High Dosage of Testosterone Propionate Increases Litter Production of the Genetically Obese Male Zucker Rat (40362)

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Since it was first described in 1961 (1) the genetically obese Zucker rat has been of considerable interest as a possible animal model of human obesity, particularly that of early-onset. Homozygous recessive (*fafa*) individuals become recognizably obese near the time of weaning and are hypercellular (2), hyperinsulinemic (3) and hypertriglyceridemic (4). In addition to their weight regulatory dysfunction, *fafa* rats are reproductively inadequate. Obese sires are rare (1), and obese dams have not been reported. Virtually all *fafa* individuals have been derived from heterozygous (*Fafa*) crosses with an expected yield of 25%. Since *Fafa* and homozygous dominant (*FaFa*) individuals are phenotypically indistinguishable, obligatory testcrossing contributes to the inefficiency of production of the *fafa* genotype. The difficulty of obtaining adequate numbers of experimental subjects has so severely restricted work on the *fafa* rat that any improvement in the efficiency of its production would be welcome.

Factors predisposing for reproductive failure in the *fafa* rat have not been identified, but work on the *fafa* rat (5) and several studies on various genetically obese strains of mice (6–8) suggest steroid insufficiency as a proximal cause of abnormal reproductive morphology and low fertility. In our breeding colony at Vassar College we have been investigating the efficacy of steroid therapy in bringing about an improved breeding performance of intact *fafa* males. Subcutaneous injection of testosterone at high dosage levels showed promise. We report below experimental confirmation of the efficacy of this treatment in substantially enhancing the fertility of *fafa* males, its suppressive effects on increments in body weight and observations on the size and genotypic composition of litters resulting from the crossing of *fafa* males with known *Fafa* females.

Materials and methods. Twenty-eight *fafa* males ranging from 83 to 106 days of age were randomly assigned to one of two treatment groups. Fourteen males received a subcutaneous injection of 20 mg testosterone propionate (TP) in 0.1 cc sesame oil for the first three consecutive days and 20 mg TP once every three days thereafter. Fourteen *fafa* males received sham injections of sesame oil on an identical schedule. Injections were continued over a 90-day period. On the third day of the experiment, two *Fafa* females were introduced to each male's cage and remained for 13 days whereupon they were removed and replaced by two other females. Thereafter, new *Fafa* females were provided each male every seven days. Thus during the 90-day experimental period each male had exposure to 24 females. Care was taken to assure that one of the females was a proven breeder whenever feasible, as we believed previous experience on the part of the female might improve the chance of impregnation. Females varied in age from three to 16 months. Males in both treatment groups were periodically weighed to detect any influence of TP on body weight.

Results. The numbers of litters sired by the two groups of males during the 90-day experimental period are summarized in Table I. The difference in production is substantial: TP-injected males sired 73 litters while sham-injected males sired 19 ($P < .001$, Chi-square test). This disparity in litter production by the two treatment groups is attributable to three factors. Eleven TP males became sexually active compared to seven sham-injected males. Mean latency to first conception for sexually active TP males was 15.9 days (range 2–36 days); for active sham-injected males: 24.1 days (range 2–56 days). The rate of impregnation was higher for active TP than active sham males: 30.6% of females placed with TP males after they had sired their first litter gave birth whereas only 10.5% of females placed with proven sham males bore young. For comparison, 90.2% of females

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placed with eleven similarly experienced non-injected *Fafa* males in an otherwise identical breeding regimen conceived. Females with prior breeding experience were no more likely than inexperienced females to conceive when placed with TP or sham males.

The breakdown of litter conception into consecutive 30-day periods (Table I) reveals a sharp drop in the number of males active and the number of litters sired for both treatment groups during the last third of the treatment period. While the number of litters remained significantly higher ($P < .01$, Chi-square test) for the TP males, it appears that the efficacy of TP attenuates with time. A separate experiment in which thirteen *fafa* males seven to eleven months of age received 20 or 30 mg TP ($n = 11$) or sham ($n = 2$) for 90 days in the regimen described above resulted in no litters. Females were provided to these older males in the same manner as for young males.

Table II provides information which makes possible a comparison of the size and composition of litters from *fafa* and *Fafa* males paired with *Fafa* females. Size and composition of litters from *FaFa* × *FaFa* crosses are included for comparison. Litter size at birth did not differ significantly among groups, nor did litter size at weaning. For *Fafa* sired litters the *fafa* pups comprised 25.5% of the offspring, which conforms to expectation. In *fafa* sired litters 44.5% of the pups were *fafa*. This is a significant departure from the expected 50% ($P < .05$, Chi-square test). Between birth and weaning *fafa* sired pups exhibit a 21.2% mortality, lean sired pups a 14.7% mortality. The difference is significant ($P < .01$, Chi-square test). Among lean pups and obese pups, regardless of parentage, there is a slightly smaller number of male pups than female pups at weaning age.

At the beginning of the experiment, the young TP-injected males had a mean body weight of 354 ± 6.7 g[1]; the young sham-injected males 334 ± 13.6 g. The difference was not statistically significant. On day 89 of treatment the mean weight of TP males was 505 ± 13.8 g; sham males 584 ± 16.2 g ($P < .001$, t test). Changes in body weight with time are shown in Fig. 1 as mean percent increase over initial body weight. The rate of weight gain was significantly reduced ($P < .01$, t test) as early as 29 days after treatment was begun.

Partial correlational analyses of litter production and body weight dynamics among the TP-injected males revealed no significant association between either latency to first conception or number of litters sired and initial body weight, final body weight, the changes in body weight or the percent increase in body weight. The same was true for sham-injected males.

Discussion. High doses of testosterone propionate clearly increase the litter production of the young *fafa* male rat. TP-injected males sired nearly four times as many litters as sham-injected controls. The improved litter production makes practical the use of *fafa* males instead of heterozygous males for breeding with heterozygous females. This should increase greatly the efficiency of producing *fafa* rats since nearly twice as many will result from a successful mating. The breeding of *fafa* males with lean females also guarantees that any phenotypically lean offspring are heterozygous. Thus, testcrossing to identify heterozygous rats is no longer necessary.

The suppressive effect of TP on rate of weight gain is attributable, at least in part, to reduced food consumption. We have preliminary data which indicate that *fafa* males given TP in the same regimen as in our breeding experiment significantly reduce their daily food intake.

The possibility that the increased obesity which accrues with age contributes to the reproductive impairment of *fafa* males is suggested by the sharp decline in litter production in both TP-and sham-injected young

TABLE I. EFFECT OF TESTOSTERONE PROPIONATE ON THE LITTER PRODUCTION OF YOUNG *fafa* MALES.

Treatment	Number of males	Numbers of litters conceived 0–90 days	1–30 days	31–60 days	61–90 days
TP[a]	14	73 (11)[b]	25 (9)	32 (11)	16 (7)
Sham	14	19 (7)	8 (5)	9 (5)	2 (2)

[a] 20 mg testosterone propionate in 0.1 cc sesame oil administered subcutaneously once every three days.

[b] Numbers in parentheses indicate the number of males responsible for the litters conceived during the above indicated span of time.

[1] S.E.M.

TABLE II. SIZE AND COMPOSITION OF LITTERS SIRED BY *fafa*, *Fafa* AND *FaFa* MALES.

Genotype of parents			Mean litter size		Phenotype and sex			
					Lean (Fa−)		Obese (fafa)	
Male	Female	Number of litters	At birth	At weaning	Male	Female	Male	Female
fafa (19)[a]	Fafa (49)	55	8.78 ±.41[b]	6.92 +.51	102	110	78	93
					55.4%[d]		*44.6%*	
Fafa (30)	Fafa (50)	55	9.40 ±.42	8.06 ±.42	159	171	54	59
					74.5%		*25.5%*	
FaFa (16)	FaFa (46)	55	9.02 ±.47	7.57[c] ±.79	70[c]	79[c]		
					100%			

[a] Numbers in parentheses indicate the number of individuals of this type involved in the production of the litters on which the data is based.
[b] S.E.M.
[c] Data based on 21 litters. Remainder of those used for determining litter size at birth were utilized in experiments before weaning age.
[d] Frequency of phenotypes at weaning expressed as a percentage.

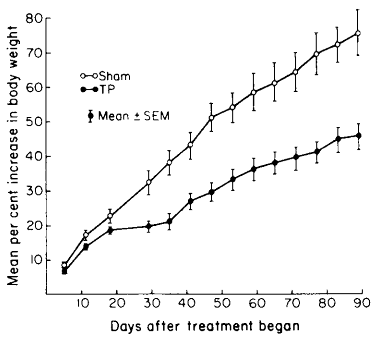

FIG. 1. Effect of testosterone propionate on the rate of weight gain in young *fafa* males.

males toward the end of the testing period and the total lack of response of the older, more obese, males to treatment. Hemmes and Hirsch (9) have recently reported that Osborne Mendel rats rendered obese by feeding a high fat diet exhibit markedly diminished sexual vigor. These findings together with the observation that substantially reducing the weight of *fafa* males improves their litter production (P. Johnson, personal communication) lead us to suspect that factors secondary to the obese condition contribute to the infertility of *fafa* males.

The efficacy of TP in increasing litter production suggests that *fafa* males may have a testosterone deficiency. Circulating levels of testosterone have not been reported for the Zucker rat. Testosterone deficiency is known to occur in morbidly obese men. Glass *et al.* (10) have suggested that aromatization of testosterone by the enlarged adipose depot may be responsible for the deficiency. Barbato and Landau (11) report that, after substantial weight loss, testosterone levels of obese men return to the normal range and that sexual performance and libido improve. Further study would reveal the extent to which adipose tissue, steroid levels, and reproductive function are causally interrelated.

Summary. A high dose of testosterone propionate increases dramatically the litter production of young genetically obese male Zucker rats. Twenty milligrams testosterone injected subcutaneously once every three days over a 90-day period resulted in a nearly fourfold increase in the number of litters sired compared to sham-injected controls. The efficacy of the treatment attenuates with time. TP was ineffective in inducing litter production in older, more obese, males. Young obese males injected with TP exhibited a significantly reduced rate of weight gain compared to sham-injected controls. The findings are consistent with the hypothesis that the reproductive inadequacy of the genetically obese male rat may be due to a deficiency of circulating testosterone. The treatment of obese males with TP greatly increases the efficiency with which the obese (*fafa*) genotype may be produced and also avoids time-consuming testcrossing for identification of heterozygous (*Fafa*) individuals.

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