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Effect of 5α-Dihydrotestosterone Implants on the Fertility of Male Rats Treated With Tamoxifen

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ABSTRACT: In adult male rats, tamoxifen (TAM) reduces circulating levels of luteinizing hormone (LH) and testosterone (T) with no effect on follicle-stimulating hormone (FSH) and prolactin (PRL). It reduces the male rat's ability to inseminate the female (potency), as well as its siring ability (fecundity). The objective of the present study was to test whether androgen supplementation could reverse all or some of the observed effects of TAM. To obviate the effects of estrogen, the study was designed to evaluate the beneficial or deleterious effect of 5α-dihydrotestosterone (DHT), a 5α-reduced, nonaromatizable metabolite of T, on the reproductive functions of TAM-treated adult male rats. Adult male rats received either saline or TAM (0.2 or 0.4 mg per day PO) for 90 days. A group of TAM-treated rats was implanted with 6 mg DHT from day 50 to day 90. A third group of untreated animals was implanted with 0-, 1-, 3-, or 6-mg DHT implants for 90 days. Mating studies were done to assess the fecundity, potency, and fertility index at the end of the treatment. Weights of testes, pituitary, and accessory sex organs were recorded, and circulating levels of LH, FSH, PRL, T, and 17-β-estradiol were estimated. DHT did not affect the fecundity or fertility index. TAM reduced fecundity, potency, and the fertility index. DHT implants improved the fertilizing ability of the TAM-treated male rat. This study discusses and reviews the role of T and 17-β-estradiol in sperm-fertilizing potential in light of these observations.

Key words: Potency, fecundity, prolactin, follicle-stimulating hormone, testosterone.

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Tamoxifen (TAM) is a synthetic nonsteroidal antiestrogen of the triphenylethylene series. Its effects range from complete estrogen antagonism to pure estrogen agonism, depending on its concentration, the sex of the animal, and the target organ. In humans and rats, TAM is predominantly antiestrogenic with residual estrogenic activity (Furr and Jordan, 1984). In adult male rats, it reduces serum luteinizing hormone (LH) and testosterone (T) with no effect on follicle-stimulating hormone (FSH) and prolactin (PRL; Gill-Sharma et al, 1993). The fecundity (siring ability of the male), fertility index (fertilizing ability of spermatozoa), and potency (ability to inseminate) are significantly reduced with exposure to TAM (Gill-Sharma et al, 1993). We inferred that sperm fertilization potential is sensitive to concentrations of androgen in plasma and is impaired on its reduction (Gill-Sharma et al, 1993). T deprivation is known to affect potency of rats (Orgebin-Christ et al, 1975). T may be exerting its effect on mating behavior via estradiol (E_2; Wood, 1996). PRL also plays an important role in the regulation of mating behavior, probably by modulating LH-mediated T synthesis (Untergasser et al, 1996). Hyperprolactinemia in male rats reduces sexual behavior (Hokao et al, 1993; Sobrinho, 1993).

5α-Dihydrotestosterone (DHT) is the active form in which T exerts its physiological action, particularly in stimulating accessory sex glands (Bruchovsky and Wilson, 1968). In normal male rats, 5α-DHT suppresses serum LH and T levels and reduces weights of seminal vesicles, ventral prostate (VP), and pituitary. In castrated rats, DHT inhibits a rise in LH and FSH if steady-state DHT levels are maintained when implanted soon after castration (Parte and Juneja, 1992). Thus DHT is biologically active in the feedback regulation of the hypothalamus-pituitary-testicular axis. Because 5α-DHT is not metabolized to E_2, it is feasible to study the effects of androgen in the absence of the E_2 that would be formed if T were used as a source of androgen. The objective of the present study was to test whether supplementation with 5α-DHT could reverse all or some of the observed effects of TAM and in the process delineate the corrective effect, if any, of supplementation with androgen on the tissue weights and hormonal profile.

Materials and Methods

Animals

Randomly bred male and female Holtzman strain rats were maintained at a temperature of 22°C to 23°C, a humidity of 50%

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to 55%, and a lighting cycle of 14 hours light to 10 hours dark. Commercial rat pellets and water were available ad libitum. Ninety-day-old male rats, each weighing 250 to 280 g, were used. For mating studies, 75-day-old female rats (200–250 g) of proven fertility were used.

Administration of Drugs

5α-DHT (Sigma Chemical Company, St Louis, Mo) was administered in the form of silastic implants. DHT implants were made in silastic medical tubing (0.078-in. inner diameter × 0.0125-in. outer diameter; Dow Corning Corporation, Midland, Mich). The tubes were filled up to 1-cm lengths with 1, 3, or 6 mg DHT. The free end was ligated with a silastic medical-grade adhesive (Dow Corning). The formed capsules were weighed to the nearest microgram and autoclaved. A DHT-containing capsule was implanted aseptically into the right thigh. Controls received an empty capsule in the left thigh. Only 1 capsule was implanted per rat, and the implant was left in situ for 90 days. The release rate of DHT was calculated as previously described (Parte and Juneja, 1992).

TAM citrate tablets containing 10 mg of TAM were obtained from Lyka (Bombay, India). The drug was suspended uniformly in water by sonication and administered daily by mouth between 1000 and 1200 hours via a rat feeding tube.

Experimental Protocol

Adult male rats received 1 of the following treatments: saline alone; 0.2 or 0.4 mg per kilogram of body weight TAM PO per day for 90 days; or 0.2 or 0.4 mg TAM PO until day 50, when they were implanted with 6 mg of DHT until day 90 of TAM treatment. At the end of the treatment, the male rats were allowed to mate with female rats. Another group of adult male rats received 1, 3, or 6 mg DHT implants for 90 days. Rats with 6-mg DHT implants were mated on days 10, 30, 60, and 90 of treatment.

Mating Studies

Mating studies were performed as described by Gill-Sharma et al (1993), and the potency, fecundity, and fertility index were determined. The mating design was either 1 male to 3 females or 1 male to 2 females. Female rats were housed with treated or control male rats in the evening of proestrus for 2 consecutive cycles. The occurrence of mating was confirmed by the presence of a copulatory plug or spermatozoa in the vaginal smear.

Potency—The ability of male rats to inseminate females was expressed as the ratio of female rats inseminated to the number of female rats exposed for mating × 100.

Percentage Fecundity—The measure of the ability of male rats to sire viable pups was expressed as the ratio of the number of males siring at least 1 viable pup to the total number of males exposed for mating × 100.

Fertility Index—The index of the ability of spermatozoa to fertilize ova was expressed as the ratio of the number of implantation sites to the number of corpora lutea (per 2 ovaries).

Autopsy of Animals

The control and treated male rats were killed by decapitation on day 90. The trunk blood was collected and allowed to clot at 4°C overnight. Serum was separated by centrifugation at 800 × *g* for 20 minutes and stored frozen at −20°C for radioimmunoassay of LH, FSH, PRL, T, and E_2. Testes, pituitary gland, and accessory sex organs were collected from each rat and weighed on the Mettler balance.

Table 1. *Effect of tamoxifen (TAM) and + DHT on fecundity, fertility index, and potency**

Medication Groups†	Mating Design, Male × Female	Fecundity	Fertility Index	Potency
Control	1 × 2	100	0.84	100
0.2 mg TAM	1 × 3	40	0.41	100
0.2 mg TAM + 6 mg DHT	1 × 3	20	1	33
0.4 mg TAM	1 × 3	20	0.08	29
0.4 mg TAM + 6 mg DHT	1 × 3	0	. . .	7

* Fecundity = [(no. of male rats siring at least one viable pup)/(no. of male rats exposed for mating)] × 100; fertility index = implantation sites/no. of corpora lutea; potency = [(no. of female rats inseminated)/(no. of female rats exposed for mating)] × 100; DHT indicates dihydrotestosterone; TAM, tamoxifen.

† n = 5 in each group; values are expressed as mean of each group.

Hormone Assays

LH, FSH, and PRL were assayed as described by Balasinor et al (1992). The standard curve for LH (NIADDK-Rat-LH-RP-2) and for FSH (NIADDK-Rat-FSH-RP-2) ranged from 10 pg to 12.5 ng per assay tube, and for PRL (NIADDK-Rat-PRL-RP-3) it ranged from 10 pg to 25 ng per assay tube. The inter- and intra-assay coefficients of variation were 9% and 6% for LH, 10% and 6% for FSH, and 14% and 5% for PRL, respectively. T and E_2 were assayed as described by Juneja et al (1991). The standard curves ranged from 3.9 to 500 pg for T and from 5 to 200 pg for E_2. Inter- and intra-assay coefficients of variation were 11% and 5% for T and 10% and 6% for E_2, respectively.

Statistical Analysis

Hormone concentration and tissue weights were subjected to analysis of variance. Significant difference between groups was determined using Student's *t* test. Data relating to the fertility index, fecundity, and potency were subjected to nonparametric Kruskal-Wallis 1-way analysis of variance.

Results

Effect of TAM and TAM + DHT on Fecundity, Potency, and Fertility Index

TAM at 0.2- and 0.4-mg dose levels reduced the fecundity to 40% and 20%, respectively. With TAM (0.2 mg) plus a 6-mg DHT implant, the fecundity was reduced to 20%. With TAM (0.4 mg) plus a 6-mg DHT implant, the fecundity was 0 (Table 1). Whereas TAM 0.2 mg itself did not affect the potency of the male rats, in combination with DHT, it reduced the potency to 33%; 0.4 mg TAM reduced the potency to 29%. In combination with DHT, the potency was further reduced to 7%.

Table 2. *Temporal effect of 6 mg dihydrotestosterone on fecundity, fertility index, and potency*

Medication Groups*	Mating Design, Male × Female	Fecundity	Fertility Index	Potency
Control	1 × 2	92	0.9	84
Day 10	1 × 3	66.67	0.86	75
Day 30	1 × 3	66.67	0.89	58
Day 60	1 × 3	83.33	0.75	75
Day 90	1 × 3	66.67	0.72	33

* n = 6 in each group; values are expressed as mean of each group.

With TAM (0.2 mg), the fertility index was reduced to 0.41, which was reversed to 1.0 when DHT was implanted in these TAM-treated rats. With TAM 0.4 mg, the fertility index was 0.08, and in combination with DHT, it was 0.

Effect of 6 mg DHT on Fecundity, Potency, and Fertility Index

DHT on average gave a 30% reduction in the fecundity of rats at days 10, 30, and 60 of implantation, whereas the fertility index was not significantly affected (Table 2). Potency was lowered by about 25%. At day 90 of implantation, potency was the most affected, as compared to fecundity and the fertility index.

Effect of TAM and TAM + DHT on Tissue Weights

TAM at 0.2 and 0.4 mg significantly reduced testicular weight to 2.43 and 2.82 g, respectively, compared to the control (3.39 g). When 6 mg DHT was implanted in the TAM-treated rats, the weight of the testis was further reduced to 0.97 and 0.82, respectively, at both the doses of TAM used. This was significant compared to the testicular weight of controls, as well as treatment with TAM alone (Figure 1a). The epididymal weight was significantly reduced at both doses of TAM. Six milligrams of DHT failed to reverse this effect of TAM. DHT implanted to TAM (0.4 mg)-treated rats further reduced the epididymal weight compared to TAM alone (Figure 1b). TAM significantly reduced the weight of seminal vesicles at both the doses studied, either alone or in combination with DHT. However, it was not significantly different when compared to TAM alone (Figure 1c). With TAM 0.2 and 0.4 mg, there was a 59% and 63% reduction in weight of VP compared to that of controls. DHT reverted this effect in the 0.2-mg TAM–treated rats but was unable to overcome the effect of 0.4-mg TAM treatment on the VP weight (Figure 1d). With TAM and TAM + DHT, no effect on pituitary weight was observed (Figure 1e).

Effect of TAM and TAM + DHT on Hormonal Profile

TAM at doses of 0.2 and 0.4 mg significantly reduced LH compared to controls. Implantation of 6 mg DHT to 0.2-mg TAM–treated animals further reduced the LH compared to TAM alone. However, when 0.4 mg TAM was given in combination with 6 mg DHT, LH was reduced significantly compared to control, but not when compared to TAM alone (Figure 2a). TAM at 0.2 and 0.4 mg did not significantly alter serum FSH, although a tendency of FSH to increase was observed. TAM + DHT significantly reduced serum FSH compared to the control and also compared to the respective TAM group (Figure 2b). TAM significantly reduced T at both of the doses studied. DHT given to TAM-treated rats also showed a significant reduction in T compared to controls, but not with respect to TAM-alone group (Figure 2c). TAM had no effect on PRL levels at both the doses studied. TAM + DHT did not show any effect on PRL levels (Figure 2d). TAM alone showed no effect on circulating E_2. DHT administered to 0.2-mg TAM–treated rats significantly increased serum E_2 levels compared to controls. Such an effect of DHT was not observed on the 0.4-mg TAM–treated rats (Figure 2e).

Effect of 5α-DHT on Tissue Weights

Weights of testis, epididymis, seminal vesicles, VP, and pituitary were unaffected with 1 mg DHT implanted for 90 days. With the 3-mg dose, the weights of testis, epididymis, VP, and pituitary, although reduced, were comparable to the control; the weights of seminal vesicles were significantly reduced. Six milligrams of DHT significantly reduced weights of testis, epididymis, seminal vesicles, VP, and pituitary (Figure 3a through e).

Effect of 5α-DHT on Hormonal Profile

Serum LH and FSH was significantly reduced with 3 and 6 mg, but not with 1 mg, of DHT. Whereas serum T was significantly reduced with 3 and 6 mg DHT, PRL was not affected at any of the doses of DHT studied. Circulating E_2 was studied only with the 6-mg DHT dose, and it was significantly reduced compared to control (Figure 4a through e).

Discussion

Our earlier studies had demonstrated an antifertility effect of TAM in the adult male rat (Gill-Sharma et al, 1993). The fecundity, fertility index, and potency (a measure of the presence of an ejaculate) were significantly reduced, with a significant reduction in circulating levels of LH and T. Copulation behavior was apparently not affected, because cohabited female rats showed a constant diestrous phase. It was inferred that sperm fertilization potential is sensitive to concentrations of T in plasma and is impaired on its reduction (Gill-Sharma et al, 1993).

Potency was reduced in TAM-treated male rats. If the reduction in potency were due to T, then supplementation

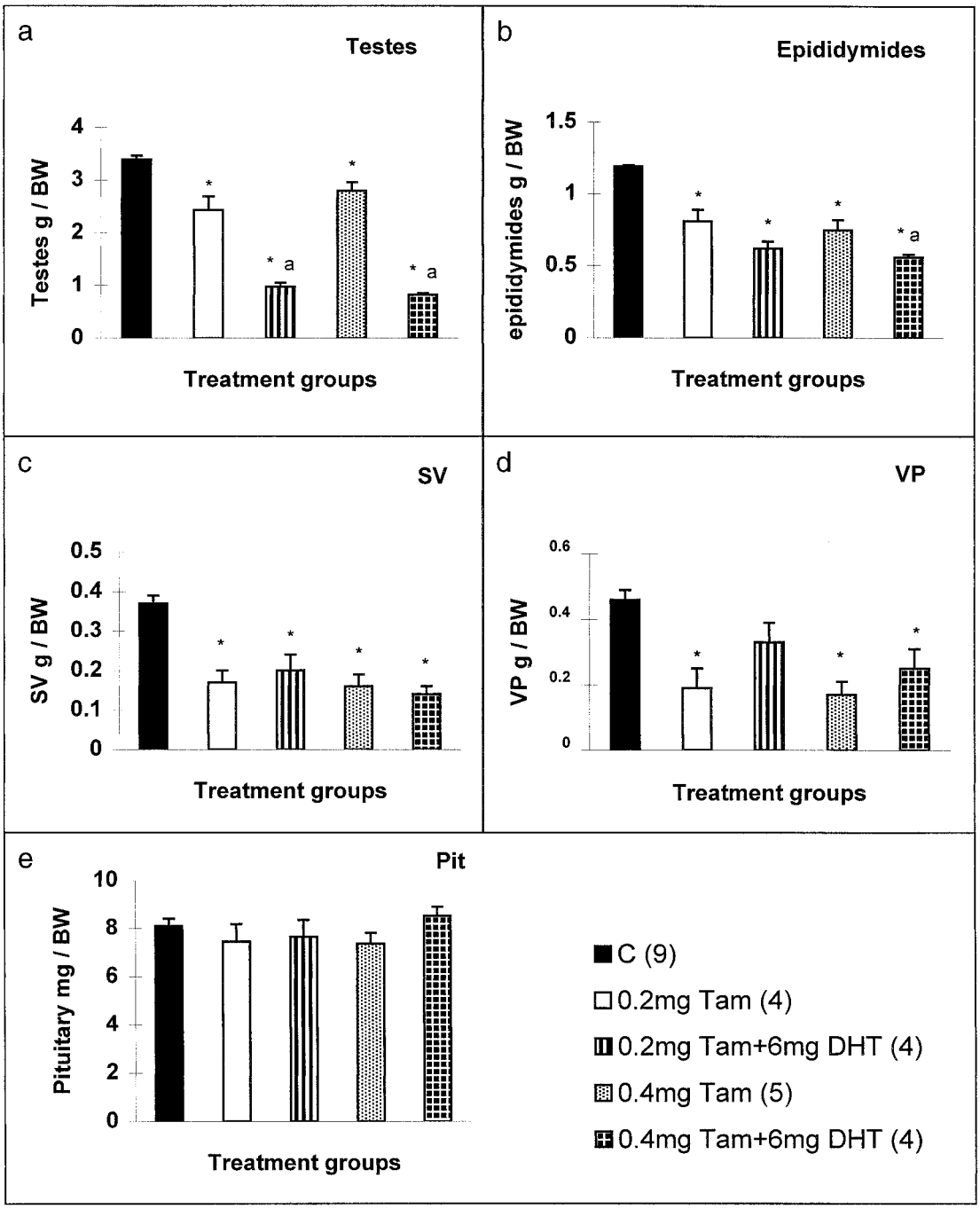

Figure 1. Effect of tamoxifen (TAM) alone or in combination with 5α-dihydrotestosterone (5α-DHT) on tissue weights of **(a)** testes, **(b)** epididymides, **(c)** seminal vesicles, **(d)** ventral prostate, and **(e)** pituitary on day 90 of treatment. Adult male rats received either saline (control) or TAM PO + subcutaneous 5α-DHT implants until day 90. Values are mean ± SEM. *Significant at $P < .05$ compared with control; a indicates significant difference compared with respective TAM-only group. Numbers in parentheses indicate the number of rats in each group.

with androgen should revert the potency to normal. DHT implants did not affect the fertility index and potency of male rats until day 60. TAM at 0.2- and 0.4-mg dose levels reduced the fertility index to 0.41 and 0.08, respectively. Supplementation with DHT implants for 40 days corrected the fertility index in 0.2 mg–treated rats but failed to overcome the effect on 0.4-mg TAM–treated rats. In fact, it reduced the fertility index nearly to 0. This suggests that the DHT 6-mg implant was able to correct the effect of 0.2 mg TAM on fertility index but was not effective against 0.4 mg TAM. Probably DHT at a higher dose could have reversed the effect of TAM at the 0.4-

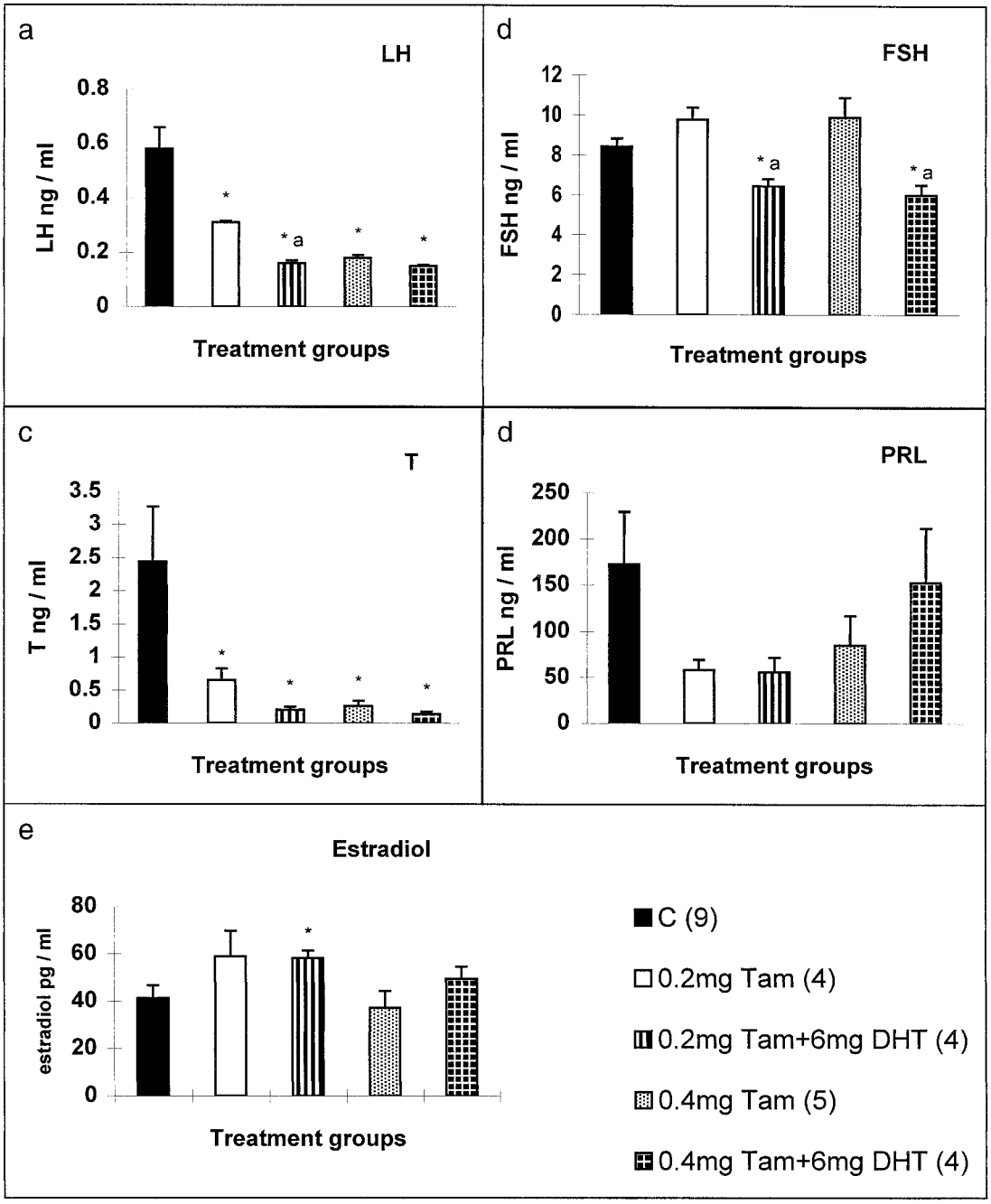

Figure 2. Effect of tamoxifen (TAM) alone or in combination with 5α-dihydrotestosterone (5α-DHT) on serum levels of **(a)** luteinizing hormone (LH), **(b)** follicle-stimulating hormone (FSH), (c) testosterone (T), **(d)** prolactin (PRL), and **(e)** estradiol (E_2) on day 90 of treatment. Adult male rats received saline as a control, TAM PO, or TAM PO + subcutaneous 5α-DHT implants until day 90. Values are mean ± SEM. *Significant at $P < .05$ compared to control; a indicates significant difference compared with respective TAM-only group. Numbers in parentheses indicate the number of rats in each group.

mg dose level. We note that DHT per se at day 60 of implantation did not have an adverse effect on fecundity and potency and reduced the fertility index to 0.75. However, although DHT did improve the sperm fertilizing potential of the rats treated with 0.2 mg TAM, it did not improve the potency and fecundity but further reduced it in these rats. In rats treated with 0.4 mg per day TAM, as the potency and fecundity were drastically reduced, DHT was unable to counteract this effect of TAM, and instead there was a complete knockout of fecundity and a further reduction in potency.

These data are intriguing because E_2 is known to affect

sexual behavior in male rats (Sodersten et al, 1980; Sinchak et al, 1996; Wood, 1996), as is PRL (Hokao et al, 1993; Sobrinho, 1993), and with TAM, neither PRL nor E_2 levels were altered. Does this imply that T itself is responsible for reducing the potency in these animals? TAM + DHT in combination drastically reduced the potency and fecundity of the male. With TAM + DHT treatment, both PRL and E_2 were unaffected, whereas LH and T were significantly reduced. Both models confirm that T is important for the ability of the male to inseminate the female (potency). T alone or along with TAM restored the potency as well as fecundity and fertility (data not shown).

The effect on weights of testis and accessory sex organs of adult male rats was studied as a preliminary indicator of whether the drugs were effective and the implants functional. The effects of TAM and 6 mg DHT individually on the weights of testis, epididymis, seminal vesicles, and VP were similar (Figures 1 and 3). In combination, a synergistic effect was observed. On treatment with TAM, DHT, or both, a significant reduction in the weights of seminal vesicles and VP was observed (Figure 1c and d; Figure 3c and d). A rise in serum DHT levels in castrated rats implanted with DHT resulted in an increase in weight of VP (Parte and Juneja, 1992). In intact rats, although steady-state levels of DHT were maintained with implants, this exogenous DHT was converted rapidly to 5α-androstane 3α-17βdiol, 5α-androstane 3β-17βdiol, and triols, respectively (Martini, 1982). Triols are dead-end products of T and are ineffective as androgens. Thus, treatment of intact rats with DHT results in a reduction of weight of VP. These data are in agreement with our earlier observations (Parte and Juneja, 1992) and also with the data of Mittler et al (1981) using doses of 18 μg DHT per day. We note that despite a severe reduction in T, 6 mg DHT restored the weights of the VP in the 0.2-mg TAM–treated rats but failed to do so in the presence of 0.4-mg TAM. Supplementation of T provides the surplus T required to be metabolized to DHT, which is the active form of androgen in the prostate (Luke and Coffey, 1994), in the TAM-treated rats, thereby restoring the weights of seminal vesicles and VP in these rats (unpublished data); 1 and 3 mg DHT did not alter pituitary weight, but a dose of 6 mg for 90 days significantly reduced weights of the pituitary (Figure 3e). Both doses of TAM, either alone or in combination with DHT, had no effect on pituitary weight (Figure 1e).

The effect of these drugs on the hormonal profile was interesting. LH and T were significantly reduced with TAM and also with TAM + DHT. But E_2 was unaltered with TAM and significantly increased with 0.2 mg TAM + DHT, but not with 0.4 mg TAM + DHT (Figure 2a, c, and e). This finding indicates that TAM probably has an estrogenic effect and acts directly at the pituitary level to reduce LH, leading consequently to a reduction in circulating T. Reduction in T activates aromatase, resulting in E_2 levels' either increasing or being maintained in these animals. Thus, although circulating DHT is present in steady-state levels (Parte and Juneja, 1992), we still see high or normal circulating E_2 levels.

Although LH and T are significantly reduced in DHT-treated rats, so is E_2 (Figure 4a, c, and e). Here DHT probably has a direct effect on the testis, thereby reducing T, which in turn reduces LH. The direct effect of DHT may also be responsible for suppressing aromatase, hence the decrease in E_2. Aromatase is known to be regulated by T and DHT (Roselli and Resko, 1984). The activity of this enzyme decreases with increasing androgen concentration (Payne et al, 1987). This result explains the significant reduction of E_2 with 6 mg DHT. When DHT is administered to TAM-treated rats, it has been unable to counteract the estrogenic effect of TAM (Figure 2e). It is important to remember that although DHT alone was implanted for 90 days, TAM-treated rats received DHT for 40 days.

FSH was unaffected with TAM, although a tendency of FSH to increase was observed. Earlier studies with intact male rats (de Jong et al, 1975) and castrated rats (Kalra et al, 1973; Swerdloff et al, 1973) have shown that E_2 suppresses release of both LH and FSH. TAM, a synthetic nonsteroidal antiestrogen, seems to have its own mechanism of action. DHT at 3 and 6 mg significantly reduced serum FSH compared to the control (Figure 4b). DHT administration to TAM-treated rats significantly reduced FSH compared to control and also compared to the respective TAM group (Figure 2b). This finding indicates that the effect observed on FSH is a DHT effect and that TAM did not contribute in any way to this effect.

In summary, analysis of the data suggests that androgen is the important factor responsible for the fertilizing ability of sperm and for the ability of the male to inseminate and sire viable pups. However, other factors besides androgen are also required for the ability of the male to inseminate and sire viable pups. The role of PRL and E_2 seems dubious, as in this study neither PRL nor E_2 was altered with TAM or TAM + DHT. Or is it that the threshold levels of androgen required for fertilizing ability may be much less than those required for the potency and fecundity in rats?

Another interesting observation that surfaced was the differential effect of TAM on LH and FSH. Although TAM was estrogenic on LH, it had no effect on FSH. Since TAM is a synthetic nonsteroidal antiestrogen with partial agonistic action (Furr and Jordan, 1984), it would be expected that TAM would either increase (antiestrogenic) or decrease (estrogenic) plasma FSH. The inability of TAM to do so warrants further investigation.

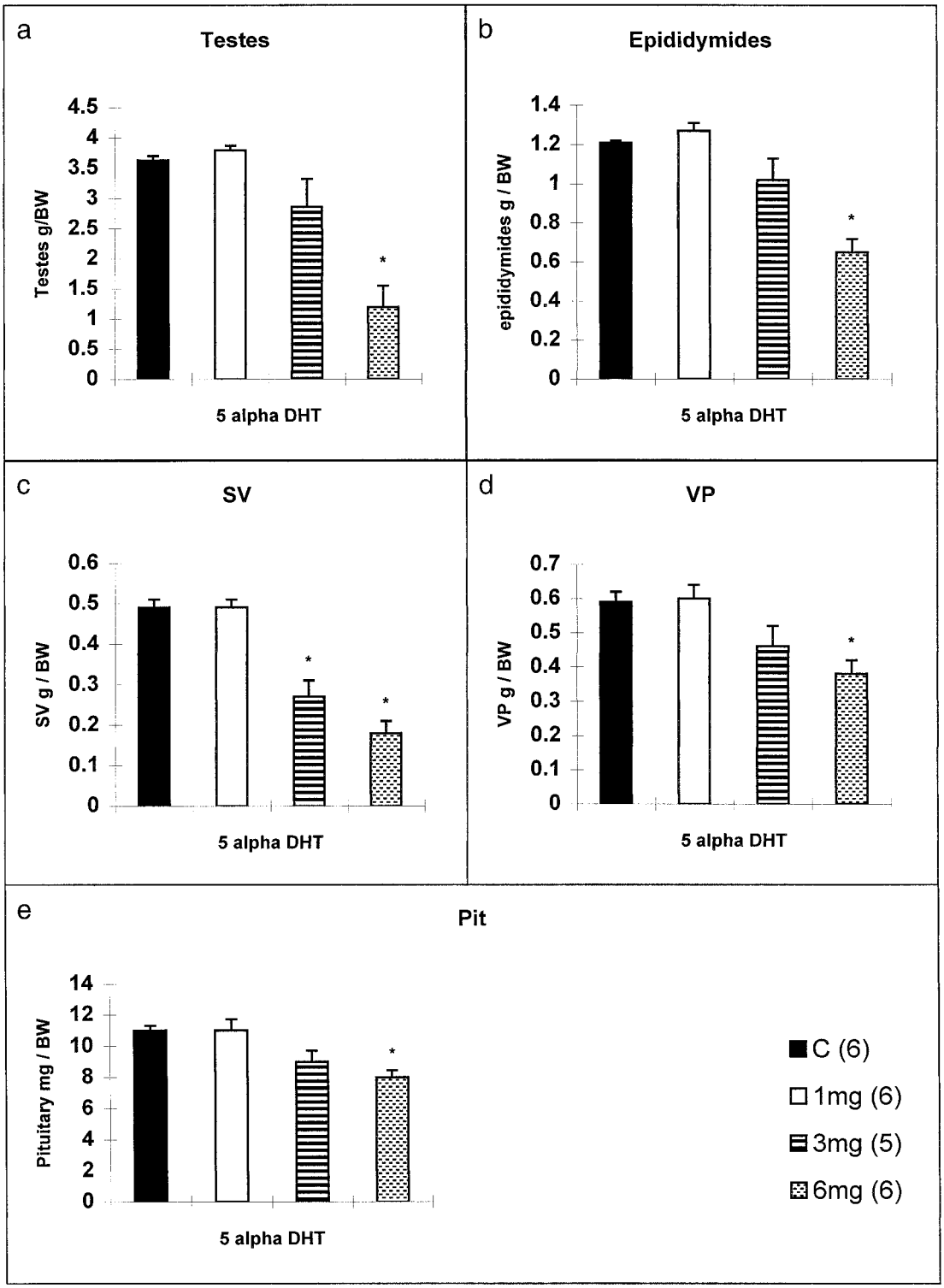

Figure 3. Effect of 5α-dihydrotestosterone (5α-DHT) on tissue weights of **(a)** testes, **(b)** epididymides, **(c)** seminal vesicles, **(d)** ventral prostate, and **(e)** pituitary on day 90 of treatment. Adult male rats were implanted subcutaneously with either empty, 1-, 3-, or 6-mg 5α-DHT implants until day 90. Values are mean ± SEM. *Significant at $P < .05$ compared to control. Numbers in parentheses indicate the number of rats in each group.

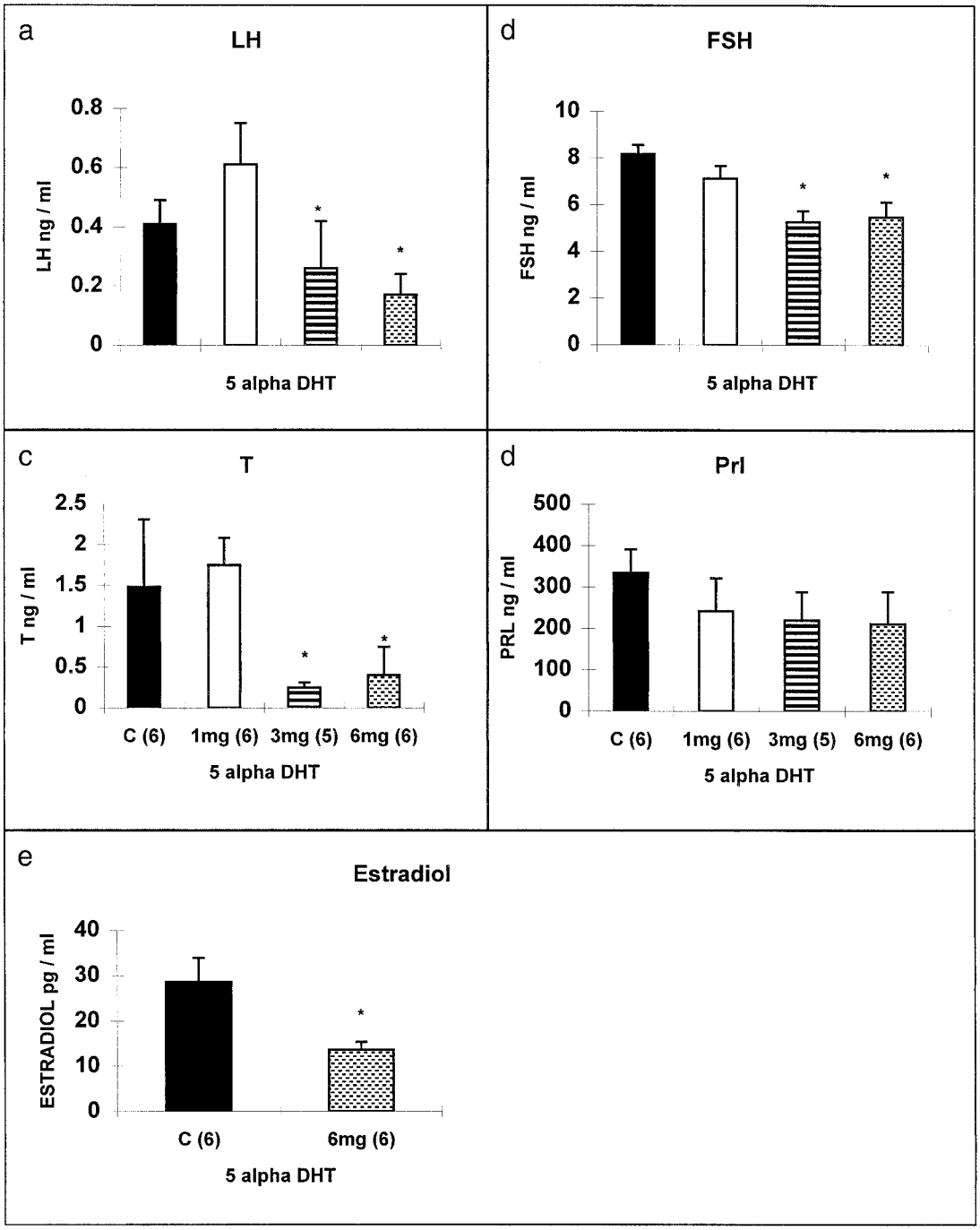

Figure 4. Effect of 5α-dihydrotestosterone (5α-DHT) on serum levels of **(a)** LH, **(b)** FSH, **(c)** T, **(d)** PRL, and **(e)** E_2 on day 90 of treatment. Adult male rats were implanted subcutaneously with either empty, 1-, 3-, or 6-mg 5α-DHT implants until day 90. Values are mean ± SEM. *Significant at $P < .05$ compared to control. Numbers in parentheses indicate the number of rats in each group.

The effect of TAM on LH appears estrogenic; that is, circulating levels of LH are reduced. In our observations, DHT also reduced circulating levels of LH. T is known to negatively regulate circulating levels of LH. Our study implies that both DHT and E_2 negatively regulate gonadotropins. If T alone can produce the same effect, then under physiological conditions, why is there a need for DHT and E_2 to act on the gonadotrophs? Do DHT and E_2 act via separate mechanisms to regulate gonadotropins, or do they act via a mechanism similar to T? Or are the effects of T mediated by DHT, E_2, or both? This issue needs further investigation.

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