Combined Treatment with an LHRH Agonist and Testosterone in Man

Reversible Oligozoospermia without Impotence

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We have previously shown that LHRH agonist [D-Trp^6,Pro^9-NEt]LHRH (LHRH$_A$) results in reversible oligozoospermia when given to normal subjects for up to ten weeks. A fall in plasma testosterone was accompanied by loss of libido and potency. We now report six subjects who were evaluated by semen analysis and hormone profile at two-week intervals during ten-week basal, 20-week treatment, and post-treatment periods lasting at least ten weeks. Treatment consisted of LHRH$_A$ (50 μg subcutaneously daily), and testosterone enanthate (100 mg intramuscularly every two weeks). Sperm density (mean basal 76.7 ± 8.7 × 10^6/ml) fell consistently in each subject to a mean nadir of 12.3 ± 4.5 × 10^6/ml ($p < 0.001$). This is similar to the mean nadir of 11.6 ± 5.8 × 10^6/ml achieved when LHRH$_A$ was given alone. In each individual subject, sperm density returned to his basal level after cessation of treatment. No consistent changes were seen in sperm motility of morphology, or in semen volume. Libido and potency were maintained in all subjects. An additional three subjects received testosterone enanthate alone in identical dosage for 20 weeks. No change in sperm density was observed. In contrast to treatment with LHRH$_A$ alone, combination treatment produces reversible oligozoospermia without attendant change in potency.

Key words: LHRH (GnRH), testosterone, spermatogenesis.

The initial descriptions of the paradoxical antifertility effects of agonist-analogues of LHRH (Belanger et al, 1980; Pelletier et al, 1978; Sandow et al, 1980; Smith et al, 1979; Tcholakian et al, 1978) suggested potential application as male contraceptive agents. We have demonstrated reversible inhibition of spermatogenesis in man by administration of the LHRH agonist [D-Trp^6, Pro^9-NEt]LHRH (LHRH$_A$) to normal volunteers for up to ten weeks (Linde et al, 1981). Oligozoospermia or azoospermia occurred in each subject. However, this method of administering the analog is impractical because of a pronounced though reversible fall in plasma testosterone concentration, together with a decline in libido or potency. In an attempt to reversibly inhibit spermatogenesis, while preserving libido and potency, we have administered LHRH$_A$ in combination with testosterone enanthate. A degree of oligozoospermia was achieved which was similar to that observed when LHRH$_A$ was given alone, and normal potency and libido were maintained.

Materials and Methods

Nine healthy male volunteers, aged 25 to 34 years, were asked to defer planned vasectomy. Subjects were seen every two weeks during a ten-week basal period, a 20-week treatment period, and a post-treatment period lasting either ten weeks or until each subject attained full recovery. During the treatment period, six of the subjects self administered 50 μg of LHRH$_A$ subcutaneously each day. Testosterone enanthate (TE), 100 mg

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intramuscularly, was administered every other week, beginning after the second treatment week. Three subjects received testosterone enanthate alone. Semen samples and hormone profiles were obtained every other week. Blood was drawn immediately before the next injection of testosterone and 12 to 18 hours after the last injection of $LHRH_A$.

Blood samples were assayed for LH, FSH, testosterone, estradiol-17β, progesterone, and 17α-hydroxyprogesterone by radioimmunoassay methods described previously (Moore et al, 1980). Semen analysis included measurement of volume, sperm density (millions of sperm per milliliter of seminal fluid), motility and morphology, and was performed by the same technologist throughout the study.

Treatment response was assessed by analysis of nadir sperm densities. A value was accepted as "nadir" if it occurred during or following the period of drug administration and if it was part of a consistent downward trend. The latter criterion was applied because of the marked variability in sperm density observed in the normal adult male. Libido and potency were assessed by noting the subjective reports of the volunteers and, where possible, of their sexual partners. Frequency of ejaculation (intercourse and masturbation) was also recorded on a weekly basis throughout all study periods.

All subjects gave informed written consent, and the study was approved by the Committee for the Protection of Human Subjects at Vanderbilt University. Statistical analysis employed Student's *t* test.

Results

Gonadotropins and Gonadal Steroids

(Tables 1 and 2). Basal levels of gonadotropins and gondal steroids were normal in all subjects. Those subjects receiving $LHRH_A$ and TE demonstrated a decline in plasma testosterone by the fourth treatment week; these levels were obtained just prior to TE injection, and therefore represent the lowest testosterone concentrations of each two-week interval. Plasma testosterone returned to basal levels within two weeks after cessation of treatment, and exceeded basal levels briefly at the fourth post-treatment week in all subjects (2.8 to 6.8 ng/ml, or 119% to 212% of individual mean basal concentration). Ten weeks after completion of treatment, mean plasma testosterone (4.97 ± 0.84 ng/ml) was not significantly different from mean pretreatment level. Similar declines were noted in plasma progesterone and 17α-hydroxyprogesterone concentrations during treatment with $LHRH_A$ and TE. Serum estradiol-17β fell to less than 10 pg/ml in each subject during treatment, and returned to basal concentration after cessation of combined treatment. No significant changes were observed in LH or FSH concentrations measured 12 to 18 hours after $LHRH_A$ injection in subjects receiving $LHRH_A$ and TE (Table 1). When measured just prior to TE injection, no change in plasma testosterone or serum gonadotropin concentrations was noted in subjects receiving TE alone (Table 2).

None of the subjects and none of the available sexual partners reported changes in libido or potency. Frequency of ejaculation varied widely between subjects, but was quite constant for a given

TABLE 1. Hormone Profile for Subjects Receiving $LHRH_A$* and Testosterone Enanthate†

Hormone‡	Basal§	Week 10‖	Week 20‖	Week 30¶
Testosterone (ng/ml)	3.76 ± 0.24	1.20 ± 0.34#	1.77 ± 0.20#	4.97 ± 0.84
Progesterone (ng/ml)	0.64 ± 0.04	0.47 ± 0.06**	0.57 ± 0.14	0.44 ± 0.05#
17α-OH Progesterone (ng/ml)	1.35 ± 0.15	0.28 ± 0.08#	0.61 ± 0.33	1.12 ± 0.30
Estradiol-17β (pg/ml)	16.3 ± 0.7	10.0 ± 0#	10.8 ± 0.8#	19.2 ± 2.4
LH (mIU/ml)	12.7 ± 1.2	10.0 ± 1.0	8.0 ± 1.0	11.7 ± 1.8
FSH (mIU/ml)	7.6 ± 0.5	5.1 ± 0.7	6.4 ± 1.2	5.4 ± 0.9

* $LHRH_A$ at 50 μg/day.
† TE at 100 mg every other week.
‡ Values are Mean ± SEM.
§ Mean of six samples from each subject.
‖ Treatment period weeks 0–20.
¶ Recovery period.
# $P < 0.005$ compared to basal mean result.
** $P < 0.025$ compared to basal mean result.

TABLE 2. Hormone Profile for Subjects Receiving Testosterone Enanthate Alone*

Hormone†	Basal‡	Week 10§	Week 20§	Week 30‖
Testosterone (ng/ml)	3.85 ± 0.26	4.50 ± 0.93	4.30 ± 0.45	4.70 ± 0.15
LH (mIU/ml)	10.9 ± 0.8	10.9 ± 0.7	12.7 ± 2.9	10.2 ± 2.1
FSH (mIU/ml)	4.2 ± 0.4	4.8 ± 0.8	5.0 ± 1.1	4.2 ± 1.1

* TE at 100 mg/every other week.
† Values are mean ± SEM.
‡ Mean of six samples from each subject.
§ Treatment period weeks 0–20.
‖ Recovery period.

subject throughout the study, and no change was noted between basal, treatment, and post-treatment periods (data not shown).

Semen Analysis

Individual basal means and ranges of sperm density are shown in Table 3, as are nadir sperm densities achieved and peak recovery values. Individual results from the study in which $LHRH_A$ was administered without TE are included for comparison.

Sperm density varied widely during the basal period. Treatment responses were variable although a consistent decline occurred in all subjects, with nadir values observed on either the final week of treatment or two weeks later. Nadir values ranged from 1.1×10^6/ml to 29.3×10^6/ml, or 3.3% to 43.4% of individual basal mean values. Azoospermia was not observed. Because of restrictions placed on assignment of nadir (see Methods), these results do not represent the lowest sperm density observed in all cases, and may thus underestimate the degree of oligozoospermia achieved. All subjects regained sperm densities comparable to the basal period following cessation of $LHRH_A$ and TE treatment, but the length of the recovery period varied from the fourth post-treatment week in subject 6 to the 34th post-treatment week in subject 4. Five of the six subjects regained previous sperm density within ten weeks after cessation of treatment. Figure 1 displays individual

TABLE 3. Sperm Density for Subjects Receiving $LHRH_A$* and Testosterone,† and $LHRH_A$ Alone*

Subject	Basal‡	Nadir	Peak Recovery
	$LHRH_A$ and Testosterone Enanthate		
1	132.6 (66.0–169.8)§	29.3	210.0
2	121.2 (59.0–161.0)	6.2	76.8
3	33.6 (18.0–55.4)	1.1	47.3
4	82.4 (42.7–172.8)	20.3	104.0
5	51.2 (21.4–84.8)	3.3	56.0
6	31.8 (9.9–58.8)	13.8	71.0
Mean ± SEM	76.7 ± 8.7	12.3 ± 4.5‖	94.2 ± 24.5
		$LHRH_A$	
A	41.7 (11.8–79)	4.5	98.2
B	148.7 (108.6–209)	46.0	110.0
C	195.3 (124.6–288)	27.2	168.0
D	145.8 (105.1–230)	2.7	102.0
E	81.5 (30–160)	4.6	71.4
F	19.7 (4.5–42.9)	1.4	27.9
G	89.3 (46–171)	0	60.4
H	33.5 (10.5–64.7)	6.0	84.8
Mean ± SEM	94.4 ± 10.4	11.6 ± 5.8‖	90.3 ± 14.5

* $LHRH_A$ at 50 μg/day.
† TE at 100 mg/every other week.
‡ All values are $\times 10^6$/ml of seminal fluid.
§ Mean of six samples. Numbers in parentheses indicate range.
‖ $P < 0.001$ compared to basal mean result.

sperm density results obtained during the basal period, 20 weeks of treatment, and in the post-treatment period.

Because of individual variability in treatment response time, we have also compared mean sperm density of the basal period with that of the last six weeks of treatment and first four weeks of recovery (weeks 14–24). An identical number of semen analyses were obtained from each subject during both ten-week intervals. Mean basal sperm density was 76.7 ± 8.7 × 10^6/ml, with a range of 31.8 to 132.6 × 10^6/ml. Mean sperm density during the ten-week period at the termination of treatment (25.2 ± 3.1 × 10^6/ml, range 3.4 to 38.8 × 10^6/ml) was significantly less than the mean pre-treatment value ($p < 0.001$).

No consistent changes were noted in semen volume or sperm morphology. Sperm motility fell in two subjects, but was unchanged in the remaining four subjects receiving $LHRH_A$ and TE.

No consistent change in sperm density was observed in the three subjects receiving only TE. Mean basal sperm density in this group was 74.8 ± 13.1 × 10^6/ml (range 25.8 to 105.5 × 10^6/ml), which was not significantly different from the mean sperm density of the ten-week interval comprising the latter six treatment weeks and first four recovery weeks (66.8 ± 22.2 × 10^6/ml, range 23.6 to 127.8 × 10^6/ml).

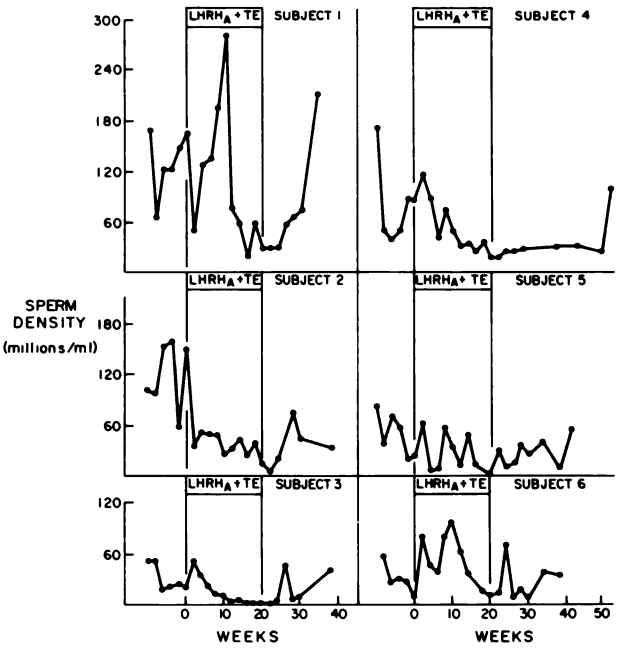

Fig. 1. Individual sperm density results (millions of sperm per milliliter of seminal fluid) for subjects receiving $LHRH_A$ (50 μg/day) and testosterone enanthate 100 mg/every other week. Results are shown for basal, treatment and post-treatment periods. Institution of treatment is designated week 0.

Discussion

Since the suppressive effect of $LHRH_A$ on spermatogenesis is accompanied by diminished libido and potency (Linde et al, 1981), we have utilized $LHRH_A$ treatment in combination with testosterone enanthate (TE) in an attempt to suppress sperm production while preserving sexual function. As a single agent, TE can produce oligozoospermia or azoospermia if administered at a dosage and frequency sufficient to suppress gonadotropin secretion (Steinberger and Smith, 1977). We chose a dose of TE which we thought would be too low to inhibit spermatogenesis if given as a single agent, yet which might prevent changes in sexual function during treatment with $LHRH_A$.

In three control subjects, we have demonstrated that TE (100 mg intramuscularly every two weeks for 20 weeks), does not produce any consistent changes in sperm density. Administration of identical doses of TE to six $LHRH_A$-treated subjects prevented loss of libido and potency, however, but did not alter the suppressive effects of $LHRH_A$ on spermatogenesis in this group (Table 3). The testosterone concentrations measured during treatment in subjects receiving $LHRH_A$ and TE represent the lowest values of each two-week treatment interval (Table 1). These values nevertheless are significantly higher than nadir values noted during treatment in subjects receiving $LHRH_A$ alone (0.81 ± 0.21 ng/ml vs. 0.24 ± 0.09 ng/ml; $p < 0.025$). The preservation of potency in the present study presumably reflects the higher testosterone concentrations present during treatment.

Libido and potency were determined by the subjective assessment of the participants and must, therefore, be interpreted cautiously. Reports by subjects receiving either $LHRH_A$ plus TE, or TE alone, however, stand in marked contrast to reports by subjects receiving $LHRH_A$ alone. In the latter group, all subjects reported diminished libido by the tenth treatment week, and five of eight subjects reported impotence, necessitating early termination of treatment. None of the subjects in the present study receiving $LHRH_A$ plus TE or TE alone reported loss of libido, and all completed the full 20-week treatment period. In addition, the fre-

quency of ejaculation was recorded weekly by subjects during all periods of the study. While this activity is potentially influenced by a number of uncontrolled factors, no change was observed for any subject.

Nadir sperm densities were observed 14 weeks after the onset of treatment with $LHRH_A$ alone and 21 weeks after the onset of combined treatment. Despite a delay in attainment of nadir sperm density when TE was added to the treatment protocol, comparable degrees of oligozoospermia were achieved (mean nadir $12.3 \pm 4.5 \times 10^6$/ml for $LHRH_A$ and TE vs. $11.6 \pm 5.8 \times 10^6$/ml for $LHRH_A$ alone). This observation differs from that of Heber and Swerdloff, who noted synergistic suppression of pituitary gonadotropin secretion and spermatogenesis in the adult rat receiving an LHRH analogue [D-Leu^6,Pro^9-NEt]LHRH, 200 ng/100 g body weight daily) and testosterone administered by implanted silastic capsules (Heber and Swerdloff, 1980; Heber and Swerdloff, 1981). Differences in the degree of suppression of spermatogenesis in these two studies may reflect differences in the dose of testosterone employed. A modest suppression of sperm count was noted in the rat with testosterone alone, while doses of testosterone used in the present study were clearly nonsuppressive; the possibility that a higher dose of testosterone enanthate might synergistically suppress spermatogenesis in the human is not excluded by the present data. The mode of administration of testosterone may also be important; intramuscular injection results in initial elevation of plasma concentration above baseline values, while stable and more physiologic plasm levels can be achieved with implantation of silastic capsules. A direct testicular effect of LHRH analogues has been observed in the rat (Clayton et al, 1980; Heber and Swerdloff, 1981; Hsueh and Erickson, 1979). A direct effect of LHRH analogues on the human testis has not been demonstrated. Differences in the degree of suppression of spermatogenesis with combined LHRH agonist and testosterone may thus reflect species differences between rodent and man.

Although the mechanism(s) by which LHRH agonists inhibit spermatogenesis remains to be established, $LHRH_A$ is nonetheless capable of reducing sperm density in man. The addition of nonsuppressive doses of TE prevented changes in libido and potency in this group of subjects and allowed long-term treatment with an LHRH agonist.

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