# The Effect of Steroids on the Follicle Stimulating Hormone (FSH) Content of Chicken Anterior Pituitary Glands

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#### Abstract

A variety of steroids were administered to cockerels and capons to note their effect on anterior pituitary gland weight and FSH content. Short-term experiments with capons indicated that FSH content was increased by testosterone propionate (TP), decreased by progesterone, while androstenedione, dehydroisoandrosterone, dexamethasone, pregnenolone, androstenediol, and small amounts of estradiol had little if any effect. Birds injected with TP for a 40-day period after caponization had pituitary weights similar to cockerel controls but the FSH content was still high above normal. Results indicate that testosterone alone does not control FSH pituitary content in the male bird.

The negative feedback relationship between the gonads and the anterior pituitary gland is still incompletely understood despite many years of experimentation. This is particularly true in the case of the male. In addition, the majority of studies have been done with mammals; therefore, even less is known of the system in the non-mammalian vertebrates. The purpose of this paper is to attempt to answer the question whether testosterone alone is responsible for the control of pituitary FSH content and weight in the male bird.

## **Materials and Methods**

The single comb White Leghorn chickens used in these experiments were obtained when 1 day old from the Indianapolis, Indiana, Farm Bureau Co-op. The female rats used to assay pituitary FSH content were obtained when 21 days old from the Holtzman Company, Madison, Wisconsin. The experimental procedure was essentially as follows. Caponization was performed before the birds were 2 weeks old and the capons and intact controls were then grown to the desired age for an experiment. The birds were then injected with various steroids. Testosterone propionate was a gift from the Schering Corporation, estradiol dipropionate a gift from CIBA Pharmaceutical Corporation, and the dexamethasone a gift from the Merck Corporation. The other steroids were purchased from Nutritional Biochemicals. All of the steroids were carried in sesame oil and injected subcutaneously in 0.1 cc amounts. At the end of the injection period the animals were killed and the pituitary glands quickly removed, weighed, and then homogenized in cold distilled water. The homogenate was centrifuged at 4°C, washed, recentrifuged and the supernatant frozen until assayed for its FSH activity. The FSH activity of the chicken pituitary glands was determined by means of the human chorionic gonadotropin (HCG) augmentation assay (4). The HCG was generously supplied by Dr. J. B. Jewell of the Ayerst Laboratory. That chicken pituitaries can be assayed by this method for their FSH activity was shown in an earlier report (5). At least 10 mg equivalent fresh pituitary material was given to each

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assay rat. Usually, 20 to 30 mg was administered. In any one experiment, however, all rats received the same amount. The end-point of the FSH assay was ovarian weight, *i.e.*, the more FSH the higher the weight. Appropriate known FSH controls were run with each assay but only the data from the chicken pituitaries are presented in the tables. The FSH was a gift from the NIH Endocrinology Study Section.

### Results

The response of cockerels and capons of several age groups to testosterone propionate (TP) is shown in Table 1. It can be seen that short term treatment in the cockerel resulted in no change in pituitary weight but a decrease in FSH content as noted by the lighter weight ovaries of the assay animals. In the capon there was some decrease in pituitary weight but increased FSH content. Table 2 presents data showing the effect of TP treatment and withdrawal on FSH content over a 2-week period. Within 2 days of TP treatment there was an increase in pituitary FSH. This continued to a peak at day 4 and was maintained through day 14. Withdrawal of TP quickly resulted in a decrease in pituitary FSH and within 7 days the level was back to that of the original untreated capons. There were no significant differences in pituitary weights between the groups. These results are similar to those obtained in the male rat (2).

	Comb		Pituitary		Rat FSH Assay
Treatment	Ν	g	mg	Ν	Ovary mg ± SE
Testosterone propionate (TP)					
82-day-old birds.					
Cock controls	46		8.7	6	$102 \pm 10$
$Cock + 400 \mu g TP x 5$	53		8.2	$\overline{5}$	$68 \pm 2^*$
Capon controls	22		13.8	6	$83 \pm 5$
Capon + 400 $\mu$ g TP x 5	<b>39</b>		11.9	6	$190 \pm 18^{**}$
105-day-old birds.					
Cock controls	18	3.23	9.3	9	$59 \pm 3$
Capon controls	15	0.60	15.3	9	$69 \pm 5$
Capon + 200 $_{\mu}$ g TP x 8	<b>14</b>	3.41	12.8	7	$118 \pm 5^{**}$
115-day-old birds.					
Cock controls	15		9.9	7	$56 \pm 4$
$Cock + 200 \mu g TP \ge 8$	14		9.8	7	$43 \pm 2$
Capon controls	8		18.1	8	$39 \pm 4$
Capon + 200 $\mu$ g TP x 4	7		14.8	7	$56 \pm 4^{**}$
Capon + 200 $\mu$ g TP x 8	8		12.8	8	$79 \pm 2^{**}$
Testosterone precursors. 55-day-	old bir	ds.			
Cock controls	46	$5.40 \pm .47$	$6.5 \pm .1$	8	$68 \pm 4$
Capon controls	26	$0.56 \pm .05$	$9.8 \pm .3^{**}$	6	$144 \pm 13^{**}$
Capons + 500 $\mu$ g steroid x 1	0 day	5.			
Androstenedione	27	$0.93 \pm .03^{**}$	$9.5 \pm .3$	8	$122 \pm 8$
Dehydroisoandrosterone	28	$0.75 \pm .06^{**}$	$10.2 \pm .4$	8	$111 \pm 5^*$
Androstenediol	<b>31</b>	$0.64 \pm .04$	$12.1 \pm .4^{**}$	9	$121 \pm 3$
Pregnenolone	29	$0.59 \pm .04$	$8.7 \pm .3^{**}$	8	$147 \pm 8$

 
 TABLE 1. The effect of testosterone propionate and testosterone precursors on cock and capon comb weight and anterior pituitary weight and FSH content.

Significance levels: \* 5%; \*\* 1%.

Because testosterone alone did not appear to return capon FSH pituitary levels to that seen in the intact chicken, the possibility that other steroids might affect FSH content was studied. This included the female sex hormones and some metabolic precursors of testosterone such as pregnenolone, androstenediol, androstenedione, and dehydroisoandrosterone. The latter two are also secretory products of the testis (1). In Table 1 it can be seen that only these two compounds had any androgenic effect as noted by the comb response. Only dehydroisoandrosterone had any effect in reducing FSH content toward the normal cockerel level.

Testosterone		Pituitary	Rat FSH Assay	%	
Treatment, days	Ν	mg	Ν	Ovary mg $\pm$ se	Increase
Capon controls	5	16.8	6	$40 \pm 2$	
200 <sub>µ</sub> g x 2	5	16.1	5	$58 \pm 3$	48
200 µg x 4	5	16.4	6	$97 \pm 7$	148
200 µg x 7	5	15.6	5	$81 \pm 4$	104
$200 \ \mu g \ge 14$	5	17.6	6	$74 \pm 8$	88
200 µg x 7, off 2	5	17.1	6	$52 \pm 5$	32
200 µg x 7, off 4	5	14.5	6	$54 \pm 4$	37
200 µg x 7, off 7	5	16.2	5	$45 \pm 1$	13

 TABLE 2. The effect of testosterone propionate on the pituitary FSH activity of 109-123

 day old capons.

The effects of estradiol and progesterone on the pituitary gland are presented in Table 3. In large amounts, estradiol dipropionate (ED) decreased pituitary weight and FSH content when given alone or in combination with TP. Progesterone decreased FSH content, a situation opposite to that reported in the mammal (3).

It is possible that the long time interval between caponization and the experimental period results in a system that is less sensitive to

 
 TABLE 3. The effect of estradiol, progesterone, and estradiol plus testosterone on chicken pituitary weight and FSH content.

Pituitary			Rat FSH Assay	
Ν	mg	N	Ovary mg $\pm$ se	
orogesterone, (	32-day-old birds	3.		
37	6.1	7	$78 \pm 5$	
16	8.1	6	$128 \pm 6^{**}$	
19	7.8	5	$115 \pm 14$	
19	8.0	6	$106 \pm 8$	
20	7.0	6	$61 \pm 5^{**}$	
18	10.0	5	$84 \pm 7^{**}$	
erone propion:	ate (TP), 68-da	y-old capo	ns.	
13	11.6	6	$78 \pm 9$	
13	9.2	4	$135 \pm 8**$	
15	9.7	6	$75 \pm 5^{**}$	
16	9.5	6	$78 \pm 5^{**}$	
	progesterone, 6 37 16 19 20 18 erone propiona 13 13 15	N         mg           progesterone, 62-day-old birds         37         6.1           16         8.1         19         7.8           19         7.8         19         8.0           20         7.0         18         10.0           erone propionate         (TP), 68-de         13         11.6           13         9.2         15         9.7	N         mg         N           progesterone, 62-day-old birds.         37         6.1         7           37         6.1         7         16         8.1         6           19         7.8         5         5         19         8.0         6           20         7.0         6         6         18         10.0         5           erone propionate (TP), 68-day-old capould capould 13         11.6         6         6         13         9.2         4           15         9.7         6	

\*\* 1% significance.

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hormone action than is found in normal birds. Therefore, an experiment was performed in which birds were caponized on day 14, then injected with 50  $\mu$ g TP every other day for 30 days. These birds then received 30  $\mu$ g TP alone or in combination with some other steriod for 10 days. Thus, the total experimental period was 40 days. The object was to try and create as normal an environment as possible as far as TP was concerned to see if it alone would maintain normal pituitary weight and FSH content, or if other steroids were also needed. The results in Table 4 indicate that the long term treatment with TP, although apparently below physiologic level as noted by comb weight, did keep the pituitary weight down, but did not maintain a normal FSH concentration. Of the other steriods, only progesterone had a depressing effect on pituitary FSH levels. Dexamethasone appeared to synergise the action of TP on the comb.

**TABLE 4.** The effect on the comb and pituitary gland of birds caponized on day 14, injected every other day for 30 days with 50  $_{\mu}g$  testosterone propionate (TP), then injected daily for 10 days with 30  $_{\mu}g$  TP alone or in combination with 200  $_{\mu}g$  TP 5  $_{\mu}g$  estradiol dipropionate (ED), 500  $_{\mu}g$  progesterone (prog), or 20  $_{\mu}g$  dexamethasone (dexa).

		Comb	Pituitary		Rat FSH Assay
Treatment	N	g	mg	N	Ovary mg $\pm$ se
Untreated controls.					
Cock controls	46	5.46	6.5	8	$68 \pm 4$
Capon controls	26	0.56	9.8	6	$144 \pm 13^{**}$
Capons + 50 $\mu$ g TP every other	day for a	30 days fo	llowed by	10-day	treatment with 30
ug TP alone or in combination w	ith other	steroids.			
30 $\mu g$ TP control	38	2.56	5.5	7	$140 \pm 3$
$+200 \mu g TP$	37	5.03	5.0	5	$147~\pm~12$
$+$ 5 $\mu g$ ED	36	2.08	5.3	6	$145 \pm 6$
$+500 \ \mu g \text{ prog.}$	33	2.38	5.9	6	$96 \pm 8^{**}$
$+$ 20 $\mu g$ dexa.	36	3.26	6.7	6	$126 \pm 11$
+ ED and prog.	38	2.06	5.3	6	$109 \pm 8^{**}$
+ TP, ED, prog., and dexa.	36	6.91	5.7	6	$111 + 7^{**}$

\*\* 1% significance level.

## Conclusion

The results of the experiments presented in this paper suggest that testosterone alone did not control FSH levels of the male chicken pituitary gland.

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