

## Triptolide: A Potential Male Contraceptive

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**ABSTRACT:** The antifertility effect of triptolide and other related compounds, isolated from *Tripterygium wilfordii*, has been demonstrated in male rats. The exact sites and mechanism of action of triptolide remain unknown. Our objectives were to determine whether triptolide at selected dose levels that induce infertility has any detrimental effects on the testes and to determine the sites and the possible mechanisms of its action. Groups of six adult male Sprague-Dawley rats were given oral administration of either vehicle (control group) or triptolide (50 or 100 µg/kg body weight) daily for 35 or 70 days. Body weight gain was normal in all treated groups. All six rats treated with a high dosage of triptolide were infertile during the second (63–70 days) mating trial. A lower dose (50 µg) of triptolide gave intermediate fertility values. Plasma levels of luteinizing hormone, follicle-stimulating hormone, testosterone, and intratesticular testosterone were not significantly different between control and triptolide-treated groups. Cauda epididymal sperm content was decreased by 68%

and the motility, which averaged 58.2% in the control rat, was reduced to almost zero. No effects of triptolide were observed on testis and accessory organs weight, volumes of tubular lumen and the total Leydig cells, tubule diameter, and the number of Sertoli cells, spermatogonia, preleptotene (PL), and pachytene (P) spermatocytes. There were, however, modest but significant decreases in tubule volume and the number of round spermatids at stages VII–VIII. No changes in the germ cell apoptotic index measured at stages VII–VIII and XIV–I were noted between controls and rats rendered infertile with a high dose of triptolide. Thus, triptolide, at a dose level that induces complete infertility in the adult rats, has minimal adverse effects on the testes and acts primarily on the epididymal sperm making triptolide an attractive lead as a post-testicular male contraceptive.

Key words: Triptolide, male contraception, testis, epididymis, germ cell apoptosis.

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The antifertility effect of a refined extract of the roots of the Chinese medicinal plant, *Tripterygium wilfordii*, has been demonstrated in male rats and mice, as well as in men (see Qian et al [1995] for a comprehensive review). Chinese men taking a daily oral doses of 20–30 mg of *T. wilfordii* extract, to treat rheumatoid arthritis and psoriasis, became infertile after 2 months, and there was a sharp decline in the epididymal sperm number and motility. Serum luteinizing hormone (LH) and testosterone (T) levels, and the libido and potency were all reported to be unaffected. Importantly, fertility returned to normal levels between 1 and 2 months after cessation of the treatment. Bioassay-directed subfractionation studies have shown that *T. wilfordii* extracts contain a number of active diterpene epoxides compounds that cause infertility in male rats (Qian et al, 1986, 1987, 1995; Matlin et al, 1993). Previous studies have demonstrated that one such

compound, triptolide, induces infertility in male rats. However, there is a striking paucity of data regarding the exact site(s) and possible mechanism(s) of action of this antifertility compound.

The objectives of the present study were (1) to assess whether triptolide at selected dose levels that induce infertility in male rats has any deleterious effects on testicular structure and function, and (2) to determine the sites and the possible mechanisms of action of this particular antifertility agent.

### Materials and Methods

#### Animals and Mating Study

Thirty-six adult male (3 months old) and 72 virgin female (60 days old) Sprague-Dawley (SD) rats purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, Indiana) were used in this study. Animals were housed in a standard animal facility under controlled temperature (22°C) and photoperiod (12 hours of light : 12 hours of darkness) at Harbor-UCLA Medical Center and provided with food and water *ad libitum*. Groups of six adult rats (350–410 g body weight [BW]) were given oral doses daily either 30% gum acacia as a vehicle control, or 50 (low dose group) or 100 µg/kg BW (high dose group) of triptolide for 35

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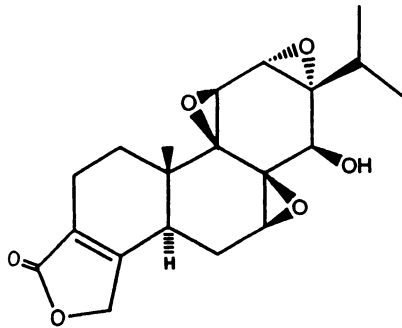


FIG. 1. Chemical structure of the triptolide.

(short term) and 70 days (long term). Triptolide was isolated by the procedure described previously (Shamon et al, 1997) and the purity of the material was >99%. The structure of this compound is shown in Figure 1. Triptolide was made available to us by Dr. David Griffin (World Health Organization, Geneva, Switzerland). The drug dosage was calculated weekly from the mean weights of the animals in each group. Fertility was assessed by pairing each test male rat with two 60-day-old virgin females for 1 week during 28–35 days in a short-term triptolide-treated group or during 63–70 days in a long-term triptolide-treated group. The successful mating was determined by the presence of sperm in morning vaginal smears. Any male that impregnated at least one of the females was considered fertile. Females were killed 1 week after mating or termination of cohabitation. The number of normal and abnormal embryos was determined at autopsy. The male rats were killed on day 36 (short-term treatment duration) or on day 71 (long-term treatment duration). Animal handling, experimentation, and killing after termination of the treatment were in accordance with the recommendation of the American Veterinary Medical Association and were approved by the Harbor-UCLA Research and Education Institute Animal Care and Use Review Committee (Project 08201-01R).

#### Blood Collection and Tissue Preparation

Control and triptolide-treated animals were injected with heparin (130 IU/100 g BW intraperitoneally [i.p.]) 15 minutes before euthanasia by a injection of sodium pentobarbital (100 mg/kg BW i.p.) to facilitate testicular perfusion using a whole-body perfusing technique (Sinha Hikim et al, 1988). Body weight was recorded at autopsy. Blood samples were collected from each animal by cardiac puncture immediately after euthanasia, and plasma was separated and stored at  $-20^{\circ}\text{C}$  for subsequent hormone assay. One testis from each rat was removed and weighed and after decapsulation, the testicular parenchyma was kept frozen at  $-70^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  until used for testicular T assay. Also one epididymis from each of these animals was dissected and weighed and used for epididymal sperm analysis (see below). The contralateral testes were fixed by vascular perfusion with 5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4), preceded by a brief saline wash. The ventral prostates and seminal vesicles were carefully dissected out and weighed. The testes were removed, cut into small ( $\sim 0.2$  cm) transverse slices, and placed into the same fixative for overnight. One slice from the middle region of the testis was processed for routine paraffin embedding for *in situ* detection of apoptosis. The adjacent tes-

ticular slice from each rat was further diced into small pieces ( $1 \times 2 \times 2$  mm), postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanols, and embedded in Epon 812. Embedded testicular specimens were sectioned with an LKB ultramicrotome at  $2.05 \mu\text{m}$  (Sinha Hikim and Swerdloff, 1993) and stained with 1% toluidine blue for light microscopic examination and for morphometric studies.

#### Morphometric Procedures

The volume density ( $V_v$ ) of seminiferous tubules, tubular lumens, interstitium, and total Leydig cells were determined by the point-counting method (Sinha Hikim et al, 1988; Sinha Hikim and Swerdloff, 1993). Five randomly selected sections per animal in each group were examined by an American Optical microscope with a  $40\times$  objective and a  $10\times$  eye piece fitted with a square lattice containing 121 intersections. The results were expressed as a percentage of the testis volume ( $V_v\%$ ). The absolute volume of each of the testis components was then obtained by multiplying its  $V_v$  by fresh testis volume (equivalent to testicular weight). The diameters of 20 randomly selected transverse sections of seminiferous tubules were measured across the minor axes of their profiles with an ocular micrometer calibrated by means of a stage micrometer.

Numerical densities ( $N_v$ ) of Sertoli and germ cells (number per unit volume of the seminiferous tubule) at stage VII–VIII of the cycle was determined by accepted stereological techniques as described previously (Sinha Hikim and Swerdloff, 1993, 1994). For each rat, 10 round cross-sections of seminiferous tubules were used. The Floderus equation  $N_v = N_a / (T + D - 2h)$ , was used to calculate the  $N_v$  of germ cell nuclei and Sertoli cell nucleoli; where  $N_a$  is the number of nuclei or nucleoli counted per unit area of the seminiferous tubule profile,  $T$  is the section thickness;  $D$  is the average diameter of a given germ cell nucleus or the Sertoli cell nucleolus, and  $h$  is the height of the smallest recognizable nuclear or nucleolar profile in the section. The nuclear profiles of each of the germ cells ( $A_1$  spermatogonia, preleptotene [PL] and pachytene [P] spermatocytes, and step 7 or 8 spermatids) and the number of Sertoli cell nucleoli (thereby cells, because only one typical nucleolus is present per nucleus or per cell) in the seminiferous tubules were counted at  $1,000\times$  magnification using an oil-immersion objective. The seminiferous tubule profile area ( $a$ ) was determined by point counting using the equation:  $a = p \times u^2$ , where  $p$  is number of points per tubular profile, and  $u$  is the distance between two neighboring points in terms of the magnification used to measure the area. The mean diameters of Sertoli cell nucleoli and germ cell nuclei were obtained by direct measurements of their largest cross-sectioned profiles in serial sections. Even though the profiles of  $A_1$  spermatogonia nuclei were somewhat ellipsoidal, their eccentricity (the average ratio of long to short axes was 0.80) did not reach levels that would produce serious error (Bolender, 1978). The height of the smallest recognizable nuclear or nucleolar profile was assumed to be one tenth of the diameter of the structure (Wing and Christensen, 1982). The  $N_v$  of a given cell type (number per unit volume of fixed tissue) was corrected for tissue shrinkage during fixation and processing by multiplying a factor of 0.855 to provide the number of cells per unit volume of the fresh tissue (Sinha Hikim and Swerdloff, 1993). The absolute

number of these cells was then determined by multiplying their  $N_c$  by the fresh volume of the testis. Cell counts were finally expressed as the number of germ cells per Sertoli cell (germ cell/Sertoli cell ratios).

### Quantitative Assessment of Germ Cell Apoptosis

*In situ* detection of germ cell apoptosis was characterized by terminal deoxynucleotidyl transferase (TdT) mediated d-UTP nick end labeling (TUNEL) technique (Sinha Hikim et al 1995) in glutaraldehyde-fixed paraffin-embedded testicular section (6  $\mu\text{m}$ ) using an Apop Tag-peroxidase kit (Oncor, Gaithersburg, Maryland). The choice of fixative was based on the results of our previous studies that showed that glutaraldehyde fixation significantly improved both the TUNEL specificity and sensitivity while maintaining excellent morphological preservation (Lue et al, 1997; Sinha Hikim et al, 1997a,b). In brief, after deparaffinization and rehydration, tissue sections were incubated with proteinase-K (20  $\mu\text{g}/\text{ml}$ ) for 15 min at room temperature, washed in distilled water, and then treated with 2% hydrogen peroxide in phosphate-buffered saline (PBS) for 5 minutes at room temperature to quench endogenous peroxidase activity. Sections were then incubated with a mixture containing digoxigenin-conjugated nucleotides and TdT in a humidified chamber at 37°C for 1 hour and subsequently treated with antidigoxigenin-peroxidase for 30 minutes at room temperature. To detect immunoreactive cells, the sections were incubated with a mixture of 0.05% diaminobenzidine and 0.01%  $\text{H}_2\text{O}_2$  for 6 minutes. Sections were counterstained with 0.5% methylgreen, dehydrated in 100% butanol, cleared in xylene, and mounted with Permount (Fischer Scientific Corporation, Fair Lawn, New Jersey).

Enumeration of viable Sertoli cell nuclei having distinct nucleoli and apoptotic germ cells was carried out using an Olympus BH-2 microscope with a 100 $\times$  oil-immersion objective at stages VII–VIII and XIV–I. For each rat, at least 10 tubules per stage group were used. These stages were identified according to the criteria proposed by Russell et al (1990) for paraffin sections. The rate of germ cell apoptosis (apoptotic index) was expressed as the number of apoptotic germ cells per 100 Sertoli cells.

### Epididymal Sperm Analysis

Epididymal sperm analyses were done, as described previously (Wang et al, 1995). In brief, the distal cauda epididymis was removed and minced with scissors to release the epididymal contents in a 35-mm petri dish containing 2 ml warm Tyrode's solution (pH 7.4). The samples were maintained at 37°C for 30 minutes before performing sperm density and motility analyses. The percentage of motile sperm was determined using a phase-contrast microscope equipped with an electrically heated (37°C) stage. The epididymal sperm content was determined from hemocytometer counts.

### Hormone Assays

The T concentrations in plasma and testicular homogenates were measured in duplicate by radioimmunoassay (RIA), as reported previously (Sinha Hikim and Swerdloff, 1993; Wang et al, 1993). Before tissue T assay, testicular tissue was homogenized in PBS (pH 7.4). All samples were then extracted with 10 vol of a mixture of ethylacetate–hexane (3:2, vol/vol) before RIA.

The minimal detection limit in the assay was 0.01 ng/ml. The intra- and interassay coefficients of variations were 11% and 15%, respectively. Plasma follicle-stimulating hormone (FSH) levels were measured by RIA, using reagents provided by NIDDK, as previously described. Rat (r)FSH RP-2 reference preparation and rFSH S-11 antiserum were used. The minimal detection limit in the assay was 0.40 ng/ml. The intra- and interassay coefficients of variations were 11% and 15%, respectively. Plasma LH levels were measured by an immunofluorometric assay for rat LH (Haavisto et al, 1993), using a combination of monoclonal antibodies to human (Medix, Kauniainen, Finland) and bovine LH (provided courtesy of Dr. J. F. Roser, University of California–Davis), as described earlier (Sinha Hikim et al, 1995). The minimal detection limit in the assay was 0.02 ng/ml. The intra- and interassay coefficients of variation were 6% and 8%, respectively.

### Statistical Analysis

Statistical analyses were performed using the SPSS program. Results were tested for statistical significance using Duncan's multiple range test after a one-way analysis of variance. When the *F*-test from analysis of variance indicated significance, differences of the means were calculated using Duncan's multiple range test. Differences were considered significant if  $P < 0.05$ .

## Results

### Mating and Fertility Data

Dose- and time-dependent effects of triptolide on male fertility are summarized in Table 1. As shown in Table 1, short-term (28–35 days) mating trial revealed no discernible effect of triptolide at the selected dose levels on fertility in male rats. By contrast, all six rats treated with a high dose of triptolide (100  $\mu\text{g}/\text{kg}$  BW) were infertile during the second (63–70 days) mating trial. Pregnancy rates, measured by exposing the individual male with two females, in control, low-dose and high-dose triptolide groups were 100%, 67%, and 0%, respectively. Accordingly, the long-term (70 days) treatment regimen was chosen in subsequent studies to evaluate the effects of triptolide on testicular structure and function as well as other reproductive organs. Also, no evidence of behavioral changes was apparent throughout this 70-day observation period.

### Body and Organ Weights

No significant differences in mean BW and testis, epididymis, ventral prostate, and seminal vesicle weights were noted among control, low-dose, and high-dose groups (Table 2). No evidence of systemic toxicity was apparent throughout this 70-day observation period.

### Hormone Levels

Hormone data from controls and rats fed with low or high dosage of triptolide are summarized in Table 3. Triptolide,

Table 1. Fertility data

Groups	Parameter					
	Short term (28–35 days cohabitations)			Long term (63–70 days cohabitations)		
	Control	T-50*	T-100†	Control	T-50	T-100
No. of males mated (%)	6/6 (100)	6/6 (100)	6/6 (100)	6/6 (100)	6/6 (100)	6/6 (100)
No. of females mated (%)	10/12 (83)	10/12 (83)	10/12 (83)	10/12 (83)	12/12 (100)	11/12 (92)
No. of fertile males (%)	6/6 (100)	5/6 (83)	5/6 (83)	6/6 (100)	5/6 (83)	0/6 (0)
No. of fertile females (%)	10/10 (100)	8/10 (80)	7/10 (70)	10/10 (100)	8/12 (67)	0/11 (0)
Normal embryos/dam	12.5 ± 0.4	13.9 ± 0.5	12.0 ± 0.6	13.7 ± 0.4	15.4 ± 0.7	0
Abnormal embryos/dam	0	0	0	0.2	0.1	0

\* T-50, 50 µg of triptolide/kg BW.

† T-100, 100 µg of triptolide/kg BW.

at a dosage of 100 µg/kg BW that induces 100% infertility, had no discernible effect on plasma levels of LH, FSH, T, and intratesticular T levels. No significant differences in these hormone levels were noted between the control and low-dose group, except for LH, which showed a significant increase when compared to the control values (though all values were within the normal range of adult male rats).

#### Testis Morphology

No differences in testicular morphology were apparent between control and triptolide-treated rats at the light microscopy level (Fig. 2). The testes of the triptolide-treated rats displayed active spermatogenesis. Tubular profiles at each of the 14 stages of the seminiferous epithelial cycle containing Sertoli cells, spermatogonia, spermatocytes, and spermatids were present in rats rendered infertile with triptolide.

#### Morphometric Observations

**Volumetric Composition of the Testis and Tubule Diameter**—Results of the stereological analysis of the volumetric composition of the testes and tubule diameters in control and triptolide treated rats are summarized in Table 4. No changes in these parameters, except for seminiferous tubule volume that showed a modest (8.2%) but significant ( $P < 0.05$ ) decrease in rats fed with high dose triptolide, were apparent between control and triptolide-treated groups.

Table 2. Long-term (70 days) effects of triptolide on body and organ weights (g) in rats

Parameter	Control	T-50	T-100
Body	518 ± 18	492 ± 10	538 ± 19
Testis	1.77 ± 0.02	1.66 ± 0.04	1.74 ± 0.05
Epididymis	0.60 ± 0.04	0.59 ± 0.02	0.56 ± 0.01
Ventral prostates	0.74 ± 0.06	0.78 ± 0.04	0.81 ± 0.08
Seminal vesicles	1.62 ± 0.06	1.63 ± 0.17	1.83 ± 0.17

Values are mean ± SEM. T-50, 50 µg of triptolide/kg BW; T-100, 100 µg of triptolide/kg BW.

**Viable Germ Cell Counts**—The effect of triptolide on spermatogenesis was assessed by enumeration of various germ cells (A<sub>1</sub> spermatogonia, preleptotene spermatocytes, pachytene spermatocytes, or step 7 and 8 spermatids) supported by an individual Sertoli cell at stages VII–VIII of the seminiferous epithelial cycle (Table 5). No significant deviation in the number of Sertoli cells, spermatogonia, PL spermatocytes, and P spermatocytes were noted among control, low-dose, and high-dose triptolide-treated groups. There was, however, a modest (15%) but significant ( $P < 0.05$ ) decrease in the number of round spermatids in the high-dose group when compared to the control values.

**Assessment of Germ Cell Apoptosis**—No germ cell apoptosis was detected in control or triptolide-treated rats at stages VII–VIII that are considered to be classically hormone responsive stages. Also, no significant changes in the apoptotic index measured at stages XIV–I were noted between controls ( $7.05 ± 0.38$ ) and rats rendered infertile with a high dose of triptolide ( $7.21 ± 0.19$ ).

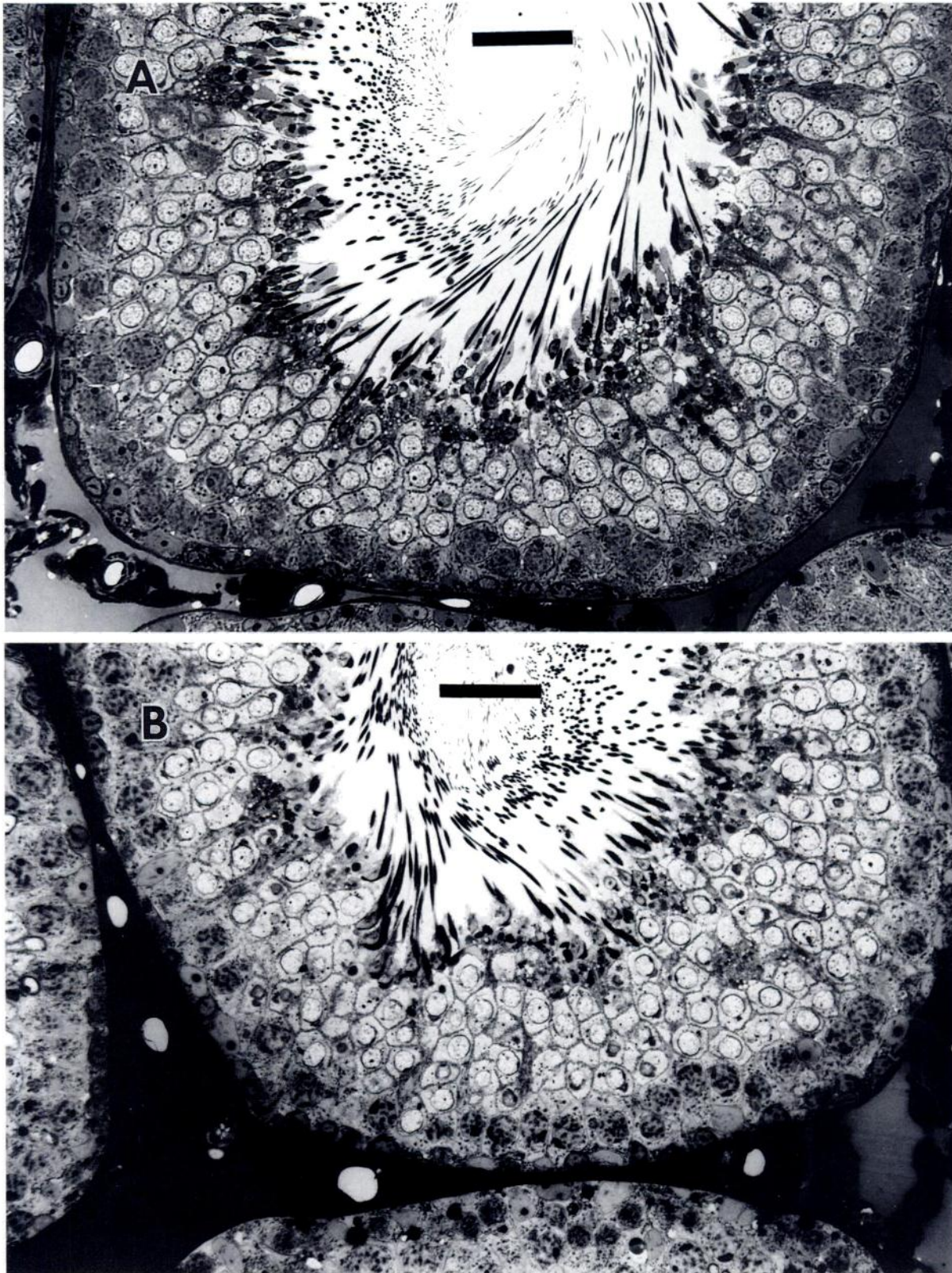
#### Epididymal Sperm Content and Motility

As shown in Figure 3, both the cauda epididymal sperm reserves, as well as the motility were significantly ( $P < 0.05$ ) reduced in rats fed with 50 or 100 µg of triptolide/kg BW for 70 days when compared to controls. Most importantly, in rats made infertile with a high dose of triptolide, cauda epididymal sperm contents were de-

Table 3. Long-term (70 days) effects of triptolide on plasma levels of LH, FSH, T, and testicular T (TT) concentrations in the adult rat

Hormone	Control	T-50	T-100
LH (ng/ml)	0.67 ± 0.07 <sup>b</sup>	1.18 ± 0.17 <sup>a</sup>	0.79 ± 0.15 <sup>ab</sup>
FSH (ng/ml)	6.48 ± 0.70	7.78 ± 0.86	6.97 ± 0.86
T (ng/ml)	1.01 ± 0.20	2.66 ± 1.10	3.10 ± 0.61
TT (ng/g)	71.4 ± 8.2	78.7 ± 16.3	102.5 ± 17.0

Values are mean ± SEM. T-50, 50 µg of triptolide/kg BW; T-100, 100 µg of triptolide/kg BW. In each row, means with unlike superscripts are significantly different.



**FIG. 2.** Light micrographs of the portions of stage VII tubules from rats fed daily with vehicle (A) or high dosage (100  $\mu\text{g}/\text{kg}$ ) of triptolide (B) showing normal spermatogenesis. These figures are representative of testicular materials used in germ cell quantitation. Magnification, 580 $\times$ . Bar = 30  $\mu\text{m}$ .

Table 4. Long-term (70 days) effects of triptolide on tubular diameter and on the volumetric composition of testis in rats

Parameter	Control	T-50	T-100
Tubule diameter ( $\mu\text{m}$ )	285.3 $\pm$ 5.7	285.4 $\pm$ 3.4	292.2 $\pm$ 2.5
$V_v$ % of testicular components			
ST	89.17 $\pm$ 0.75	90.77 $\pm$ 0.46	89.40 $\pm$ 1.18
LU	13.83 $\pm$ 0.74	13.73 $\pm$ 1.14	12.83 $\pm$ 0.46
IT	10.83 $\pm$ 0.75	9.01 $\pm$ 0.38	10.60 $\pm$ 1.18
LC	2.61 $\pm$ 0.06	2.63 $\pm$ 0.15	3.10 $\pm$ 0.14
Volume/testis			
ST (ml)	1.58 $\pm$ 0.02	1.53 $\pm$ 0.04	1.45 $\pm$ 0.04*
LU ( $\mu\text{l}$ )	245.05 $\pm$ 10.58	232.92 $\pm$ 23.09	207.47 $\pm$ 5.76
IT ( $\mu\text{l}$ )	192.95 $\pm$ 15.41	152.47 $\pm$ 7.61	172.73 $\pm$ 21.77
LC ( $\mu\text{l}$ )	46.37 $\pm$ 1.64	44.36 $\pm$ 2.43	48.81 $\pm$ 2.64

Values are mean  $\pm$  SEM. T-50, 50  $\mu\text{g}$  of triptolide/kg BW; T-100, 100  $\mu\text{g}$  of triptolide/kg BW. ST, seminiferous tubule; LU, tubular lumen; IT, interstitium; LC, Leydig cells.

\* Significantly different from controls.  $V_v$  %, volume density (which is the volume of a given testicular component per unit volume of the testis) expressed as a percentage of the testis.

creased by 68% and the motility, which averaged 58.2% in control rats, was reduced to almost zero.

## Discussion

A variety of active diterpen epoxides, including the triptolide have reported to cause infertility in male rats (Qian et al, 1986, 1995; Matlin et al, 1993). Such compounds are of special interest because of their potential application as male contraceptive agents in humans. Preliminary toxicological studies further revealed that triptolide, at dose levels that induce infertility, is not toxic, although at much higher doses (5–12 times higher than its antifertility doses) this compound can have an immunosuppressive effect (Yang et al, 1994; Gu et al, 1995; Qian et al, 1995). These studies have led to a resurgence of interest on triptolide as a potential male contraceptive.

The present study confirms and extends those previous observations by demonstrating that triptolide at a daily dosage of 100  $\mu\text{g}/\text{kg}$  BW completely inhibited the fertility of male rats during 63–70 days after administration. The loss of fertility during the second mating (63–70 days) but not at the earlier (28–35 days) mating trial is consistent with the possibility that this compound most likely

exerts its effect on the testis. To explore this possibility, we first examined whether triptolide at selected dose levels that induce infertility has any deleterious effects on spermatogenic and steroidogenic function of the testis. Data reported herein clearly demonstrate that triptolide, at least at selected doses and time points, had little or no detrimental effect on various structural and endocrine parameters of the testis. There were, however, a modest (<15%) but significant ( $P < 0.05$ ) decrease in the number of round spermatids at stages VII–VIII in rats fed a high dose of triptolide. This indicates some form of interference by triptolide during spermiogenesis. It is of note that the number of round spermatids in the high-dose group did not differ from those obtained from the low-dose group, in which five out of six males were fertile. This may imply that triptolide's modest interference with the spermiogenic process does not appear to be related to its antifertility effect. Taken together, these results suggest that triptolide-induced sterility in male rats is not mediated by alterations in germ cell development. The lack of any discernible changes in the incidence of germ cell apoptosis between controls and rats rendered infertile after triptolide treatment is consistent with this suggestion. We cannot, however, rule out an effect that occurs during

Table 5. Long-term (70 days) effects of triptolide on the Sertoli cell number and germ cell–Sertoli cell ratios at stages VII–VIII in rats

Cell types	Control	T-50	T-100
Sertoli cell ( $\times 10^6$ testis)	22.28 $\pm$ 0.86	22.32 $\pm$ 1.41	21.05 $\pm$ 0.95
Germ cell–Sertoli cell ratios			
Spermatogonia	0.19 $\pm$ 0.01	0.19 $\pm$ 0.02	0.18 $\pm$ 0.01
Preleptotene spermatocytes	3.26 $\pm$ 0.11	3.51 $\pm$ 0.23	3.06 $\pm$ 0.18
Pachytene spermatocytes	4.22 $\pm$ 0.13	4.09 $\pm$ 0.34	3.93 $\pm$ 0.21
Round spermatids	13.42 $\pm$ 0.32	12.19 $\pm$ 0.66	11.44 $\pm$ 0.32*

Values are mean  $\pm$  SEM. T-50, 50  $\mu\text{g}$  of triptolide/kg BW; T-100, 100  $\mu\text{g}$  of triptolide/kg BW.

\* Significantly ( $P < 0.05$ ) lower than controls.

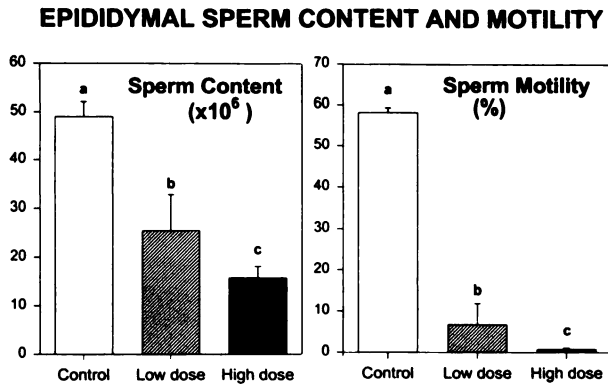


FIG. 3. Cauda epididymal sperm content and motility in rats fed daily with vehicle (control), 50 (low dose group) or 100  $\mu\text{g}/\text{kg}$  (high dose group) of triptolide for 70 days. Values are the mean  $\pm$  SEM of six rats per group. Means with unlike superscripts differ significantly.

germ cell maturation but is delayed in its manifestation or triggered at the epididymal level.

In striking contrast, triptolide at both doses caused a marked reduction in cauda epididymal sperm content and sperm motility. In rats rendered infertile with a high dose of triptolide, sperm content was decreased by 68%. Importantly, sperm motility was even more severely affected: almost no motile sperm were seen in this group. Triptolide's antifertility effect thus seems to be a consequence of cessation of cauda epididymal sperm motility and reductions in sperm number.

The mechanism(s) by which triptolide induces oligozoospermia and asthenozoospermia is not known. There is evidence from *in vitro* studies that triptolide can inhibit  $\text{Ca}^{2+}$  influx in human sperm (Qian et al, 1995). Intracellular  $\text{Ca}^{2+}$  is also known to play a major role in sperm motility (Suarez, 1996). Thus, it is possible that triptolide-induced asthenozoospermia could be attributed, at least in part, to the inhibition of  $\text{Ca}^{2+}$  influx. However, the validity of this interpretation remains to be tested in the rat. Studies are now underway in our laboratory: (1) to examine whether the antifertility effect of this compound is accompanied by any ultrastructural defects at epididymal sperm and/or epithelium; (2) to determine whether the triptolide-induced loss of epididymal sperm occurs via apoptosis; and (3) to determine whether loss of sperm motility is related to decreased sperm ATP and oxygen uptake and/or excessive generation of reactive oxygen species, which in turn affect fertility (Chan and Wang 1987; De-Lamirande and Gagnon, 1992; Sikka et al, 1995; Gomez et al, 1996). Results of these studies should provide insight to the underlying mechanism(s) of the antifertility effect of this compound.

In conclusion, triptolide, at a dose and duration of treatment that induces complete sterility, has little or no demonstrable detrimental effect on spermatogenesis. Its antifertility effect seems to be due to a reduction in epididymal

sperm number and almost complete loss of sperm motility. It is our belief that triptolide could be an extremely promising lead compound as a post-testicular male contraceptive. Future studies will assess the effects of longer term treatment and determine reversibility.

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