TESTOSTERONE EFFECTS ON GONADOTROPIN RESPONSE TO GNRH: COWS AND PONY MARES

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Summary
Effects of testosterone propionate (TP) treatment on plasma concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) before and after an injection of gonadotropin releasing hormone (GnRH) were studied using ovariectomized cows and pony mares. An initial injection of GnRH (1 µg/kg of body weight) was followed by either TP treatment or control injections for 10 (cows) or 11 (ponies) d. A second GnRH injection was administered 1 d after the last TP or oil injection. Concentrations of LH and FSH were determined in samples of plasma taken before and after each GnRH injection. Control injections did not alter the response to GnRH (area under curve) nor the pre-GnRH concentrations of LH and FSH in ovariectomized cows or ponies. Testosterone treatment increased (P<.01) the FSH release in response to GnRH in ovariectomized mares by 4.9-fold; there was no effect in cows, even though average daily testosterone concentrations were 59% higher than in pony mares. Testosterone treatment reduced the LH release in response to GnRH by 26% in ovariectomized mares (P<.05) and by 17% in ovariectomized cows (P=.051). These results are consistent with a model that involves ovarian androgens in the regulation of FSH secretion in the estrous cycle of the mare, but do not support such a model in the cow.

(Key Words: Testosterone, Mare, Cow, Luteinizing Hormone, Follicle-Stimulating Hormone, Gonadotropin Releasing Hormone.)

Introduction
Androgens are produced by ovarian follicles of the cow and mare (Hansel and Fortune, 1977; Ginther, 1979) and their concentrations in blood vary with follicular growth over the estrous cycle (Shemesh and Hansel, 1974; Kanchev and Dobson, 1976; Kanchev et al., 1976; Silberzahn et al., 1978). Several biological activities have been demonstrated for androgens in the female, including stimulation (Radford and Wallace, 1971; Pant, 1977) as well as inhibition (Beyer et al., 1972, 1974) of gonadotropin secretion. Active immunization against androgens resulted in increased gonadotropin secretion in ewes (Martensz et al., 1976; Martensz and Scaramuzzi, 1979), a greater ovulation rate in ewes (Scaramuzzi et al., 1981) and the formation of polycystic ovaries in rats (Hillier et al., 1974). Thompson et al. (1983b) reported that administration of testosterone to estrous mares resulted in a greater secretion of follicle-stimulating hormone (FSH) during the subsequent diestrus. Administration of testosterone propionate (TP) to mares fed the progesterone altroneost resulted in a 50% reduction in FSH concentrations within 24 h (Thompson et al., 1983a).

In geldings, TP administration caused a gradual but variable reduction in FSH secretion (Thompson et al., 1979); subsequent treatment with gonadotropin releasing hormone (GnRH) revealed a large accumulation of FSH in the pituitaries of TP-treated geldings compared with control animals. Similar stimulatory effects of testosterone on pituitary FSH content have been reported for ewes (Radford and Wallace, 1971) and rats (Bogdanove, 1967; Bogdanove and Gay, 1967).

The cow and mare differ in their response to estradiol treatment. That is, administration of...
estradiol results in a biphasic luteinizing hormone (LH) surge in ovariectomized cows (Beck and Convey, 1977), but produces a gradual and sustained increase in LH secretion in ovariectomized pony mares (Ginther, 1979). Such differences indicate that basic differences exist between these species in the hypothalamo-hypophyseal control of gonadotropin secretion. The present experiments were designed to determine the gonadotropin responses of ovariectomized cows and pony mares treated with TP and to relate these responses to possible physiologic roles of androgens in the cycling female.

**Materials and Methods**

Eight long-term ovariectomized pony mares (8 to 20 yr old) and eight long-term ovariectomized Hereford and Hereford x Angus cows (4 to 7 yr old) were used between May 15 and September 1. All animals were maintained on pasture during the experiment.

Two separate experiments were performed, one with pony mares and one with cows, according to the following protocol. Daily blood samples were drawn via jugular venipuncture for 2 d (d -2 and -1). On the third day (d 0), the eight animals were restrained in a chute and one jugular vein was catheterized. Two samples of jugular blood (10 ml) were drawn 15 min apart, and then GnRH was administered (1 μg/kg of body weight) through the jugular catheter. Heparinized saline was used to flush the catheter after GnRH administration. Samples of blood were drawn at 15, 30, 45, 60, 90, 120, 180 and 240 min after GnRH injection. Plasma (heparinized) was harvested by centrifugation and frozen for storage.

After the final blood sample was drawn after GnRH administration on d 0 (GnRH I), four animals were assigned randomly to one of two groups. Treated cows received daily sc injections of TP (175 μg/kg of body weight) for 10 consecutive days beginning on d 0. Control cows received an equivalent volume of vehicle (safflower oil). Treated ponies received 350 μg/kg of TP on alternate days for a total of six injections; control ponies received oil. Testosterone was measured by radioimmunoassay (Gay and Kerlan, 1978) in daily blood samples (drawn before treatment on any given day) to determine the relative concentrations in treated cows and ponies (table 1).

One day after the last TP or oil injection, all animals were catheterized, bled and administered GnRH (GnRH II) as described for d 0. To standardize the GnRH doses, enough GnRH was prepared several days in advance of GnRH I for all animals and both injections and then aliquots were frozen until needed.

**Radioimmunoassay of Gonadotropins.** All blood samples were placed on ice after collection and centrifuged within 30 min. Heparinized plasma was stored frozen at -15 C until assay. Concentrations of LH in pony plasma were determined by radioimmunoassay as described by Thompson et al. (1983a) and concentrations of FSH were determined as described by Thompson et al. (1983b). Concentrations of LH and FSH in cow plasma were measured in the same assays as for horse plasma and the validation procedures for bovine plasma and pituitary extracts were identical to those reported for horse tissues (Thompson et al., 1983a,b). This included 1) generation of parallel inhibition curves for bovine plasmas, pituitary extracts and standards (NIH-LH-B8 or NIH-FSH-B1), 2) determination of cross-reactivities of other glycoprotein hormones (Thompson et al., 1983a,b) and 3) electrophoresis of bovine pituitary extract to show that only one immunoreactive peak occurred for each specific radioimmunoassay. Intra- and interassay coefficients of variation were virtually identical to those reported for the equine assays (< 10 and 13%, respectively; Thompson et al., 1983a,b).

**Statistical Analysis.** Data for hormonal concentrations for each experiment were analyzed separately by analysis of variance in a double split-plot design (Gill and Hafs, 1971). In addition, the area under the GnRH-response curve (described by Mongkonpunya et al., 1974) was used as an index of the amount of hormone secreted. Areas were calculated for the first 2.0 h of blood sampling as the sums of the products of each post-GnRH concentration (minus mean pre-GnRH concentration) x the respective time interval. Areas (ng·ml⁻¹·h) were calculated for each animal for each hormone after each GnRH injection. Data for each species were analyzed in 2 x 2 factorially arranged analyses of variance (Steel and Torrie, 1960) or in paired t-tests (when variances were equal).
unequal) to determine the significance of differences between GnRH I and II within TP-treated and control groups.

Results

Testosterone. Concentrations of testosterone (table 1) resulting from daily injections of TP at 175 μg/kg of body weight in cows were 59% higher when averaged over the entire injection period than those in pony mares that received alternate day injections (350 μg/kg). Thompson (1978) showed that alternate day injection of TP in geldings resulted in a gradual increase in testosterone concentrations over 12 h, and concentrations remained elevated for 48 h. Thus, daily blood samples should be sufficient to characterize the mean testosterone concentrations in these cows and ponies.

FSH. Effects of TP treatment on FSH concentrations before and after GnRH in ovariectomized cows are shown in figure 1. There was a highly significant change in FSH concentrations in response to GnRH averaged over all cows and both GnRH injections. Although cows were allotted randomly to treatment groups, there was an initial difference (P<.05) in absolute concentrations of FSH between groups before treatment. Removal of this difference by subtracting each cow's mean values from all her individual values and then using the residuals in a second analysis of variance resulted in the same conclusions and interpretations that had already been drawn with the unadjusted data. Moreover, calculation of areas under the curve eliminated the pretreatment differences, indicating that no adjustment of the data was necessary.

As seen from the virtually superimposable response curves for both groups, TP treatment did not affect pre-GnRH concentrations of FSH nor did it affect the response to GnRH in cows. Areas under the curve for the first vs second

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<th>Table 1. Mean Concentrations of Testosterone in Daily Samples of Plasma from Testosterone Propionate-Treated Cows and Pony Maresa</th>
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aTestosterone concentrations in control cows and pony mares averaged 11 ± 1 and 10 ± 1 pg/ml, respectively. Limit of detection of assay was 8 pg/ml, and 85% of control levels were undetectable.

bGnRH I was given on d 0 and the first TP injection followed the last blood sample on that day. GnRH II was given on d 10 (cows) and 11 (ponies).

cPooled SE from analysis of variance was 40.6 pg/ml for TP-treated animals. Averaged over all periods, values were higher (P<.05) for cows than for pony mares.

Figure 1. Concentrations of FSH in plasma of ovariectomized cows in response to an iv injection of GnRH before (solid line) and after (dashed line) treatment with testosterone propionate (upper panel) or oil (lower panel). The GnRH was administered at 1 μg/kg of body weight at time 0. The single point on left denotes ± the pooled SE from analysis of variance. Bar graphs indicate the area under the response curve (first 2 h) for the first (I) and second (II) GnRH. Vertical lines on bars indicate one SE.
GnRH injections did not differ within either group. Two conclusions can be drawn: 1) the first GnRH injection had no influence on the release of FSH to the second injection in control cows and 2) TP treatment did not affect systemic concentrations nor amount of FSH released in treated cows.

The effects of TP on FSH concentrations in ovariectomized pony mares are presented in figure 2. Averaged over all ponies and both GnRH injections, GnRH resulted in a highly significant increase in FSH concentrations. Testosterone treatment resulted in a greater (P<.01) FSH release after GnRH. Area under the FSH curve was increased (P<.01) by an average of 4.9-fold over pretreatment response. Treatment of ovariectomized ponies with vehicle (safflower oil) did not alter the response to GnRH, as indicated by the similar pre-GnRH concentrations and areas under the FSH curve (figure 2).

**LH.** Release of LH in response to GnRH before and after TP treatment in ovariectomized cows is presented in figure 3. There was a highly significant increase in LH concentrations after GnRH averaged over all cows. Concentrations of LH before the second GnRH injection were not affected by TP or control treatment. Testosterone treatment did result in a decrease (P=.051) in area under the curve for LH; there was no difference in response to the first vs second GnRH injections in control cows. Thus, the first GnRH injection did not influence the response to the second injection 10 d later, whereas TP-treatment reduced the response by approximately 17%.

The influence of TP-treatment on LH concentrations in ovariectomized pony mares is presented in figure 4. Testosterone treatment reduced (P<.05) the area under the LH curve by approximately 26% (GnRH II vs I); control treatment did not alter the response to GnRH.

![Figure 2](image1.png)  
**Figure 2.** Concentrations of FSH in plasma of ovariectomized pony mares in response to an iv injection of GnRH before (solid line) and after (dashed line) treatment with testosterone propionate (upper panel) or oil (lower panel). The GnRH was administered at 1 μg/kg of body weight at time 0. The single point on left denotes ± the pooled SE from analysis of variance. Bar graphs indicate the area under the response curve (first 2 h) for the first (I) and second (II) GnRH. Vertical lines on bars indicate one SE; asterisks indicate difference between responses (paired t-test; P<.01).

![Figure 3](image2.png)  
**Figure 3.** Concentrations of LH in plasma of ovariectomized cows in response to an iv injection of GnRH before (solid line) and after (dashed line) treatment with testosterone propionate (upper panel) or oil (lower panel). The GnRH was administered at 1 μg/kg of body weight at time 0. The single point on left denotes ± the pooled SE from analysis of variance. Bar graphs indicate the area under the response curve (first 2 h) for the first (I) and second (II) GnRH. Vertical lines on bars indicate one SE; asterisk indicates difference between responses (paired t-test; P=.051).
Figure 4. Concentrations of LH in plasma of ovariectomized pony mares in response to an iv injection of GnRH before (solid line) and after (dashed line) treatment with testosterone propionate (upper panel) or oil (lower panel). The GnRH was administered at 1 μg/kg of body weight at time 0. The single point on left denotes ± the pooled SE from analysis of variance. Bar graphs indicate the area under the response curve (first 2 h) for the first (I) and second (II) GnRH. Vertical lines on bars indicate one SE; asterisk indicates difference between responses (paired t-test; P<.05).

Pre-GnRH concentrations of LH were reduced (P<.01) by 88% in TP-treated mares but were unaffected in control mares.

Discussion

Testosterone concentrations produced in cows and pony mares by TP treatment in the present experiment were generally higher than those reported in intact animals (cows: 15 to 45 pg/ml, Shemesh and Hansel, 1974; mares: 12 to 75 pg/ml, Silberzahn et al., 1978). However, the average testosterone concentrations produced in pony mares were similar to concentrations of androstenedione found in mares 1 d before ovulation (380 pg/ml, Noden et al., 1975). Moreover, Kanchev and Dobson (1976) reported androstenedione concentrations of approximately 80 to 120 pg/ml in cyclic heifers; they also reported testosterone peaks in these heifers of 1 to 4 ng/ml approximately 7 d before estrus. Thus, even though testosterone concentrations in the present experiment were considered supraphysiologic, they were not beyond the realm of concentrations that could possibly be experienced by the cyclic female.

Secretion of FSH in response to GnRH was 4.9-fold greater in TP-treated mares compared with control mares. This stimulation of FSH secretion in response to GnRH by testosterone is identical to that observed for geldings (Thompson et al., 1979). Moreover, TP treatment of geldings resulted in an eightfold increase in pituitary concentration of FSH (Thompson et al., 1979); thus, we suspect that this same effect occurred in the pituitaries of these pony mares. Similar effects of testosterone on pituitary FSH stores have been reported for the ewe (Radford and Wallace, 1971) and rat (male: Bogdanove, 1967; Gay and Bogdanove, 1969; female: Bogdanove and Gay, 1967).

In contrast to the horse, sheep and rat, FSH secretion in response to GnRH in the cow was not affected by testosterone treatment. Neither systemic concentrations of FSH nor response to GnRH was affected by treatment. This lack of response occurred even though concentrations of testosterone in systemic plasma of cows were 59% higher on the average than in pony mares (table 1). Thus, the cow differs from the mare in its response to androgens just as it differs from the mare in its response to estrogens (Beck and Convey, 1977; Ginther, 1979).

Thompson et al. (1983b) suggested that androgens from the ovary of the mare during estrus may stimulate FSH secretion during the subsequent diestrus. A similar potential role of androgens in the bovine estrous cycle seems unlikely, due to the lack of response in the cow to the supraphysiologic concentrations of testosterone in the present experiment.

Effects of testosterone on FSH secretion in any species can be confounded by its conversion to metabolites such as estradiol and dihydrotestosterone (DHT). Thompson et al. (1980) reported a significant increase in serum concentrations of estradiol in geldings treated with TP, indicating a systemic aromatization of the injected testosterone. Moreover, the metabolism of testosterone may occur within the target tissue itself, such as brain (Naftolin et al., 1975) or pituitary (Jaffe, 1969). Thus, further studies are necessary to determine if the effect of TP on FSH secretion in the mare is due to testosterone's androgenic properties (or DHT) or if the effect is due to estrogens formed from the injected testosterone. In geldings, estradiol
and testosterone had widely differing effects on FSH secretion after GnRH (Thompson et al., 1979); estradiol alone or in combination with testosterone suppressed FSH secretion after GnRH compared with testosterone treatment. Therefore, we suspect that the effect of testosterone on FSH secretion in mares is an androgenic rather than estrogenic effect.

The relatively small effect of TP treatment on the LH release in response to GnRH in ovariec tomized cows was likely an androgenic effect rather than an estrogenic effect because estrogens generally increase the production and secretion of LH by bovine pituitary cells (Padmanabhan et al., 1978) and increase the in vivo response to GnRH (Beck and Convey, 1977). Systemic concentrations of LH before GnRH injection were not affected by TP treatment. The reduced release of LH after GnRH due to testosterone was not likely due to a depletion of LH during treatment because LH concentrations in daily blood samples were similar for TP-treated and control cows (data not shown). That is, there was no evidence of a latent LH surge due to TP treatment as is seen approximately 18 h after estradiol treatment (Beck and Convey, 1977).

The reduction in LH concentrations before GnRH due to TP treatment in ovariec tomized pony mares was dramatic compared with ovariec tomized cows. Testosterone is a potent inhibitor of LH secretion and production in geldings (Thompson et al., 1979) and the degree of response in these pony mares was very similar to the response of geldings treated for 15 d with the same dosage of TP (Thompson et al., 1979). Thus, in the gonadectomized horse, there appears to be little sexual differentiation of the hypothalamo-hypophysial axis with regards to its response to gonadal steroids. In addition to the similar responses to testosterone reported herein, both ovariec tomized mares and geldings respond to estradiol treatment with an increase in LH secretion and a decrease in FSH secretion (Ginther, 1979; Thompson et al., 1979, S. I. Reville-Moroz and D. L. Thompson, Jr., unpublished data).

In conclusion, TP treatment of pony mares resulted in an apparent accumulation of FSH in the pituitary gland similar to that observed in geldings. In contrast to horses, FSH in ovariec tomized cows was unresponsive to TP treatment. Testosterone treatment decreased LH secretion in cows and pony mares. These data are consistent with a model that includes ovarian androgens in the regulation of FSH secretion in mares over the estrous cycle (Thompson et al., 1983b), but do not support such a physiologic role of androgens in the cow.

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