

# The Effect of $\Delta^9$ -tetrahydrocannabinol *In Utero* Exposure on Rat Offspring Fertility and Ventral Prostate Gland Morphology

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Male rats exposed *in utero* to  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) had lower levels of testosterone (T) and luteinizing hormone (LH) prior to puberty ( $P < 0.01$ ). At puberty, the levels returned to within the normal range. Ultrastructural examination of the ventral prostate gland at puberty revealed alterations suggestive of degenerative changes. A drastic reduction in secretory granules and acini reflected depressed androgen production and function during the developmental period. The fertility of the F<sub>1</sub> and F<sub>2</sub> male offspring was decreased by 30 to 40%. It is concluded that THC exposure *in utero* caused a permanent reduction in fertility and altered ventral prostate gland morphology.

**Key words:** marihuana, testes, ventral prostate gland, testosterone.

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The effects of  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), the principal psychoactive component of marihuana, on male and female reproductive function in several species, including man, have been extensively reported (Symons et al, 1976; Kolodny et al, 1976; Ayalon et al, 1977; Bloch et al, 1978; Purohit et al, 1979). In males, blood testosterone (T), luteinizing hormone (LH), follicle-stimulating hormone (FSH) levels, and accessory sexual organ weights are decreased (Marks, 1973; Ayalon et al, 1977; Dalterio et al, 1978; Purohit et al, 1979). In females, THC has been shown to suppress gonadotropin secretions in rats (Tyrey, 1978), leading to abolishment of the estrous cycle (Ayalon et al, 1977), and in subprimates, to induce anovulatory cycles (Smith et al, 1979; 1980; Asch et al, 1981).

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THC is known to cross the placental barrier (Vardaris et al, 1976), and prenatal cannabinoid exposure alters the hypothalamo-pituitary-gonadal axis and sexual behavior in adult male mice (Dalterio and Bartke, 1979). Postnatal exposure of male mice to cannabinoids decreased their fertility and increased the incidence of chromosomal abnormalities (Dalterio et al, 1982). It is clear that prenatal and postnatal THC exposure induces alterations in endocrine function, but whether the effects are transitory or permanent is not clear. A study was designed in which low level  $\Delta^9$ -THC was given to pregnant rats, and their subsequent offspring were examined to determine the effects on fertility, sex gland morphology, and endocrine function. The ventral prostate was chosen for the morphologic studies because a previous report showed that it is the site of THC effect (Purohit et al, 1980).

## Materials and Methods

### *Animals*

Adult female rats of the Sprague-Dawley strain between the ages of 55 and 60 days were used. The animals were housed in an air-conditioned environment with the temperature set at 76 F and a 14-hour light: 10-hour dark cycle. Food and water were given *ad libitum*. Vaginal smears were examined daily for two complete estrus cycles. Only those animals having cycles of 4 or 5 days were used. During proestrus, the female rats were placed in a cage with a proven male breeder. The animals were kept together for 36 to 48 hours to ensure the maximum time for mating, and the morning following was considered to be day 0 of pregnancy if a vaginal plug and spermatozoa were present.

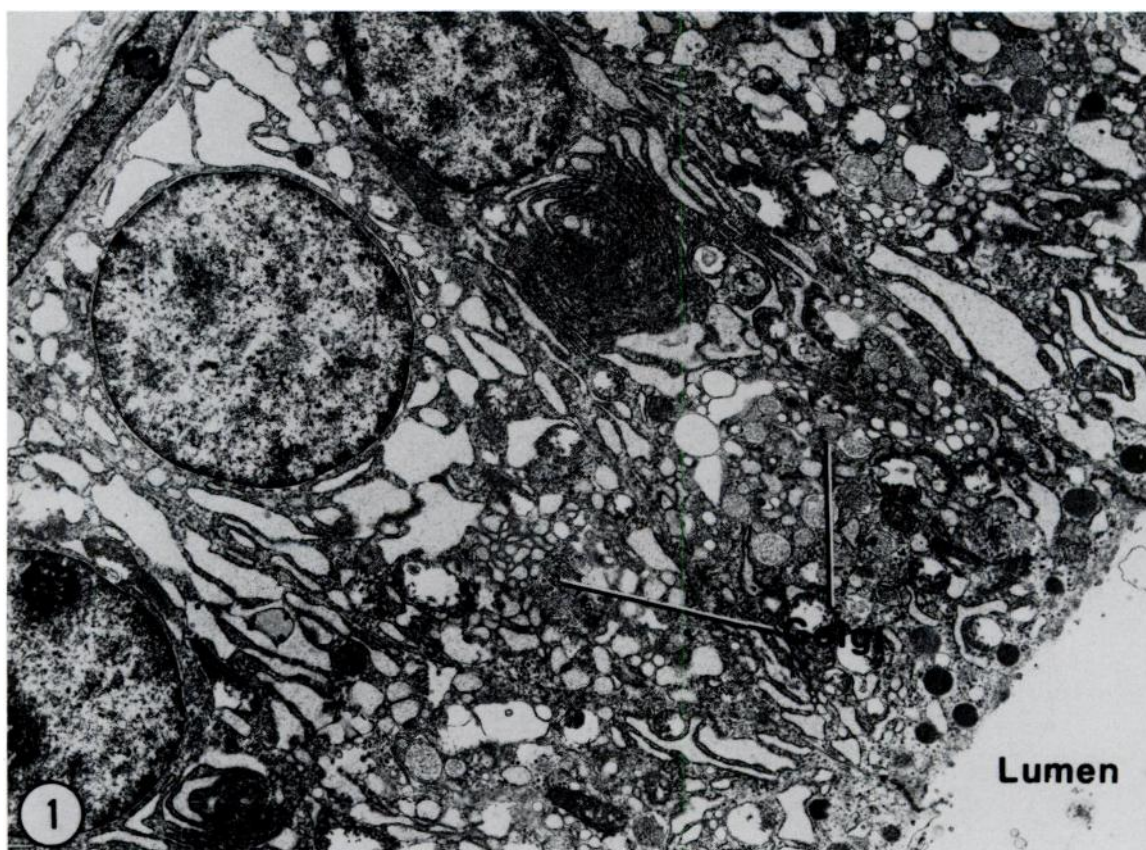


Fig. 1. Electron micrograph of ventral prostate gland from untreated rats (control). Note abundance of Golgi apparatus and dilated cisternae of granular endoplasmic reticulum ( $\times 6480$ ).

#### Route of Injection and THC Dose Level

$\Delta^9$ -THC was obtained from The National Institute on Drug Abuse.  $\Delta^9$ -THC was injected intraperitoneally (IP) mixed in sesame oil at a dose level of 6 mg/kg body weight. This is the minimum level at which IP injection significantly decreases blood T levels in adult male rats (Purohit et al, 1979). The injections were given from day 14 through day 19 of gestation. Care was taken to assure that the needle neither entered the uterus nor penetrated the

fetal membranes. The pups were surgically removed, and the males were used for the study. The controls were treated with an equivalent volume of sesame oil.

#### Serum Levels of T and LH

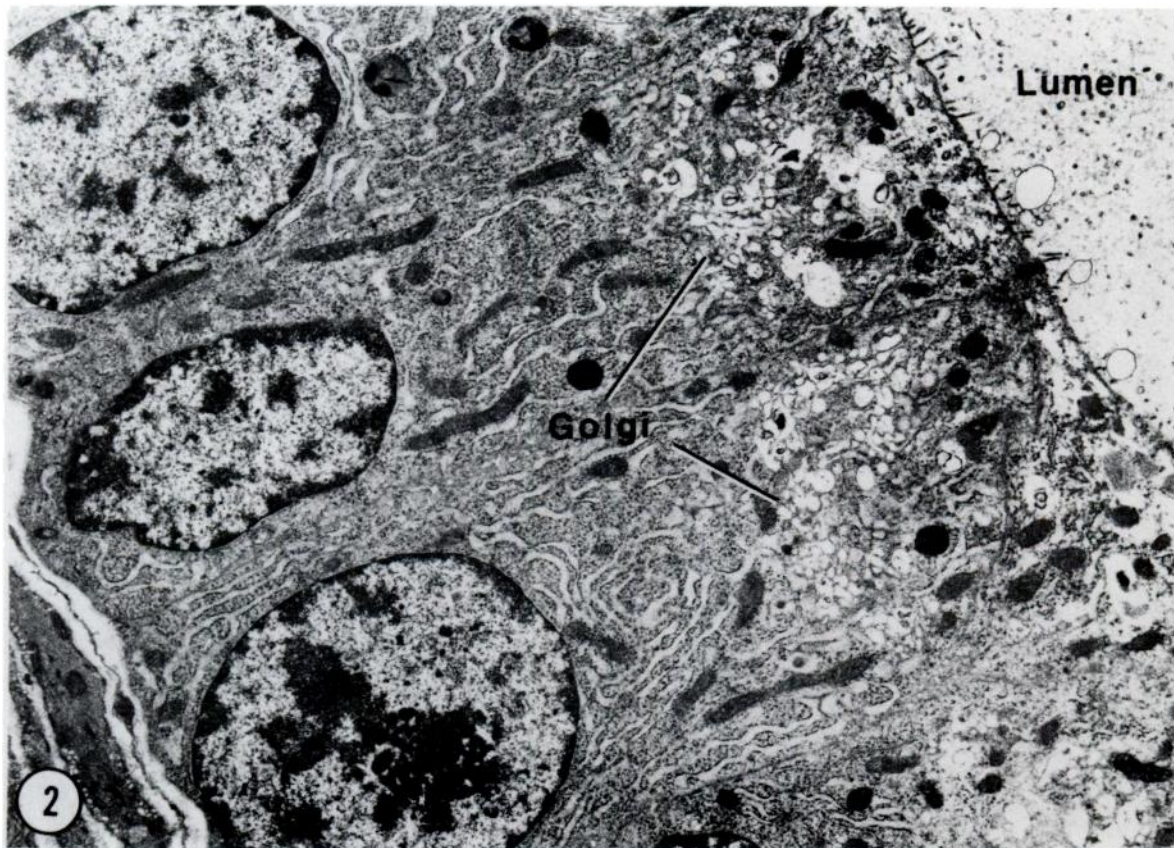
On days 1, 5, 25, and 60, blood was obtained by killing the animals, and the serum was used to determine T and LH levels by radioimmunoassay (Niswender et al, 1969 and Midgley and Niswender, 1970).

TABLE 1. Serum Testosterone (ng/100 ml) and Luteinizing Hormone (ng/ml) Levels in Male rats Exposed *In Utero*  $\Delta^9$ -THC\*

Group	Age (Days)			
	1	5	25	60
Controls (T)	85 $\pm$ 10 (10)	52 $\pm$ 07 (8)	48 $\pm$ 10 (8)	450 $\pm$ 75 (10)
Treated (T)	35 $\pm$ 12 (10)†	26 $\pm$ 10 (8)†	28 $\pm$ 5 (8)†	380 $\pm$ 102 (10)
Controls (LH)	70 $\pm$ 15 (10)	62 $\pm$ 12 (8)	55 $\pm$ 8 (8)	95 $\pm$ 7 (10)
Treated (LH)	45 $\pm$ 8 (10)†	26 $\pm$ 8 (8)†	25 $\pm$ 10 (8)†	88 $\pm$ 10 (10)

\*Blood was drawn at days 1, 5, 25 and 60 after birth, and this serum was used to measure T and LH levels by radioimmunoassay.

†Significantly different from controls.  $P < 0.01$ . Note that at day 60, differences were not significant.



**Fig. 2.** Ventral prostate gland of 50-day-old rats born to  $\Delta^9$ -THC-treated mothers (6 mg/kg). Golgi apparatus is scarce, the cisternae of the granular endoplasmic reticulum are slightly distended. Mitochondria are slender and numerous ( $\times 6480$ ).

### *Morphologic Studies*

The animals were anesthetized with nembtal and were perfused through the left ventricle with saline followed by 5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 (Karnovsky, 1965). After 10 to 15 minutes of whole-body perfusion, the prostate gland was ligated and carefully dissected from the surrounding tissues, cleared of fat and 1- to 2-mm sections were cut from several parts of the gland. Tissue sections were placed in ice cold fixative for 1 to 2 hours, postfixed for 1 hour in cold 1%  $\text{OsO}_4$ , dehydrated in an ethanol series, and embedded in Epon. The Epon-embedded tissues were sectioned at 1 to 2  $\mu\text{m}$  and stained with 2% Toluidine blue for light microscopic examination (Luft, 1961). For electron microscopy (EM) study, thin sections were cut with a diamond knife, stained in uranyl acetate and lead citrate, and examined with a Zeiss EM-10 microscope.

### *Fertility Studies*

On day 60, the males in the treated and control groups were tested for fertility. Adult, normal-cycling females

during proestrus were placed with males, and in order to allow the maximum time for mating, the animals were left together for 36 to 48 hours.

Females that failed to become pregnant when placed with the treated males were mated with control males to confirm their fertility. The pregnant females were monitored throughout their pregnancies, and the pups were removed on day 20 of pregnancy ( $F_1$ ). The number of pups, and the male-to-female ratio were recorded. The  $F_2$  generation males were similarly tested for fertility.

### **Results**

Shown in Table 1 are the results of serum T and serum LH levels in the treated males from days 1 to 60. The results showed that T and LH levels were significantly lower ( $P < 0.01$ ) during the prepubertal period. At puberty, the T and LH levels were within

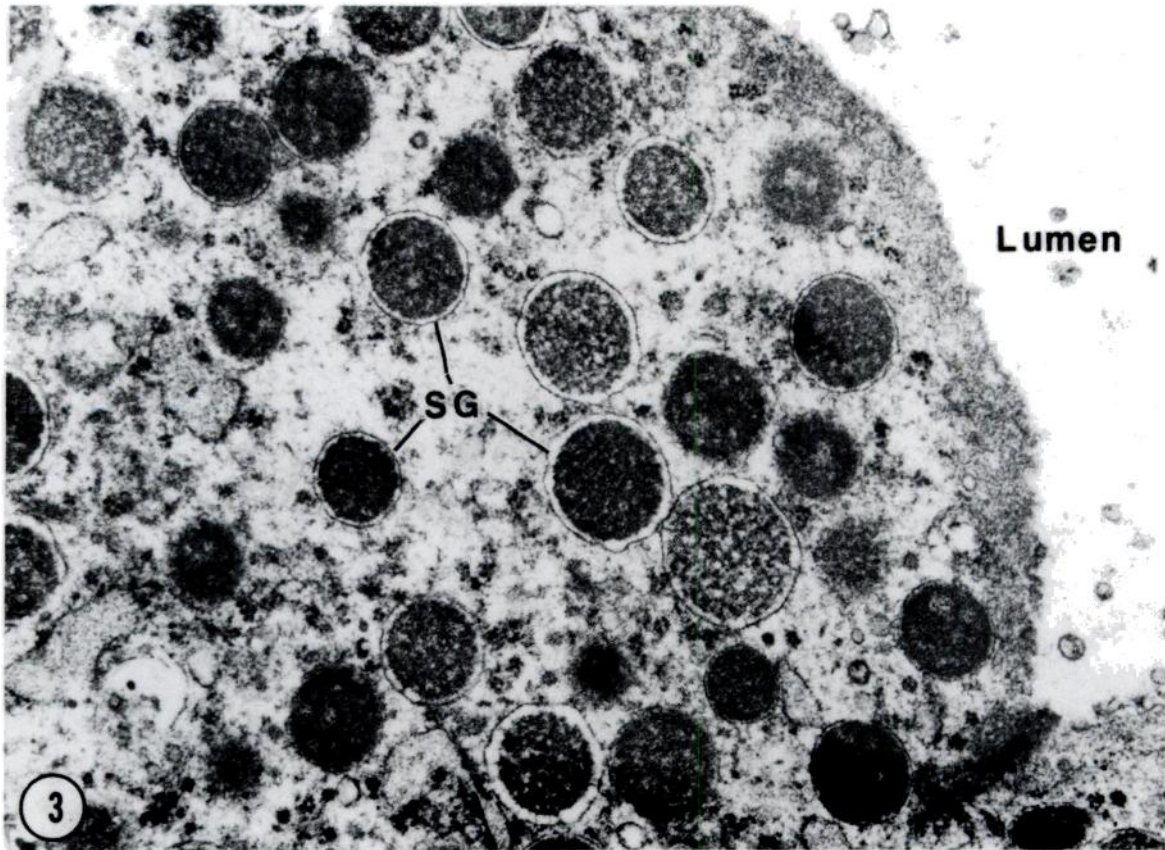


Fig. 3. Electron micrograph of ventral prostate gland from untreated rats (control) at 50 days of age. Note numerous secretory granules (SG) in the apical region ( $\times 23,750$ ).

the normal range. No significant differences were found in the number of litters born per birth or in the ratio of male to female pups from each birth in the treated animals and the controls. The results of the EM studies of the ventral prostate at day 60 are shown for the controls (Figs. 1 and 3) and treated (Figs. 2 and 4) rats. In Fig. 2 the secretory granules (SG) are vestigial and agranular, suggestive of degeneration. The Golgi cisternae are small, and both smooth and rough endoplasmic reticula showed evidence of decreased activity (Fig. 4) when compared with the controls (Figs. 1 and 3).

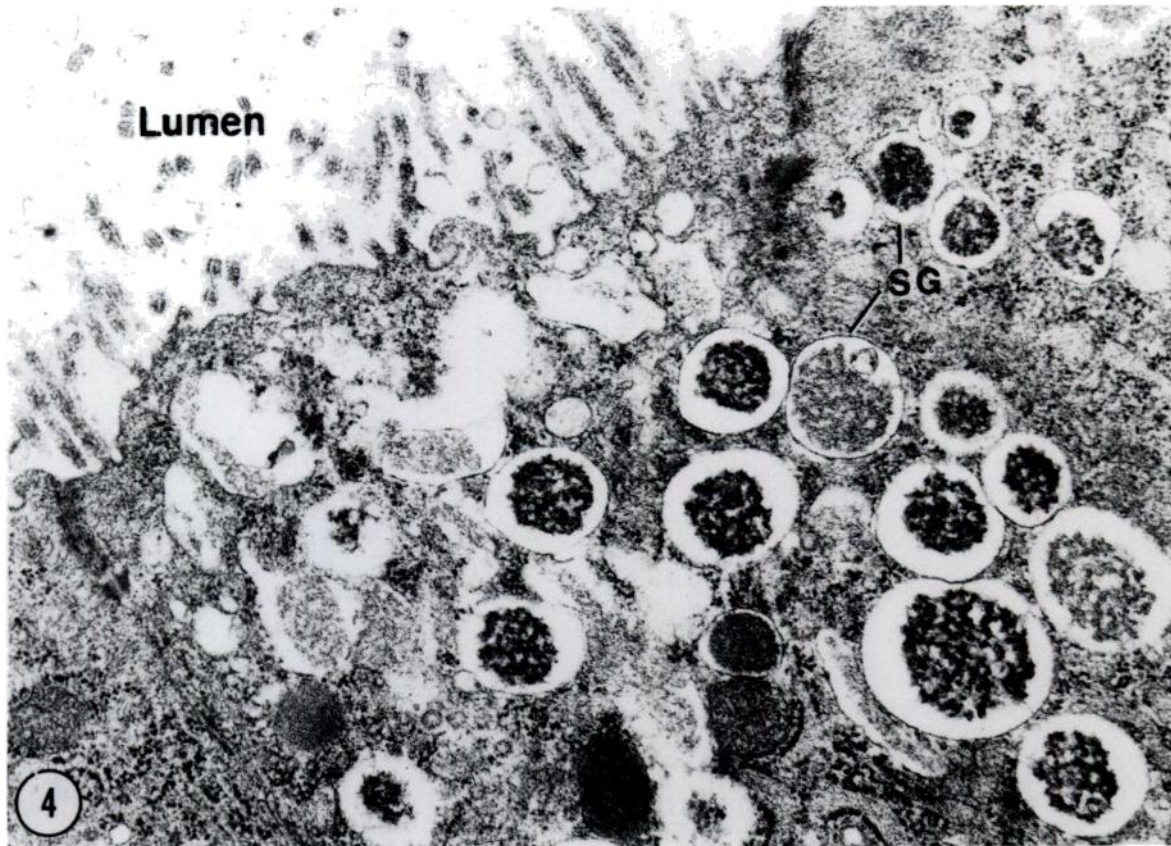
Data from the fertility studies are shown in Fig. 5 for the THC-exposed males in the  $F_1$  and  $F_2$  generations. In the treated group, there was a 30 to 40% decrease ( $P < 0.01$ ) in the number of females they were able to impregnate. Prolonged exposure of the males to the females did not improve fertility. When

those females that did not become pregnant by the treated males were housed with proven studs, approximately 80 to 90% of them became pregnant, suggesting that the treated males achieved lower pregnancy rates.

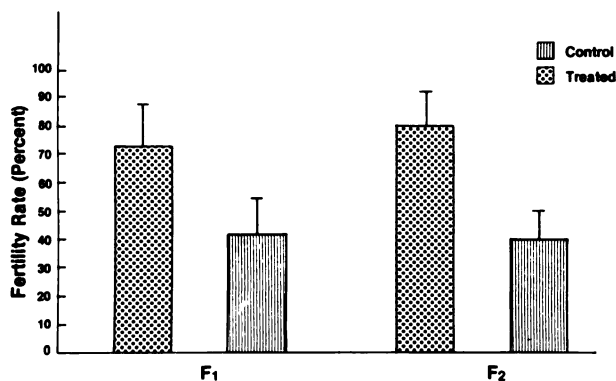
No significant differences were found in the number of pups born and in the ratio of males to females born in the treated and control groups. For this reason, the data are not presented here.

### Discussion

Our finding that a significant reduction in fertility in male rat pups born to mothers exposed to THC during pregnancy extends similar studies in mice (Dalterio and Bartke, 1979) that showed a decrease in copulatory activity in males whose mothers had



**Fig. 4.** Ventral prostate gland of rats born to mothers treated with 6 mg/kg  $\Delta^9$ -THC during pregnancy. Note the vestigial secretory granules (SG) ( $\times 23,750$ ).



**Fig. 5.** Fertility study of F<sub>1</sub> males treated *in utero* with  $\Delta^9$ -THC. These animals were housed with normal females. Note an approximately 40% decrease in the number of pups sired by these  $\Delta^9$ -THC-treated males. The F<sub>2</sub> males that received no treatment also showed decreased fertility.

received THC. The cause of decreased fertility is speculative. Dalterio et al (1984) reported alterations in the function of the hypothalamo-hypophyseal-gonadal axis in adult male mice following prenatal exposure to cannabinoids. Other authors have reported reduction in copulatory behavior in male rats after acute exposure to THC (Symons et al, 1976) and altered sexual behavior and neurochemical effects in rats (Corcoran et al, 1974). The major findings of this study are the morphologic changes in the ventral prostate that persisted following puberty, in contrast to the endocrine changes, which returned to normal at puberty. This is not surprising in view of the known plasticity of the hypothalamic-hypophyseal axis in the regulation of gonadotropins. Based on our results, the decreased fertility following THC exposure *in utero* is not due to altered endocrine function but perhaps is caused by permanent damage to parts of the reproductive tract, for example, the prostate gland. The secretory granules in the ventral

prostate gland resemble those which follow castration (Helminen et al, 1975; Merk et al, 1980).

The role of the secretory granules in prostatic secretory activity is a subject of debate. It has been reported that the secretory granules are the source of phosphatase activity (Helminen et al, 1975), and phosphatase secretion is primarily under androgen control (Mann and Lutwak-Mann, 1981). It is possible that decreased T levels during the early developmental period in these rats cause degenerative changes in ventral prostate gland morphology. The low T levels, coupled with a decrease in the number of androgen binding sites in the THC-treated ventral prostate (Purohit et al, 1980), provide a reasonable explanation for the morphologic alterations in the ventral prostate gland. The physiologic role of phosphatase in overall semen quality is not clear; evidence presented by several workers, however, suggests that choline liberated by phosphatase is linked to the phospholipid metabolism in spermatozoa (Mann and Lutwak-Mann, 1981). Our results indicate that biochemical evidence, together with morphologic evaluation, is necessary to evaluate the long-term effects of drugs.

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