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The Effect of Tamoxifen on the Reproductive Traits in White Leghorn Cockerels

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ROZENBOIM, I., O. DGANY, B. ROBINZON, E. ARNON AND N. SNAPIR. *The effect of tamoxifen on the reproductive traits in White Leghorn cockerels.* PHARMACOL BIOCHEM BEHAV 32(2) 377–381, 1989.—Fifty White Leghorn male chicks were divided into five equal groups of ten chicks each. Beginning at two weeks of age they were injected on each alternate day as follows: corn oil as a vehicle control or 0.5, 1.0, 5.0, or 10.0 mg tamoxifen/kg/b.wt. The whole experimental period lasted until twelve weeks of age. The two lowest doses of tamoxifen (TAM) enhanced comb growth, while the highest dose suppressed it. The two lowest doses of TAM also caused an earlier increase in sexual activity of the chicks, and precocious production of semen. At nine weeks of age the 0.5 and 1.0 mg doses of TAM increased plasma testosterone to a level three times higher than in the controls. This effect was not observed with the highest dose of TAM. At 12 weeks of age the chicks treated with 1 mg TAM had larger testes than the controls and produced three times more sperm per ejaculation. At this stage chicks treated with the highest dose of tamoxifen produced less sperm than the control and had smaller testes and adenohypophyses.

Chicken Mating behavior Tamoxifen Semen Testosterone

ESTROGEN, secreted by the ovary in the female, or produced by aromatization of testosterone in the male, is considered as the main blocker of gonadotropins secretion, acting at the central nervous system (CNS) and the pituitary (11).

Compounds that reduce or alleviate the physiological effects of hormones or drugs can be used as tools to probe the cellular processes underlying agonist-induced responses (8). Antisteroids are competitive inhibitors of steroids binding to target tissues or organs. The most popular antiestrogen at this time is tamoxifen (TAM).

TAM is a trans isomer of a triphenylethylene (11) and was found to have a wide spectrum of activities in animals, depending on the species in which it was assayed. In the rat uterotropic assay TAM was found to have some agonistic and some antagonistic effects on estrogenic functions. In a similar assay in the mouse, TAM was found as a pure estrogen antagonist. Similarly, in the chicken oviduct, TAM was characterized as a pure antiestrogen (18). TAM is routinely used for suppression of human estrogen-responsive breast cancer (13), for induction of ovulation in anovulatory women (20), and to increase sperm count in oligospermic men (21).

It was postulated that TAM functions as a nonsteroidal antiestrogen by competition with estrogen on its binding sites. Thus, at the hypothalamus and the pituitary gland, TAM reduces the ability of the sex steroids to inhibit the gonadotropin secretion and, via increase in their release, enhances gonadal activity (11,20).

TAM administration to juvenile male broiler chicks (White Rock × White Cornish) significantly increased testes weight and enhanced the spermatogenesis process (14). White Leghorn (WL) male chicks treated similarly produced semen at nine weeks of age which, following insemination, resulted in normal descendants (15).

Precocious sexual puberty, as well as increase in sperm count in ejaculate, are both of great economic value in aves farming. The present study assayed the rate of gonadal system and sexual behavior maturation, as affected by TAM administration to juvenile WL male chicks.

METHOD

Experimental Animals

Forty-five male WL chicks were kept in a brooder until 4 weeks of age and then moved into individual cages for the rest of the experimental period (age of 12 weeks). During the whole experimental period they were illuminated by 40-W tungsten light bulbs providing 14 hr light in a 24-hr cycle. The chicks were provided feed and water ad lib.

Experimental Procedure

At two weeks of age, the chicks were divided into five groups and were injected IM, each alternate day, as follows: 1) 0.5 mg TAM/kg/b.wt.—Tam-0.5. 2) 1.0 mg TAM/kg/b.wt.—

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TABLE 1

AVERAGE BODY, COMB, TESTES, AND ADENOHYPOPHYSIAL WEIGHTS, SEMEN VOLUME, SPERMATOCRIT, AND TOTAL SPERMS PER EJACULATE (MEAN ± SEM), AND PLASMA TESTOSTERONE (MEAN AND RANGE) OF COCKERELS OF THE VARIOUS EXPERIMENTAL GROUPS

Treated Group n	Tam-0.0 11	Tam-0.5 8	Tam-1.0 7	Tam-5.0 9	Tam-10.0 10
Body weight (g)	1139 ± 36^{a,b}	1008 ± 30^{c}	1177 ± 44^{a}	1214 ± 32^{a}	1063 ± 31^{b,c}
Comb weight (g)	22.3 ± 3.4^{a,b}	23.8 ± 2.6^{a,b}	28.0 ± 2.7^{a}	21.3 ± 2.9^{a,b}	16.1 ± 2.5^{b}
Testes weight (g)	5.8 ± 1.2^{b}	6.2 ± 0.8^{a,b}	9.6 ± 0.9^{a}	7.4 ± 1.3^{a,b}	3.9 ± 0.9^{b}
Adenohypophysial weight (mg)	12.6 ± 1.0^{a}	12.0 ± 1.0^{a,b}	10.0 ± 1.0^{a,b}	10.3 ± 1.0^{a,b}	9.7 ± 1.0^{b}
Semen volume (ml)	0.21 ± 0.04^{a,b,c}	0.26 ± 0.04^{a,b}	0.31 ± 0.03^{a}	0.16 ± 0.03^{b,c}	0.105 ± 0.02^{c}
Spermatocrit (%)	2.61 ± 0.74^{b,c}	3.12 ± 0.51^{b}	5.71 ± 0.83^{a}	2.58 ± 0.61^{b,c}	1.1 ± 0.31^{c}
Total sperm per ejaculate (millions)	212.2 ± 60^{b}	316.6 ± 90^{b}	678.9 ± 110^{a}	159.8 ± 60^{b,c}	44.7 ± 20^{c}
Testosterone (pg/ml)	310 (130–900)	1060* (720–2500)	1170* (270–3100)	722 (110–2400)	290 (40–900)

^{a,b,c}—Figures which are not marked by the same letter are significantly different from each other ($p<0.05$).
*Significantly different from the control ($p<0.05$).

Tam-1.0. 3) 5.0 mg TAM/kg/b.wt.—Tam-5.0. 4) 10.0 mg TAM/kg/b.wt.—Tam-10.0. 5) 0.1–1.0 ml (according to age) of the corn oil vehicle—Tam-0.0.

Body weight (b.wt.) was measured once a week from hatching to twelve weeks of age. Comb factor (9) was measured weekly from three to twelve weeks of age. Hematocrit was determined weekly, from seven to twelve weeks of age. In order to monitor the development of sexual behavior, each male was placed twice a week for a two-min period, in a 2×3 m room with a female of the same age and the number of mating attempts was counted. Attempts to ejaculate the cockerels by the abdominal massage procedure (7) were carried out twice a week from seven weeks of age and on. The response to the massage procedure was graded from 0 to 8 as follows: 0—no erection of the phallus, 1—erection with no fluid, 2—erection with secretion of fluid only, 3–8—secretion of semen with increasing amount of spermatozoa.

At 12 weeks of age semen volume and sperm concentration (19) were measured, and total sperm cells/ejaculate was calculated (12).

Heparinized blood samples, for testosterone assay, were drawn from the brachial vein at 9 weeks of age.

Autopsy Procedure

At 12 weeks of age the cockerels were killed by cervical dislocation. The comb, testes and adenohypophysis were immediately removed, cleaned of adhering tissues and weighed.

Radioimmunoassay for Testosterone

All samples were tested in a single assay using a kit (Diagnostic Products Corporation, USA). The specific binding of the kit's antibody as defined by the supplier were 100% testosterone, 34% 5α-DHT and 5β-DHT, 4% 5β-androstan-3β, 17β-diol, 3.3% 11-hydroxytestosterone, 2.9% 5α-androstan-3α, 17β-diol, 2.7% 5α-androstan-3β,17β-diol, 2.1% androsterone. The intraassay variance was 3.6% and the sensitivity of the test was 5 pg/tube.

Statistical evaluation of the data was made using ANOVA and Duncan's Multiple Range Test (6). The results of the RIA were tested in a nonparametric Mann Whitney U-test (17).

RESULTS

There was a slight inhibition in growth rate in the Tam-0.5 and the Tam-10.0 cockerels (Table1).

Comb growth (Fig. 1) was initially accelerated in the cockerels receiving the two lowest doses of TAM. However, following the seventh week of age it remained so only in the TAM-1.0 birds. The highest dose of TAM (Tam-10.0) reduced comb growth from the fifth week of age and on (Fig. 1, Table 1).

The two lowest doses of TAM significantly enhanced the response to the abdominal massage procedure (Fig. 2). The first observation of sperm cells in the ejaculate was made at 8 weeks of age in 3 cockerels of the Tam-0.5 group. At nine weeks of age, 50% of the ejaculate samples collected from the Tam-0.5 and Tam-1.0 groups contained sperm cells. The highest dose of TAM suppressed semen production (Fig. 2, Table 1) and most of the Tam-10.0 birds did not show any sperm production until the end of the experimental period.

TAM at the two lowest doses caused an early increase in mating activity (Fig. 3). In the Tam-0.5 cockerels, this activity decreased to meet that of the controls, at the ninth week of age, but the Tam-1.0 cockerels manifested higher sexual activity until the end of the experiment. The two highest doses of TAM suppressed sexual activity and the Tam-10.0 cockerels did not attempt to mate at all.

The hematocrits of cockerels treated with the lowest dose of TAM increased before that of the rest of the birds (Fig. 4). However, towards the end of the period in most birds, except Tam-10.0, hematocrits increased to a similar level. In the latter hematocrits were significantly depressed.

At nine weeks of age, the two lowest doses of TAM increased plasma testosterone at about three-fold over that of the controls. In the Tam-5.0 birds there was also some, but not significant, increase in plasma testosterone (Table 1).

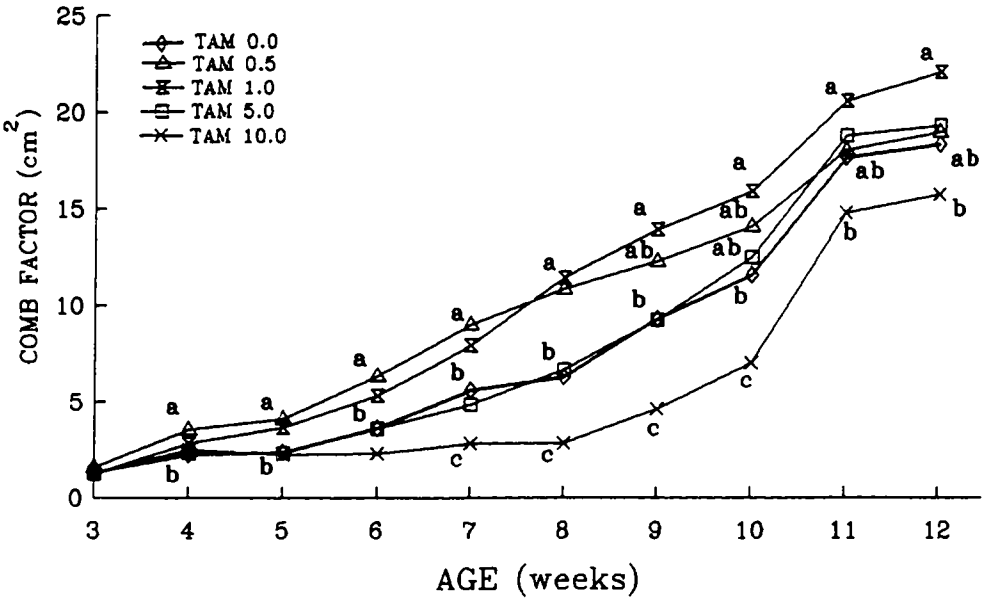

FIG. 1. Weekly change in mean comb factor of the Tam-0.0, Tam-0.5, Tam-1.0, Tam-5.0 and Tam-10.0 chicks. a, b, c—Values having different letters are significantly different ($p<0.05$).

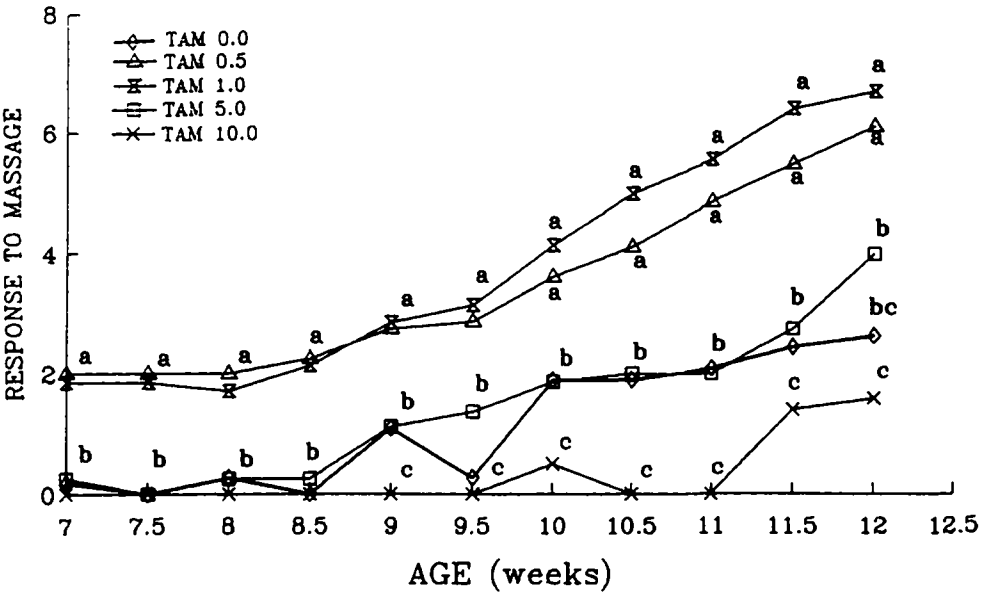

FIG. 2. Mean response to the abdominal massage procedure, from seven weeks of age and on, Tam-0.0, Tam-0.5, Tam-1.0, Tam-5.0 and Tam-10.0 chicks. a, b, c—Values having different letters are significantly different ($p<0.05$).

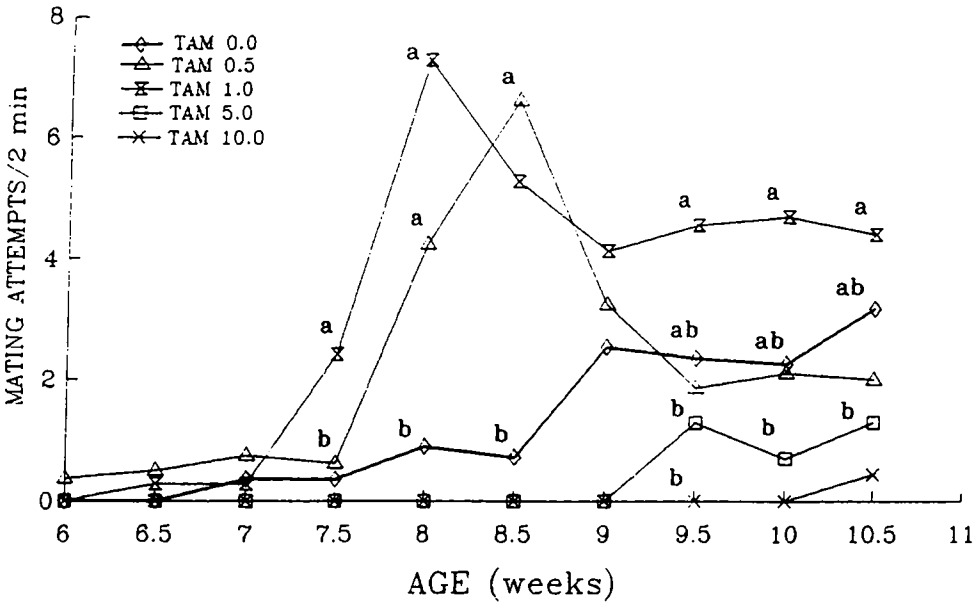

FIG. 3. Mean mating attempts/2 min, from six weeks of age and on, in Tam-0.0 Tam-0.5, Tam-1.0, Tam-5.0 and Tam-10.0 chicks. a, b—Values having different letters are significantly different ($p<0.05$).

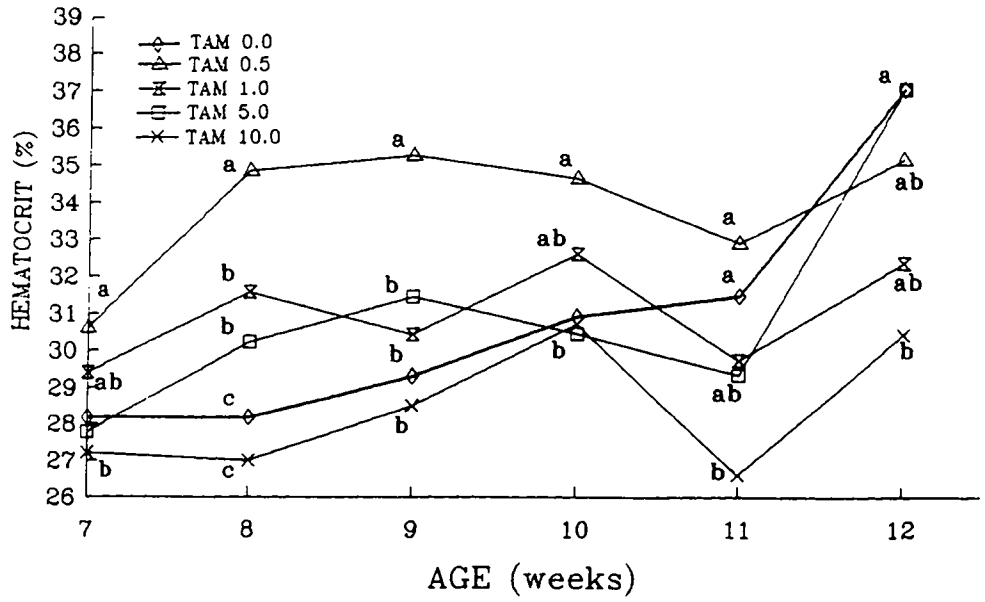

FIG. 4. Weekly mean hematocrit values, from seven weeks of age and on, in Tam-0.0, Tam-0.5, Tam-1.0, Tam-5.0 and Tam-10.0 chicks. a, b, c—Values having different letters are significantly different ($p<0.05$).

Tam-1.0 increased testes, and comb weights, semen volume, spermatocrit, and total sperm per ejaculate (Table 1). Most of these parameters and the adenohypophysial weight were reduced in the Tam-10.0 cockerels.

DISCUSSION

The present experiment suggests that administration of low doses of TAM to young cockerels induces precocious semen production, increased plasma testosterone, hematocrit, and testes weight, enhanced comb growth and precocious mating behavior. Similarly, in oligospermic men, administration of this antiestrogen enhanced spermatogenesis and increased plasma testosterone and LH (16).

Testosterone increases mating behavior in young chicks (10). Plasma testosterone was elevated in those chickens treated with the low doses of TAM, where mating activity rose as well. It was suggested that it is the estrogen that triggers the hypothalamic activation of mating behavior in cockerels (10) and that testosterone increases mating only following its aromatization to estrogen. In accordance with this hypothesis, antiestrogen such as CI-628 and aromatase inhibitor suppressed copulation in castrated male quail treated with testosterone (1,2). However, in the present study, low doses of TAM increased sexual activity. One can argue that at these doses, although TAM increases testicular functions, it is insufficient to block neural estrogen receptors involved in the triggering of mating activity. Thus, the increased plasma testosterone should, by its aromatization, lead to the activation of the estrogen-dependent neural mechanism that initiates mating behavior. It is also possible that TAM at these low doses acts as an estrogen agonist in this neural system. On the other hand, these low doses of TAM did activate the gonadal system, presumably due to their antiestrogenic effect in the neural system sites involved in the regulation of gonadotropin secretion. At the present time there is no reason to speculate that these neuronal components react differently to TAM than those involved in triggering of mating activity. Thus, the increased mating activity under the antiestrogen supplementation may suggest it to be the direct result of the rise in plasma testosterone induced by this substance. In that case, it may be assumed that in the cockerel mating activity may be induced by a direct neuronal action of testosterone or its androgenic metabolites. This suggestion is supported by the following observations: 1) In the chicken hypothalamus the level of 5β-reduction of testosterone is high (4). In juvenile male chicks, 5β-dihydrotestosterone, which cannot be aromatized, elicited sexual behaviors (4). 2) Flutamide is an antiandrogen that does not affect estradiol binding to its receptors. Still, flutamide moderately reduced the reproductive behavior of castrated quails treated with testosterone (3). 3) The synthetic androgen, methyltrienolone (R1881), is not metabolized in target tissues and binds only to the androgen but not to the estrogen receptors. In castrated male quails, R1881 did trigger sexual behavior to the same extent as testosterone (5).

The high doses of TAM suppressed gonadal activity as well as sexual behavior. By these, TAM mimics the effects of high doses of estradiol. Thus, estrogen agonistic activity for high doses of TAM may be suggested.

The present study and previous findings (14,15) suggest that precocious sexual puberty can be achieved in young cockerels by supplementation of TAM at low doses. The economic value of this effect should be evaluated.

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