group were in the normal male range throughout the treatment period. Sperm counts returned to the pretreatment range in all animals during the recovery period. We conclude that the combination of a GnRH antagonist plus T can induce azoospermia reversibly in this nonhuman primates species, and that a similar combination may be an effective contraceptive regimen in men. The GnRH antagonist alone may be an effective treatment for androgen-dependent neoplasia. (*J Clin Endocrinol Metab* 73: 465-469, 1991)

**A**LTHOUGH oral hormonal regimens provide effective reversible contraception for women, no effective hormonal contraceptive regimen has yet been developed for men. Testosterone (T) decreases gonadotropin secretion by exerting negative feedback at both the hypothalamus and pituitary (1-3), thereby inhibiting spermatogenesis. Administration of exogenous T causes azoospermia in only 50-70% of men, however (4). Agonist analogs of GnRH can inhibit gonadotropin secretion and gonadal function (5), but they do not consistently induce azoospermia in primates when given alone or in combination with androgen (6-9).

GnRH antagonists are synthetic analogs of GnRH that compete with endogenous GnRH for pituitary binding sites, thereby inhibiting the secretion of LH and FSH (5). In short term studies in humans and nonhuman

primates, these antagonists reversibly suppress plasma levels of LH, FSH, T, and inhibin (10-12). When given without androgen replacement, GnRH antagonists can induce azoospermia in adult monkeys (13-15). An androgen must be administered with a GnRH antagonist in a contraceptive regimen to maintain the normal androgen milieu. However, in previous studies of concomitant antagonist and androgen administration in experimental animals, induction of azoospermia was inconsistent (15, 16). The present study was undertaken to determine the effects of daily injections of the GnRH antagonist Deterelix [*N*-Ac-D<sup>1</sup>Nal(2)-D<sup>2</sup>pCl-Phe<sup>2</sup>-D<sup>3</sup>Trp<sup>3</sup>-DhArg(Et2)<sup>6</sup>-DAla<sup>10</sup>-GnRH], alone and in conjunction with simultaneous T replacement, on sperm production and serum T levels in adult male monkeys.

## Materials and Methods

### Animals

Adult male monkeys, *Macaca fascicularis*, were housed under controlled conditions of temperature (21 ± 2 C) and light (lights on at 0600 h, off at 1800 h) in individual cages at the Regional

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Primate Center at the University of Washington. In addition to monkey chow, the animals received fresh fruit, chewable vitamins, and iron injections. The animals were aged 8–15 yr (as assessed by dental radiographs).

#### Experimental drugs

The GnRH antagonist (supplied courtesy of Drs. Brian Vickery and John J. Nestor of the Syntex Corp., Palo Alto, CA) was dissolved at a concentration of 4  $\mu\text{g}/\text{mL}$  in a vehicle containing glacial acetic acid, benzyl alcohol, sodium hydroxide, and sterile water. The vehicle was supplied by the Syntex Corp. and contained 0.02 M sodium acetate buffer, 0.9% benzyl alcohol preservative, and 0.02 M glacial acetic acid. Antagonist was added to this solution and was filtered through a 0.8- $\mu\text{m}$  nucleopore filter. Aliquots of 20 mL were frozen at  $-20\text{ C}$  until use. During the study period, either the antagonist or the vehicle was injected sc daily between 0800–1200 h.

All animals in the experimental groups received Silastic capsule implants sc 5 days before the first injection of GnRH antagonist. The capsules were 0.33 cm id  $\times$  0.46 cm od and 5.5 cm in length. These implants contained either crystalline T or were empty, depending on the treatment regimen. Capsules were sterilized in Zepheran (Winthrop Pharmaceuticals, New York, NY) and rinsed in sterile saline before implantation. Implants were removed when injections of GnRH antagonist were completed.

#### Hormone measurements and sperm counts

Serum T was measured by RIA, using methods previously described (1). The minimum detectability of the assay was less than 0.35 nmol/L. The intra- and interassay coefficients of variation were 5.1% and 9.8%, respectively. GnRH antagonist levels were measured in groups 2, 3, and 4 by RIA at the Syntex Corp.

Seminal fluid was obtained by rectal electroejaculation. Sperm counts were performed in the Seminal Fluid Core Laboratory (C. Alvin Paulsen, Director) of the Population Center for Research in Reproduction.

#### Experimental design

All animals ( $n = 22$ ) were studied for an initial 4-month control period, during which baseline measurements were obtained. The animals were then divided into four groups: group 1 ( $n = 5$ ) received antagonist (250  $\mu\text{g}/\text{kg}\cdot\text{day}$ ) plus sham implants for 12 weeks, group 2 ( $n = 5$ ) received GnRH antagonist (250  $\mu\text{g}/\text{kg}\cdot\text{day}$ ) plus T via implants for 20 weeks, group 3 ( $n = 5$ ) received antagonist (750  $\mu\text{g}/\text{kg}\cdot\text{day}$ ) plus T via implants for 16 weeks, and group 4 ( $n = 7$ ) received vehicle alone for 20 weeks. Animals were monitored daily for any physical or behavioral effects of the drug treatment. Throughout the control and experimental periods, seminal fluid, blood samples, and body weights were determined every 2 weeks.

#### Testicular biopsies

Two animals in each treatment group were chosen randomly to undergo testicular biopsy during the treatment period and

again after recovery. Open biopsies were performed using sterile technique after the animals had received general anesthesia. Tissue samples were fixed in Cleland's solution, sectioned, and stained with hematoxylin and eosin.

#### Statistics

Differences among control, treatment, and recovery period values for sperm counts, body weight, and T levels were determined by analysis of variance with repeated measures and multiple comparison procedures. Differences between groups were determined by analysis of variance. For each group, a  $\chi^2$  test was used to determine differences between baseline, treatment, and recovery periods.

## Results

#### T levels

In group 1 (antagonist alone) serum T levels decreased significantly, from  $21.2 \pm 6.6$  to  $1.4 \pm 0.4$  nmol/L (Fig. 1) during 12 weeks of treatment ( $P < 0.05$ ). Within a

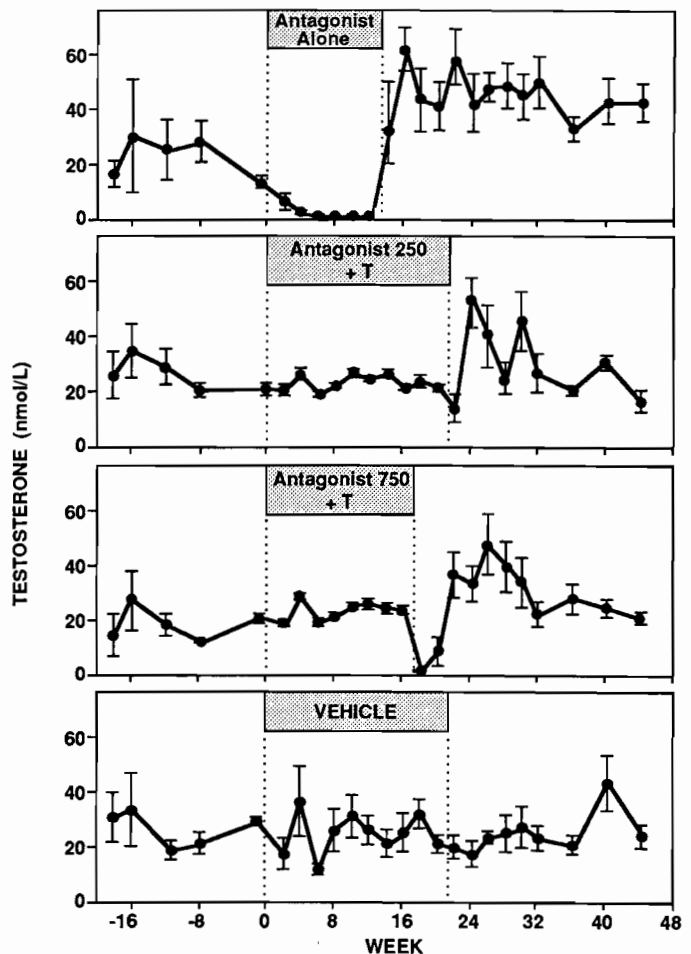


FIG. 1. Mean serum T levels in normal adult male monkeys before, during, and after administration of a GnRH antagonist alone (*top panel*), GnRH antagonist in two dosages plus T (*middle panels*), or vehicle alone (*bottom panel*).

week after the end of injections, the mean serum T levels had increased to  $35.0 \pm 14.9$  nmol/L and remained elevated at the end of the recovery period. Serum T levels in groups 2 [antagonist (250  $\mu\text{g}/\text{kg}\cdot\text{day}$ ) and T] and group 3 [antagonist (750  $\mu\text{g}/\text{kg}\cdot\text{day}$ ) and T] did not change significantly during drug administration. After the end of injections and removal of the T implants, serum T levels decreased transiently, but by the end of the recovery period, T levels in both groups were similar to the baseline levels. T levels in group 4 (vehicle) did not change significantly throughout the course of the study.

#### Sperm counts

Sperm counts in the antagonist-treated groups dropped markedly by 8 weeks, and by 12 weeks all of the animals in groups 1 and 3 were azoospermic (Fig. 2). At 16 and 20 weeks of antagonist administration, only one animal in group 2 failed to become azoospermic, and this

animal's sperm counts were very low (50,000–100,000/ejaculate). At the end of the recovery period, sperm counts in all groups were comparable to pretreatment values. The mean sperm count in the group receiving vehicle alone did not change significantly during the study period.

#### Testicular histology

Representative sections demonstrated normal testicular histology in animals receiving placebo (Fig. 3A) and marked regression of spermatogenesis in animals receiving the antagonist (Fig. 3B). Antagonist-treated animals (at both doses and including those animals receiving T) demonstrated a marked decrease in tubular diameter with loss of spermatocytes and spermatids, but preservation of spermatogonia and Sertoli cells. In the antagonist-treated animals, histology returned to normal during the recovery period.

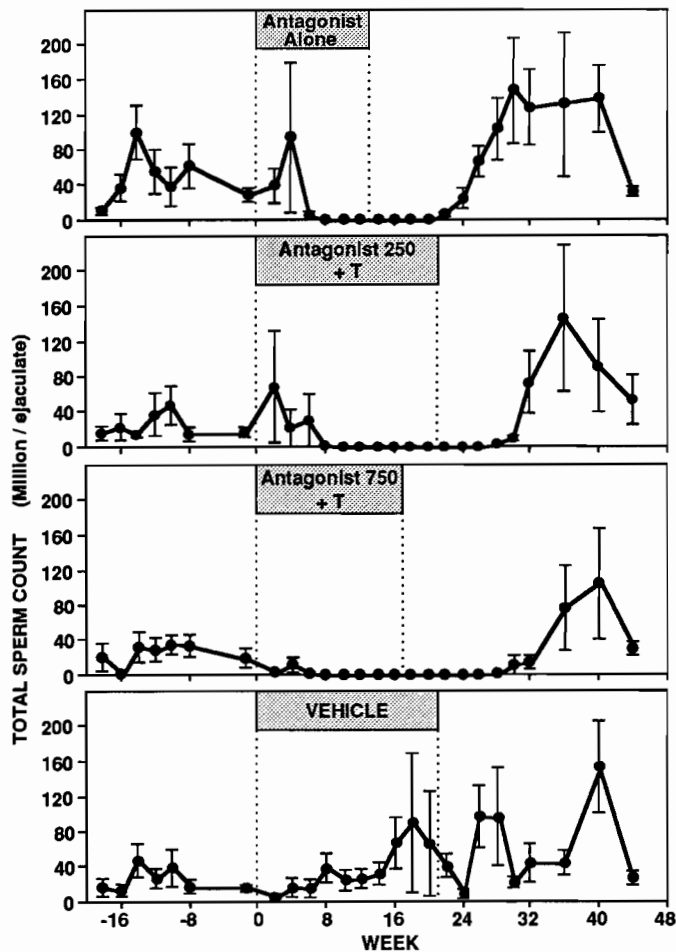


FIG. 2. Mean total sperm counts in normal adult male monkeys before, during, and after administration of a GnRH antagonist alone (*top panel*), a GnRH antagonist in two dosages plus T (*middle panels*), or vehicle alone (*bottom panel*).

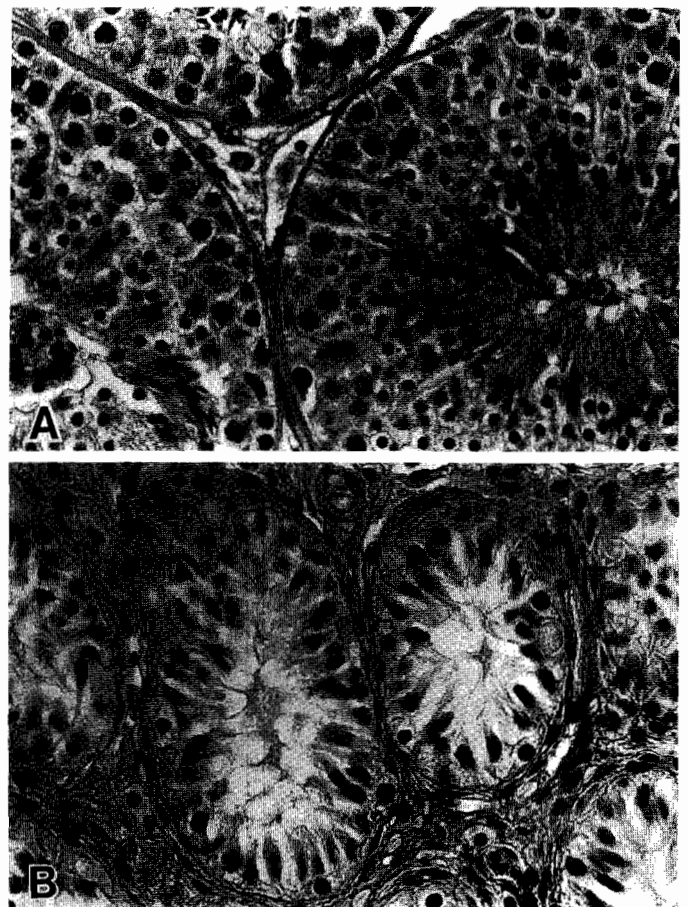


FIG. 3. Testicular histology during administration of vehicle (A) or GnRH antagonist (750  $\mu\text{g}/\text{kg}\cdot\text{day}$ ) plus T (B). During antagonist plus T administration, tubular diameter decreased, and spermatids and spermatocytes were absent, leaving only Sertoli cells and spermatogonia in the tubules.

### Body weights

Animals in group 1 (antagonist alone) lost weight during the treatment period ( $5.3 \pm 0.4$  to  $4.7 \pm 0.3$  kg;  $P < 0.05$ ). By the end of the recovery period, the animals had regained the lost weight and gained some additional weight; mean weight at the end of the recovery period was  $5.7 \pm 0.3$  kg. Animals receiving T replacement (groups 2 and 3) neither lost nor gained a significant amount of weight during the study. In group 4 (vehicle alone), body weights increased slightly during the treatment period ( $5.1 \pm 0.2$  to  $5.4 \pm 10.2$  kg;  $P < 0.05$ ). This increase was maintained to the end of the recovery period.

### GnRH antagonist levels

Mean serum levels of antagonist increased after injections and were maintained at an elevated level until the next injection (Table 1). Animals in group 4 had antagonist levels that were undetectable before and 6 h after the injection of vehicle.

## Discussion

We administered a GnRH antagonist with and without T replacement to sexually mature male monkeys for periods of 12–20 weeks. The antagonist alone consistently led to suppression of serum T levels to the castrate range and azoospermia. When combined with T, the antagonist caused azoospermia in all animals receiving the 750  $\mu\text{g}/\text{kg}$  dose and in four of the five animals receiving the 250  $\mu\text{g}/\text{kg}$  dose. These findings were associated with testicular histology showing lack of progression of spermatogenesis beyond spermatogonia in the antagonist-treated animals. These effects were reversible by the end of the recovery period. These results suggest that the combination of a GnRH antagonist plus androgen replacement may be an effective male contraceptive regimen and that the antagonist alone may be an effective treatment for T-dependent neoplasia.

GnRH antagonists without androgen replacement have been shown by others to induce azoospermia in male monkeys (13–15); our work confirms this finding

TABLE 1. GnRH antagonist plasma levels in monkeys receiving daily antagonist injections

| Treatment  | GnRH antagonist plasma levels (ng/mL) |                     |
|--|---------------------------------------|---------------------|
|  | Before injection                      | 6 h after injection |
| Group 2 (250 $\mu\text{g}/\text{kg} \cdot \text{day} + \text{T}$ ) | $59 \pm 11$                           | $131 \pm 20$        |
| Group 3 (750 $\mu\text{g}/\text{kg} \cdot \text{day} + \text{T}$ ) | $228 \pm 38$                          | $428 \pm 69$        |
| Group 4 (vehicle)  | Undetectable                          | Undetectable        |

and extends it to include androgen supplementation as part of the experimental design. Since long term androgen deficiency would have unacceptable behavioral and physiological sequelae for normal men, androgen replacement would be an important component of a hormonal contraceptive regimen. In contrast to previous studies (15, 16), we found that azoospermia could be induced when a GnRH antagonist and T were administered concomitantly. We used antagonist doses of 250 and 750  $\mu\text{g}/\text{kg} \cdot \text{day}$ , while Weinbauer *et al.* (15, 16) used doses of 400–460  $\mu\text{g}/\text{kg} \cdot \text{day}$ . The Nal-Glu analog used by Weinbauer *et al.* in one of their studies is similar in potency to Deterelix (5), which was used in their other work (16) and in our study reported here. Since four of the five animals receiving the lower antagonist dose became azoospermic in our study, and the fifth was nearly azoospermic, the magnitude of the dose cannot explain the difference in results. We administered the antagonist in daily sc injections, while Weinbauer *et al.* used osmotic minipumps (15). The injections were very effective in maintaining high blood levels of the antagonist; this is a plausible explanation for the more consistent suppression of spermatogenesis in our animals compared to those of Weinbauer *et al.* (15). It is not clear why Weinbauer *et al.* (16), when using daily injections of a potent antagonist, were unable to induce azoospermia. Their explanation that the concurrent administration of T stimulated spermatogenesis directly seems unlikely, since we were able to induce azoospermia using a GnRH antagonist and T in this study.

Azoospermia was achieved within 12 weeks in all animals in groups 1 and 3, while in group 2, azoospermia was reached in four animals at 14–16 weeks, and the fifth animal became severely oligospermic. Thus, 14 of 15 animals treated with antagonist became azoospermic, including 9 of 10 receiving T replacement. These results suggest that the combination of a GnRH antagonist and T may be an effective contraceptive in men, leading to more consistent induction of azoospermia than is true with T alone regimens (4). We have recently shown that in normal men, the combination of the antagonist plus T causes a greater suppression of FSH, LH, and inhibin than either T alone or antagonist alone (12).

As expected, serum T levels in group 1 declined to castrate levels during antagonist administration. During this treatment period, serum T levels in the T-replaced groups were equal to or slightly higher than those during the pretreatment period and were in the same range as those in the control group. These T levels are higher than those achieved by Weinbauer *et al.* in their 1987 study (15) and are in the same range or slightly lower than those found in a later study by these researchers (16). These data suggest that the level of T replacement employed is not the most important factor in determining

whether azoospermia is achieved. On the other hand, determining an optimal level of T replacement will be very important in preventing the adverse effects of either insufficient or excessive androgen levels (e.g. effects on behavior, lipids, and bone mass).

Body weights decreased significantly in animals receiving the antagonist alone and slightly but not significantly in animals receiving antagonist plus T. These effects were not accompanied by noticeable decreases in appetite or food intake during the study. The mechanism of this weight loss is unknown, but could be due in part to antagonist-induced T deficiency. No other physical or behavioral effects of the experimental regimens were noted.

In conclusion, we have shown that the combination of a GnRH antagonist and T can successfully induce azoospermia in a nonhuman primate species. These data suggest that a similar hormonal regimen might be effective as a contraceptive regimen for the human male. The effectiveness of the antagonist alone in achieving prolonged and profound suppression of serum T levels suggests that this peptide could be an effective treatment of androgen-dependent neoplasia, particularly those of the prostate.

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