Testicular Function of Actively Immunized Male Rats with LH Releasing Hormone (LHRH): A Possible Role of Prolactin on Regulation of Spermatogenesis

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Abstract

The effect of testosterone propionate (TP) on the recovery of spermatogenesis was compared in 3 types of experimentally induced gonadotropin-deficient rats; 1. hypophysectomized rats, 2. rats actively immunized to LHRH and 3. hypophysectomized and immunized rats bearing a pituitary isograft. In order to immunize animals to LHRH, deamidated LHRH conjugated with BSA (LHRH-BSA) was injected intradermally to male rats 4 times at 2 week intervals and additional booster injections continued once a month.

Anti-LHRH titer was the highest on the 12th week and the titer remained at a relatively high levels thereafter. A remarkable decrease in testicular weight and testosterone production was achieved in 10 weeks associated with a drop in serum LH and FSH levels. In long term immunized rat (LIM rats) which had been immunized for more than 3 months, testicular atrophy advanced to a similar extent to that in long term hypophysectomized rats (HX rats).

The administration of TP (1 mg/day) sc for 30 days restored spermatogenesis in LIM rats. The simultaneous administration of anti-LH and -FSH sera in addition to TP did not affect the restoration of spermatogenesis in LIM rats. The same TP treatment in HX rats, however, failed to restore spermatogenesis. Spermatogenesis was reinitiated after TP treatment if immunized-hypophysectomized rats received a pituitary isograft. Though the serum PRL level in LIM rats was one fifth of that in normal rats, a 2.5-fold rise in the PRL level was observed after TP treatment.

These results suggest that PRL is involved in the process of spermatogenesis.

Hypophysectomy eliminates all hypophyseal hormones including, LH, FSH and prolactin (PRL) and impairment of spermatogenesis subsequently ensues. LH and

FSH have a stimulatory effect on the restoration of spermatogenesis but spermatogenesis is not fully restored by treatment with both hormones, suggesting the involvement of other hypophysial hormones in the regulation of spermatogenesis. The stimulatory effect of PRL was reported on the male reproductive function in rats (Hafiez *et al.*, 1971, 1972a, b; Bartke *et al.*, 1977; Negro-Vilar *et al.*, 1977), seasonally breeding animals (Ravault, 1976), golden hamsters (Bex and Bartke, 1977; Bex *et al.*, 1978; Bartke *et al.*, 1980) and hereditary dwarf mice (Bartke and Lloyd,

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1970; Hafez *et al.*, 1971). The regulation of gonadal LH receptor by PRL was noted in male and female animals (Richards and Williams, 1976; Aragoma *et al.*, 1977; Zipf *et al.*, 1978; Morris and Saxena, 1980) and the mechanism of the gonad-stimulating action of PRL is considered to reside in the ability of PRL to increase number of LH receptors. However, the presence of other mechanisms of PRL action without mediating LH receptors were suggested (Crisp, 1977) and an inhibitory action of PRL on reproductive function was also reported (Fang *et al.*, 1974; Winters and Lariaux, 1978). The significance of PRL in male animals is still debatable.

Hypophysectomized animals have been widely used in the study of hormonal control of spermatogenesis. As with hypophysectomy, the elimination of endogenous LHRH by active immunization against LHRH causes a deficiency of circulating LH and FSH (Makino *et al.*, 1973; Fraser *et al.*, 1974; Jeffcoat *et al.*, 1976; Takahashi *et al.*, 1978) and consequently lessened secretion of testicular androgen, and testicular androgen plays a primary role both in spermatogenesis and PRL secretion (Kalra *et al.*, 1973; Shin *et al.*, 1974; Ojeda *et al.*, 1977).

Hypophysectomized and LHRH immunized rats, present differ in the ability to secrete hypophysial hormones other than LH and FSH (Jeffcoat *et al.*, 1976; Takahashi *et al.*, 1978). In this report testosterone propionate was chronically administered to androgen deficient rats prepared by hypophysectomy or immunization to LHRH and the restoration of spermatogenesis was compared to investigate the role of hypophysial hormones in the process of spermatogenesis.

Materials and Methods

Animals

Adult male Wistar rats bred in our laboratory were maintained on a commercial diet and tap water *ad libitum* under a light regimen of 14 h of light (0500–1900 h) and 10 h of darkness.

Immunization of rats with LHRH and collection of samples

Animals began to be immunized at 2 months old with LHRH conjugated with bovine serum albumin (BSA). The conjugate (LHRH-BSA) was prepared from 200 µg deamidated LHRH from Dr. Fujino (Takeda Chemical Industries, Japan) by the carbodiimide method described before (Makino *et al.*, 1973), dissolved in 0.3 ml of 0.15 M NaCl, emulsified with 0.3 ml Freund's complete adjuvant and injected intradermally into each animal at more than 10 sites on his dorsal skin. The first immunization was followed by 4 injections of the same amount of LHRH-BSA at weeks 2, 4, 6, and 10. Anti-LHRH titer and the serum LH, FSH and testosterone levels were monitored at intervals throughout the experimental periods. Blood samples were collected by heart puncture under ether anesthesia. Testicular testosterone content, the weight of the testis and seminal vesicles were also determined using another group of animals subjected to the same schedule of immunization. A part of the testis samples harvested in week 12 was subjected to a histological study. They were fixed in a formalin solution and paraffin sections were stained with hematoxylin and eosin.

For the experiment using the long term immunized rats (LIM rats), the immunized rats received additional booster injections of the same amount of LHRH-BSA at 1-month intervals and were used between the 1st and 4th booster injections. The weight of the thyroid and adrenal glands in LIM rats was compared with that of hypophysectomized rats operated 2 months before at 3 months old.

Serum PRL measurement in LIM and castrated rats after testosterone propionate (TP) or estradiol benzoate (EB) administration

LIM and 5-month-old castrated rats operated 6 days before were injected sc with TP (1 mg/0.1 ml sesame oil) or EB (50 µg/0.1 ml sesame oil). The injections were repeated twice at 1600 and 1400 h the next day. An hour after the second injection, blood samples for PRL assay were collected by heart puncture under light ether anesthesia.

Table 1. Protocol of Morphological Study for Spermatogenesis

Abbreviation & Animal Condition	Treated			Control		
	Injection*	Start of Injection	Collect. of Testis	Injection*	Start of Injection	Collect. of Testis
Intact	—	—	5 month old	—	—	—
HX (hypophysectomized)	TP 1 mg/day	immediately after hypox	1 day after final inj.	—	—	—
		2 weeks after hypox		—	—	2 weeks after hypox
		1 month after hypox				1 month after hypox
		3 months after hypox				3 month after hypox
LIM (immunized)	TP 1 mg/day		1 day after final inj.	Sesame oil		1 day after final inj.
	TP 1 mg/day + Anti-LH**				—	—
	TP 1 mg/day + Anti-FSH**					
LIMX (immunized- hypophysectomized)	TP 1 mg/day	immediately after hypox	1 day after final inj.	—	—	—
LIMIX (immunized- hypophysectomized- AP-grafted)	TP 1 mg/day	immediately after hypox	1 day after final inj.	Sesame oil	Immediately after hypox (1 week after graft)	1 day after final inj.

* sc injection for 30 days. TP was dissolved in sesame oil (1 mg/0.1 ml).

** Anti-LH serum, 0.4 ml/day; Anti-FSH serum, 1.2 ml/day.

Morphological study for spermatogenesis

As listed in Table 1, intact, hypophysectomized (HX), immunized (LIM), immunized-hypophysectomized (LIMX) and immunized-hypophysectomized-pituitary-grafted (LIMIX) rats were subjected to morphological observation for spermatogenesis. Immunization for the LIM, LIMX and LIMIX groups, and hypophysectomy for the HX group were done at 2 months of age. TP was dissolved in sesame oil (1 mg/0.1 ml) and injected sc once a day for 30 days. In HX rats, TP injection started either immediately after hypophysectomy, or 2 weeks, 1 month or 3 months later. The unilateral testis was removed before the injection as the control specimen. LIM rats received either sesame oil (control), TP, TP together with anti-LH serum (0.4 ml/day) or TP together with anti-FSH serum (1.2 ml/day). Antiserum for LH or FSH was generated in rabbits by repeated injections of ovine LH (NIH-LH-S15) or porcine FSH (Armour Pharmaceutical Co.) emulsified with Freund's complete adjuvant, respectively. The amount of serum used in this study was proven to inhibit the occurrence of spontaneous ovulation in female rats if injected iv in the morning of proestrous for anti-LH or in the morning of metestrous for anti-FSH, respectively. LIM rats were

hypophysectomized (LIMX) and received TP from the day of operation. The anterior pituitary removed from adult male rats was placed under the kidney capsule of the recipient-LIM rats anesthetized with ether. One week after the implantation, the *in situ* pituitary was removed (LIMIX) and TP injection was started. The vehicle was injected as a control. In HX, LIMX and LIMIX rats, hypophysectomy was performed by a parapharyngeal approach under ether anesthesia. The animals were autopsied at the end of the experiment to verify the complete removal of the pituitary gland.

One day after the final injection, animals were sacrificed and the testis was removed, weighed and fixed in Bouin's solution, and paraffin sections were stained with hematoxylin and eosin.

Measurement of anti-LHRH titer

Binding activity of the antiserum to tritiated LHRH (New England Nuclear, Specific Activity; 30 Ci/mmole) was measured according to the method described previously (Takahashi *et al.*, 1978). The titer was expressed as percent binding at 1/100 dilution to [³H]-LHRH. The blank values in each assay about 10% were subtracted.

Measurement of hormones

Duplicated aliquots of 50 to 200 μ l of sera were used for either LH, FSH or PRL. Samples were measured by each double antibody radioimmunoassay using the kits kindly donated by NIAMDD, according to the method described by Niswender *et al.* (1968) for LH and by Daane and Parlow (1971) for FSH or by Murakami *et al.* (1979) for PRL. Iodinated rat PRL (Specific Activity: 48.3 μ Ci/ μ g) was purchased from New England Nuclear. Each result was expressed in terms of NIAMDD-rat-LH-RP-1, NIAMDD-rat-FSH-RP-1 and NIAMDD-rat-PRL-RP-1, respectively. The assay sensitivity was 5 ng/ml for LH, 100 ng/ml for FSH or 2 ng/ml for PRL.

Testosterone was extracted from either peripheral serum or testicular homogenate with ethylether and measured by radioimmunoassay as described before (Shiota *et al.*, 1976) using the antibody purchased from Dr. G. E. Abraham (S 741 #2).

Statistical analysis was carried out by Student's *t*-test.

Results*(1) Changes in anti-LHRH titer and the organ weight*

Fig. 1 shows the time course study of anti-LHRH titer and the organ weight during the course of immunization. A significant rise in the anti-LHRH titer was observed 2 weeks after the 1st injection of

LHRH-BSA ($p < 0.05$). The titer reached the maximum level at week 12 and a relatively high titer was maintained until week 18 without further injections of LHRH-BSA. Binding activity to [3 H]-LHRH was not observed in any sera taken from the control rats throughout the experimental period. On week 10, atrophy of the testis was detectable by palpation in all the immunized rats and testicular weight decreased to less than 300 mg. The testicular weight remained suppressed until week 18. A weight decrease in the seminal vesicle was recognizable earlier than that in the testis.

Table 2 shows the weight of the adrenal and thyroidal glands in immunized rats later than week 10 of immunization (LIM rats) together with that in normal and hypophysectomized HX rats. Each body ($p < 0.01$), adrenal ($p < 0.01$) and thyroidal ($p < 0.05$) weight was significantly lower in HX rats than the control, while a slight but significant increase in body and thyroidal weight ($p < 0.05$) was observed in immunized rats. There was no significant difference between the adrenal weight in immunized and control rats.

Fig. 1. Change in anti-LHRH titer and organ weight during the course of immunization. The titer was expressed as percent binding of each serum at 1/100 dilution to [3 H]-LHRH. The number of animals is shown in parenthesis. Each vertical line represents S. E. of the mean.

Table 2. Weight of body, adrenal and thyroidal glands in long term immunized rats and hypophysectomized rats

	Body wt. (g)	Adrenal wt. (mg)	Thyroid wt. (mg)
Intact control	334.0 ± 4.8*	46.0 ± 3.2	20.3 ± 0.7
LHRH-Immunized	394.8 ± 22.2**	41.4 ± 3.4	24.6 ± 1.4**
Hypox (2 month)	246.3 ± 11.0***	9.5 ± 1.2***	15.7 ± 1.2**

* Mean ± SEM. Five rats were used for each group.

** $p < 0.05$ vs. control

*** $p < 0.01$ vs. control

(2) *Testosterone concentration in peripheral and testicular tissue in immunized rats.*

The testosterone concentration in peripheral blood during the course of immunization was measured and correlated with the antibody titer individually (Fig. 2). There was a distinct negative correlation between the two parameters. Testosterone content of the testis in immunized and control rats is shown in Fig. 3. In testes collected at weeks 4 and 6 in immunized rats, testosterone decreased to a much lower level than that in control rats though testicular weight (around 1,000 mg) still remained at 76.3% of the control. Testicular testosterone levels decreased further and then a marked reduction took place in the testicular weight in week 10.

(3) *Serum LH and FSH levels in immunized rats.*

The serum LH and FSH levels tended to continue to decrease during the course of immunization, though a wide range of variations was associated with the LH value (Fig. 4). A slight elevation in LH in week 2 was not significant ($p > 0.05$). Sera collected at weeks 4, 6 and 10 showed significantly lower LH levels than those collected at Week 2 ($p < 0.05$).

(4) *Serum PRL level in LIM, castrated and normal rats before and after TP or EB treatment.*

The serum PRL concentration in intact, castrated or LIM rats is shown in Table 3. The PRL level in castrated and LIM rats was significantly lower ($p < 0.05$) than that in normal rats. The PRL concentration in LIM rats remained at about a half of that in normal rats. The treatment with TP or EB resulted in 2.5- and 4.5-fold increase in LIM and castrated rats, respectively.

(5) *Effects of TP treatment on spermatogenesis of LIM and hypophysectomized rats under various hormonal milieu.*

As shown in Plate 1, the involution of seminiferous tubules and Leydig cells was evident in immunized rats on week 12 (Fig. 6) and hypophysectomized rats (Figs. 7-9). Degeneration proceeded in the testis of hypophysectomized rats with the lapse of time after the operation.

Testicular specimens from LIM and hypophysectomized rats treated with TP are presented in Plate 2. In hypophysectomized rats, TP treatment failed to restore spermatogenesis (Figs. 12-14), while in LIM rats the restoration was significant and mature spermatozoa were found in the lumen (Fig. 11). Vehicle treatment to LIM rats had no effect on spermatogenesis (Fig. 10). Simultaneous administration of anti-

Fig. 2. Correlation between anti-LHRH titer and serum testosterone concentration. Blood samples were collected during the course of immunization until week 28.

LH or -FSH serum did not affect the restoration of spermatogenesis in TP-treated LIM rats (Plate 3, Figs. 15 and 16).

When LIM rats were hypophysectomized (LIMX rats) before TP treatment, spermatogenesis was not restored (Plate 4, Fig. 17). In LIMX rats which were previously transplanted with the anterior pituitary ectopically under the kidney capsule (LIMIX rats), however, TP treatment again was effective in reinitiating spermatogenesis (Plate 4, Fig. 19). Without TP treatment, a slight increase in tubule diameter was observed in LIMIX rats Plate 4, Fig. 18) compared with LIM rats Plate 2, Fig. 12).

(6) Testicular weight in LIM and hypophysectomized rats after TP treatment.

Fig. 20 shows the testicular weight in various groups mentioned above at the end of experimental manipulation. TP treatment failed to increase the testicular weight in 2-week-hypophysectomized rats and slightly increased it in 1- or 3-month-hypophysectomized rats. If the treatment started on the day of hypophysectomy, the testicular weight remained at the normal level. In contrast, TP treatment resulted in a significant testicular weight gain ($p < 0.05$) in immunized rats so long as the anterior pituitary was kept either *in situ* or ectopically.

Discussion

The present study confirms previous observations (Fraser *et al.*, 1974; Jeffcoat *et al.*, 1976; Takahashi *et al.*, 1978) that immunoneutralization of exogenous LHRH resulted in a decrease in the secretion of LH and FSH. The deficiency of LH and FSH in our preparation lasted for a long period after the final treatment with LHRH-BSA. Serum FSH concentrations continued to decrease in an inverse relationship with the LHRH-titer, whereas those of LH showed 2 rises at weeks 2 and 6 of immunization, though they were not statistically significant. This apparent difference could partly be attributed to the difference in the magnitude of the discharge in the release of each hormone (Desjardins, 1981). LH pulses of greater magnitude than those of FSH may be evaluated as higher variability of mean values in the infrequent sampling schedule employed in this study.

The weight of the seminal vesicle decreased to a minimal level at week 6 to 10 of immunization. In contrast to a fast and steady decrease in the weight of the seminal vesicle in immunized animals, that

Fig. 3. Testosterone content in testicular tissue and weight of the testis in immunized and control rats. In immunized rats, testes were classified into 2 groups according to weight. The testes collected at weeks 4 and 6 were heavier than 1000 mg and those collected at weeks 10 and 12 were less than 300 mg. The number of animals is shown in parenthesis. Each vertical line represents S.E. of the mean.

in the testis was much slower; the testicular weight at week 6 to 10 still remained at 76% of control. Serum FSH during the same experimental period was at 50% of the control level and a marked reduction in testicular weight was associated with a decrease in serum FSH to less than 25% of the control level. Thus, the slow decrease of testicular weight seemed mostly due to the decrease in the serum FSH concentration during the course of immunization. It is suggested that the secretion of FSH resists the reduction of LHRH stimuli in comparison to that of LH (Knobil, 1981).

In LIM rats which continued to receive monthly injections of LHRH-BSA since week 10, serum PRL concentrations were significantly lowered. This is in accord with previous reports (Fraser, 1976; Takahashi *et al.*, 1978) showing that active immunization with LHRH results in the lowering of serum PRL levels in female or male rats. There have been reports (Kalra *et al.*, 1973; Shin *et al.*, 1974; Ojeda *et al.*, 1977) indicating that castration lowers the PRL concentration in the pituitary and peripheral blood and that these decreases are prevented by the administration of estradiol or testosterone. In the present experiment, serum PRL level was elevated by TP or EB treatment in LIM rats as in orchidectomized animals, suggesting that lowered PRL levels in LIM rats were caused by a reduced outflow of testicular steroids. Since the testis secretes estrogen under FSH control (Dorrington *et al.*, 1978), the decrease in the serum PRL concentration in LIM rats is considered to be caused by a deficiency of FSH as well as LH. The serum PRL level in LIM rats was significantly lower than in the rats orchidectomized 6 days before, and may result from the longer steroid-deficient period than that in castrated rats.

Impairment of spermatogenesis, involution of the seminiferous tubule and atrophy of Leydig cells in LIM rats occurred to the same extent as in long term (for 3 months) hypophysectomized (HX) rats. Administration of TP for 30 days reinitiated spermatogenesis and increased testicular weight in LIM rats. When LIM rats were hypophysectomized before the start of TP treatment, however, the treatment could induce neither spermatogenesis nor testicular weight gain. The serum FSH concentration was still above the nondetectable level on week 10 of immunization. Synergistic action of FSH and androgen on the restoration of spermatogenesis is known (Steinberger, 1971). To minimize the possibility that the minute

Fig. 4. Serum LH and FSH concentrations during the course of immunization. The results were expressed in term of NIAMDD-rat-LH-RP-1 and NIAMDD-FSH-RP-1. The number of animals is shown in parenthesis. Each vertical line represents S. E. of the mean.

Table 3. Prolactin concentration after treatment of TP or EB in long term immunized and castrated rats

	Prolactin Conc. (ng/ml serum)	※ Increase Ratio (Treated/Control)
Intact (5)*	8.48 × 0.70	—
Castrated (5) (Control)	3.09 × 0.29**	—
Castrated (5) + TP	7.31 × 1.70***	2.36
Castrated (5) + EB	13.48 × 1.49***	4.36
LIM (control) (4)	1.70 × 0.41	—
LIM TP (4)	4.53 × 0.40***	2.67
LIM + EB (4)	8.34 × 1.35***	4.91

LIM and castrated rats were injected sc with TP (1 mg) or EB (50 ug). The injections were repeated twice at 1600 and 1400 hr the next day. An hour after the second injection, blood samples were collected.

* Number of rats

** Mean × SEM

*** $p < 0.05$ vs. control

※ NIAMMD-rat-PRL-RP-1

amount of LH and FSH secreted in LIM rats, if any, might still be effective on those restoring events, anti-LH or -FSH serum was administered simultaneously with TP. The extent of the restration was not altered by both anti-hormone injections. The hypophyseal hormones other than LH and FSH, therefore, seemed to contribute to the restration of spermatogenesis in TP-treated LIM rats.

In immunized-hypophysectomized rats once transplanted with the anterior pituitary ectopically, recovery of spermatogenesis and testicular weight gain became evident by TP treatment. The pituitary graft under the kidney capsule has been used for the continuous and selective supply for PRL, though associating secretion of slight amount of LH, FSH and growth hormone were reported (Hjalmarsen and Ahren, 1965; Lam *et al.*, 1976). The secretion of gonadotropins from the grafted pituitary is considered to depend on circulating LHRH (Evans and Nikitovitch-Winer, 1969; Ari-mura *et al.*, 1973) but LHRH in the immunized rats was continuously neutralized

Plate 1. Comparison of testicular morphology in intact, immunized and hypophysectomized rats. Fig. 5, intact 5 month old rats; Fig. 6, immunized rat testis at week 12; Fig. 7-9, Hypophysectomized rat testis, which was collected 2 weeks (HX 2w), 1 month (HX 1mo) or 3 months (HX 3mo) after hypophysectomy.

in the circulation. Therefore, the synthesis and the release of LH and FSH from the grafted pituitary in LIM rats should be minimized. Thus, the most probable candidate that stimulated spermatogenesis in LIM rats in the presence of testosterone was PRL.

A possible involvement of pituitary hormones other than LH and FSH has been widely accepted in the regulation of testicular function. Among them, the presence of the specific receptor for PRL (Aragona

and Friesen, 1975; Aragona *et al.*, 1977) and the regulation of LH receptor by PRL in the testis were reported (Zipf *et al.*, 1978; Morris and Saxena, 1980). PRL stimulation of 3β -hydroxysteroid dehydrogenase activity (Hafez *et al.*, 1971), incorporation of acetate to testosterone (Hafez *et al.*, 1972a) and testosterone production (Hafez *et al.*, 1972b) were demonstrated using hypophysectomized LH-treated rats. In contrast, the inhibition of testicular function in hyperprolactinemic condition

Plate 2. Effect of TP treatment (1 mg in 0.1 ml sesame oil/day sc for 30 days) on the recovery of spermatogenesis in LIM and hypophysectomized rats. Fig. 10, LIM vehicle control. LIM rats received sc 0.1 ml of sesame oil (LIM+oil); Fig. 11, LIM rats received TP (LIM+TP); Figs. 12-14, TP injection was initiated 2 weeks (HX 2w+TP), 1 month (HX 1mo+TP) or 3 months (HX 3mo+TP) after hypophysectomy. The testes were collected one day after the final injection.

was reported (Fang *et al.*, 1974; McNeilly *et al.*, 1978; Winters and Loriaux, 1978), but this inhibition might be mostly due to an aberration of gonadotropin secretion. PRL was also reported to stimulate spermatogenesis in LH-treated hypophysectomized rats (Wood and Simpson, 1961; Bocca-bells, 1963) in hereditary dwarf mice (Bartke and Lloyd, 1970) and in hypophysectomized mice (Bartke, 1971). Though these authors claimed that the spermatogenic action of PRL was effected via augmentation of LH action rather than potentiation of androgen

action on spermatogenesis, it seems plausible that TP treatment alone is unable to restore spermatogenesis in long term hypophysectomized animals from the present study and others (Woods and Simpson, 1961). Success in the recovery of spermatogenesis in LIM rats with only TP treatment, thus, strongly suggests that PRL has a permissive role in the completion and maintenance of spermatogenesis by androgen. Further studies to elucidate this permissive action in detail are in progress.

Plate 3. Effects of TP injection (1 mg in 0.1 ml sesame oil/day sc for 30 days) together with anti-LH serum (0.4 ml/day, Fig. 15) or anti-FSH serum (1.2 ml/day, Fig. 16) on the recovery of spermatogenesis. The testes were collected one day after the final injection.

Plate 4. Effects of TP injection (1 mg in 0.1 ml sesame oil/day sc for 30 days) on the recovery of spermatogenesis in immunized-hypophysectomized and immunized-hypophysectomized-pituitary isografted rats. Fig. 17, LIM rats were hypophysectomized before TP treatment (LIMX+TP); Figs. 18 and 19, LIM rats were transplanted with the anterior pituitary under the kidney capsule and the *in situ* pituitary was removed before the injection of TP (LIMIX+TP) or sesame oil (LIMIX+oil). The testes were collected one day after the final injection.

Fig. 20. Testicular weight in immunized (LIM), hypophysectomized (HX) immunized-hypophysectomized (LIMX), immunized-hypophysectomized-pituitary grafted (LIMIX) rats after TP treatment (1 mg/day sc for 30 days).

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