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Androgen and Progesterone Effects on Follicle-stimulating Hormone and Luteinizing Hormone Secretion in Anestrous Mares

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ABSTRACT

Anestrous lighthorse mares were treated in December with dihydrotestosterone (DHT; 150 μg/kg of body weight), progesterone (P; 164 μg/kg), both DHT and P (DHT+P), testosterone (T; 150 μg/kg), or vehicle (n = 4/group). Daily blood sampling was started on Day 1, and on Day 4 all mares were administered a pretreatment injection of gonadotropin-releasing hormone (GnRH) and were bled frequently to characterize the responses of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentrations. Treatment injections were given on Day 4 and then daily through Day 17. On Day 18, all mares were again administered GnRH and were bled frequently. Treatment of mares with DHT, P, or T increased ($p<0.01$) plasma concentrations of these steroids to approximately 1.5 ng/ml during the last 10 days of treatment. There was no effect ($p>0.10$) of treatment on LH or FSH concentrations in daily blood samples. Relative to the pretreatment GnRH injection, mares treated with T or DHT+P secreted approximately 65% more ($p<0.01$) FSH in response to the post-treatment GnRH injection; FSH response to the second GnRH injection was not altered ($p>0.10$) in control mares or in DHT- or P-treated mares. There was no effect of any steroid treatment on LH secretion after administration of GnRH ($p>0.10$). Averaged over all mares, approximately 94 times more FSH than LH was secreted in response to injection of GnRH. We conclude that: 1) anestrous mares respond to T treatment in a manner similar to intact cyclic mares and ovariectomized mares with regard to FSH secretion; 2) there is no effect on FSH of DHT or P treatment alone in anestrous mares; but 3) there is an interaction between DHT and P treatment on FSH secretion after exogenous GnRH.

INTRODUCTION

Treatment of intact cyclic mares with testosterone propionate (TP) increased the amount of follicle-stimulating hormone (FSH) secreted in response to administration of gonadotropin-releasing hormone (GnRH) relative to control mares (Thompson et al., 1983c). Similar increases in FSH secretion were reported for intact mares treated with testosterone (T) during estrus and for ovariectomized pony mares treated with TP (Thompson et al., 1983d, 1984b; Reville-Moroz et al., 1984). Reville-Moroz et al. (1984) showed that the increase in FSH secretion after exogenous GnRH in TP-treated ovariectomized pony mares was due partially to increased de novo production of FSH and partially to reduced daily secretion of FSH. The reduction in daily secretion of FSH in intact mares by TP treatment appeared to be an estrogenic effect because it could be mimicked by treatment with estradiol benzoate but not by treatment with dihydrotestosterone (DHT) benzoate (Thompson et al., 1983c). Treatment of those intact mares with DHT benzoate did increase the amount of FSH secreted in response to exogenous GnRH, as did TP, thereby confirming the androgenic nature of that response (Thompson et al., 1983c).

In contrast to intact mares, long-term ovariectomized pony mares did not respond to treatment with DHT benzoate with an increase in FSH secretion after exogenous GnRH (Garza et al., 1985). Thus, it appeared that the ability of DHT alone to increase FSH secretion was dependent upon other factor(s) present in the intact cyclic mare but absent in the ovariectomized mare. This factor is not estradiol because treatment with a combination of DHT and estradiol benzoates did not produce any effect

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different than treatment with estradiol benzoate alone (Garza, 1985).

Other than androgens, progesterone (P) has been reported to increase FSH secretion when administered to anestrous mares (Evans and Irvine, 1979). The artificial progestogen Altrenogest has also been shown to increase FSH secretion in mares (Squires et al., 1983; Thompson et al., 1984a). Because the hypothalamic input to the pituitary appears to differ markedly between the breeding and nonbreeding seasons in mares (Hart et al., 1984), it could not be predicted how LH and FSH secretion might respond to androgen and P administration in the winter. Thus, the present experiment was designed to determine: 1) if T treatment of anestrous mares would increase FSH secretion after exogenous GnRH as it does in ovariectomized and intact cyclic mares; 2) if DHT treatment would produce results similar to T (as in cyclic mares) or have no effect on FSH secretion (as in ovariectomized mares); and 3) if P or the combination of P and DHT would increase FSH secretion in anestrous mares in a manner similar to T.

MATERIALS AND METHODS

Twenty grade, lighthorse mares between 3 and 15 yr of age were selected from a larger herd based on three criteria for anestrus: 1) lack of cyclic displays of estrus, 2) lack of elevated P concentrations in plasma, and 3) lack of significant follicular development based upon rectal palpation of the ovaries. The 20 mares were maintained on pasture and were supplemented with grass hay to maintain body condition during the winter months. Once selected, mares were randomly allotted to one of five groups (n=4/group): control mares (vegetable shortening at 0.006 ml/kg BW), DHT-treated mares (150 μg/kg BW), P-treated mares (164 μg/kg BW), DHT+P-treated mares (at the above dosages), and T-treated mares (150 μg/kg BW). On December 19 (Day 1), daily blood sampling via jugular venipuncture was initiated; daily sampling was continued through Day 18. On Day 4, a 14-gauge catheter was placed into one jugular vein of each mare and three samples of blood (10 ml) were drawn at 15-min intervals. Immediately after the third sample was drawn from a mare, that mare was administered GnRH (1.0 μg/kg BW in 155 mM saline) through the catheter. Blood samples were then drawn at 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min after injection of GnRH.

Immediately after the 240-min sample was drawn from a mare, that mare was injected subcutaneously with the appropriate treatment and blood samples were then drawn at 1, 2, 4, 8, 12, 16, 20, and 24 h after injection. Treatment injections were repeated immediately after the 24-h sample was drawn and then daily each morning (after blood sampling) through Day 17. On Day 18, mares were again catheterized, administered GnRH, and bled as described for Day 4.

All plasma samples were assessed for LH and FSH concentrations by radioimmunoassay as described previously (Thompson et al., 1983b,d). Gonadotropin values were calculated from standards that were based on highly purified, iodination-grade equine LH and FSH (supplied by Dr. Harold Papkoff, University of California, San Francisco). The LH preparation (E98A) had a biopotency of approximately 2.97 NIH-LH-S1 units/mg in the ovarian ascorbic acid depletion assay and the FSH preparation (E99B) had a biopotency of approximately 24.5 NIH-FSH-S1 units/mg in the Steelman-Pohley ovarian augmentation assay (calculated from data of Licht et al., 1979). Daily samples and those drawn during the 24-h period after the first treatment injection were assessed for concentrations of P (Thompson et al., 1983a), T, and DHT. Concentrations of T and DHT were assessed by a radioimmunoassay based on antiserum generated against T-3-oxime-bovine serum albumin (Erlanger et al., 1959) and tritiated T. Cross-reactivities of T and DHT in this assay were 100% and 50.5%, respectively; cross-reactivities of other androgens were <4% and of nonandrogens <0.01%. A thin-layer chromatogram of an extract of plasma from a T-treated mare indicated one major peak (T) and one minor peak (DHT) of immunoreactivity. Testosterone accounted for 96% of this immunoreactivity; thus it was assumed that the immunoreactivity in the androgen assay for a given mare was attributable to the steroid (T or DHT) that was injected. Authentic standards of T and DHT were run in each assay and the concentrations of androgen were calculated according to which steroid the mare received.

Hormonal data were analyzed by analysis of variance that took into account the repetitive nature of sampling (split-plot design; Steel and Torrie, 1960; Gill and Hafs, 1971). The response of mares to the two injections of GnRH was assessed by calculating the net areas under each 4-h response curve for each mare and each hormone (Thompson et al., 1983c). Areas were analyzed by analysis of variance; the significance of differences between the first and second GnRH injection within each group was evaluated by orthogonal contrasts (Steel and Torrie,

1960), which accounted for any random variation that existed among groups before treatment. The total amount of LH and FSH secreted in the first 1.0 h after injection of GnRH was calculated from the net change in hormonal concentration (maximum post-GnRH concentration within the first 60 min minus average pre-GnRH concentration) multiplied by the estimated plasma volume of each mare (5% of BW; Sack and Sadler, 1982) to compare the relative secretion rates of the two gonadotropins during the anestrous season. Apparent half-times and disappearance constants of each gonadotropin were calculated for each mare by regression analysis (time vs. natural log of concentration) of the 45- to 240-min samples after the post-treatment GnRH injection and were analyzed by analysis of variance. The apparent disappearance constant (λ) was the calculated least-squares slope of the semilogarithmic curve and the apparent half-time was calculated from $0.693/\lambda$ (Wang et al., 1975).

RESULTS

Treatment of anestrous mares at the described dosages elevated ($p<0.01$) concentrations of the appropriate steroid within 1–2 h (Fig. 1). Maximal concentrations immediately after the first treatment injection on Day 4 occurred at the 2- or 4-h sampling period for all three steroids. Moreover, concentrations of all three steroids were maintained at approximately 1.5 ng/ml during the last 10 days of treatment (Fig. 2). Because daily blood samples were drawn before treatment injections, these averages represent the minimal concentrations of steroid occurring during the treatment period.

There was no effect ($p>0.10$) of steroid treatment or day of sampling nor was there any treatment × day interaction in the analysis of variance for concentrations of LH and FSH in daily blood samples (data not shown). Average concentrations (ng/ml) were 0.56 ± 0.04 for LH and 12.7 ± 0.9 for FSH.

Concentrations of FSH in plasma increased ($p<0.01$) in mares of all treatment groups after injection of GnRH (Fig. 3). The magnitude of the FSH response to the post-treatment GnRH injection, as assessed by areas under the curves (Fig. 3), was greater ($p<0.01$) than for the pretreatment injection for DHT+P-treated and T-treated mares. The pretreatment and post-treatment responses were virtually identical for the control, DHT-treated, and P-treated mares (Fig. 3).

Concentrations of LH in plasma increased ($p<0.01$) in mares of all treatment groups after injection of GnRH (Fig. 4). However, there was no effect ($p>0.10$) of any steroid treatment on LH concentrations after GnRH injection or on areas under the curve; thus only the pooled data are presented (Fig. 4).

The amount of LH and FSH secreted in the first 1.0 h after GnRH injection, based on highly purified equine standards, averaged 0.014 mg and 1.33 mg, respectively. When amount of hormone released in the first 1.0 h after injection of GnRH was analyzed by analysis of variance, the results (treatment effects and relative size of means) were virtually identical to those for area under the curve (which an index of the amount of hormone secreted over the 4-h period). Thus, only means for the areas are presented (Figs. 3 and 4).

Apparent half-times and disappearance constants after the post-treatment GnRH injection were not affected ($p>0.10$) by steroid treatment. Mean half-times (min) were 380 ± 45 for LH and 170 ± 11 for

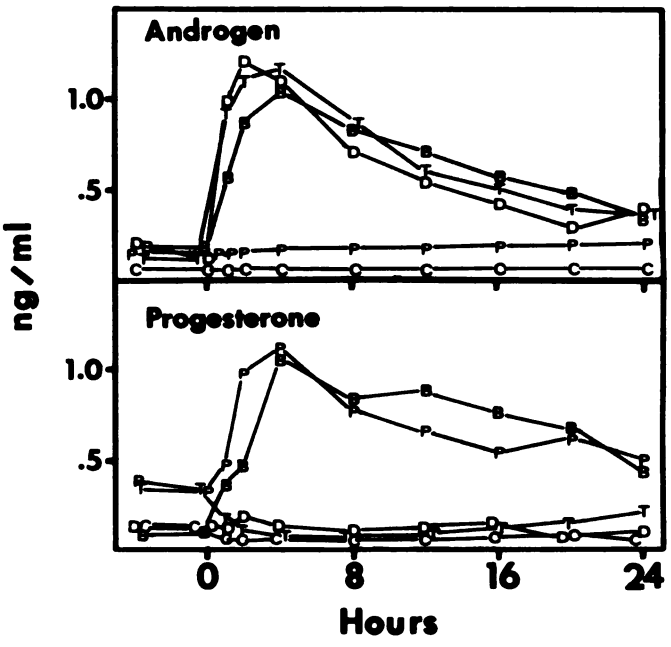

FIG. 1. Mean concentrations of androgen and progesterone in plasma of control mares (*C*) and mares treated with DHT (*D*), P. DHT+P (*B*), or T. These data are for the first treatment injections given on Day 4 (Time 0). Androgen values for control, T-treated, and P-treated mares were based on authentic T standard; values for DHT- and DHT+P-treated mares were based on authentic DHT standard. Pooled SEM from the analyses of variance were 0.11 ng/ml for androgen and 0.12 ng/ml for progesterone.

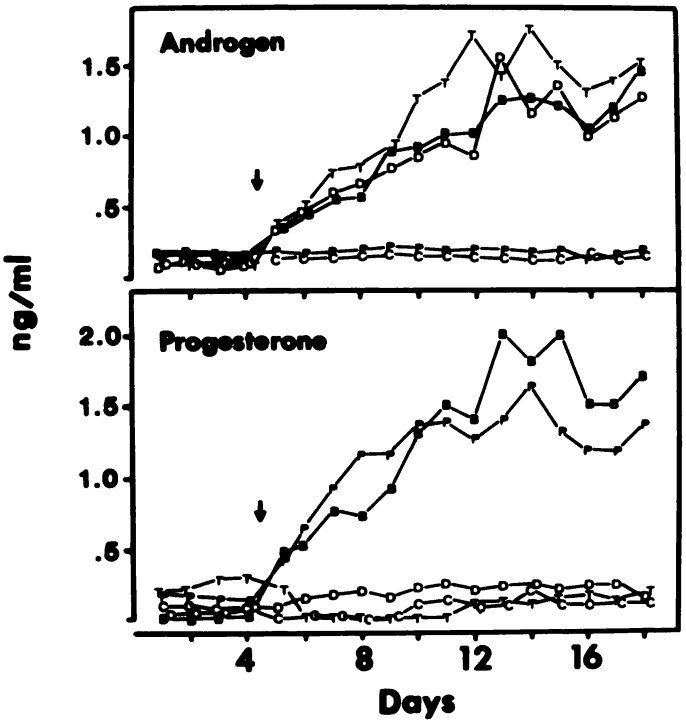

FIG. 2. Mean concentrations of androgen and progesterone in plasma of control mares (*C*) and mares treated with DHT (*D*), P, DHT+P (*B*), or T daily for 14 days during anestrus. First treatment injections were on Day 4 (*arrows*). Androgen values for control, T-treated, and P-treated mares were based on authentic T standard; values for DHT- and DHT+P-treated mares were based on authentic DHT standard. Pooled SEM from the analyses of variance were 0.11 ng/ml for androgen and 0.12 ng/ml for progesterone.

FSH; mean disappearance constants ($10^{-3} \cdot min^{-1}$) were 2.44 ± 0.31 for LH and 4.27 ± 0.18 for FSH. The correlation coefficients associated with the regression analyses for the 20 mares were all >0.96 for FSH and were between 0.46 and 0.98 for LH.

DISCUSSION

Treatment of intact anestrous mares with testosterone increased the FSH response to exogenous GnRH just as treatment with TP has been shown to do in intact cyclic mares, ovariectomized mares, and geldings (Thompson et al., 1979, 1983c, 1984b; Reville-Moroz et al., 1984). All the previous experiments were performed in the breeding season (summer) when hypothalamic input to the pituitary appears to be maximal (Hart et al., 1984). Thus, it appears that the difference in hypothalamic input to the pituitary between the breeding and nonbreeding seasons does not influence the FSH response to T administration, just as season does not appear to affect the concentration of FSH in the pituitary of the mare (Hart et al., 1984). In contrast, season has a marked effect on LH concentration in the pituitary of mares (Hart et al., 1984) and the lack of effect of T treatment on LH secretion in the mares in the present experiment was in contrast to previous studies (Thompson et al., 1983c, 1984b; Reville-Moroz et al., 1984) in which LH secretion was reduced. It is possible that the low amount of LH secreted by these anestrous mares represented a minimal secretion rate that was independent of steroid feedback. This concept is supported by the fact that P treatment of ovariectomized mares in summer suppressed LH secretion but simi-

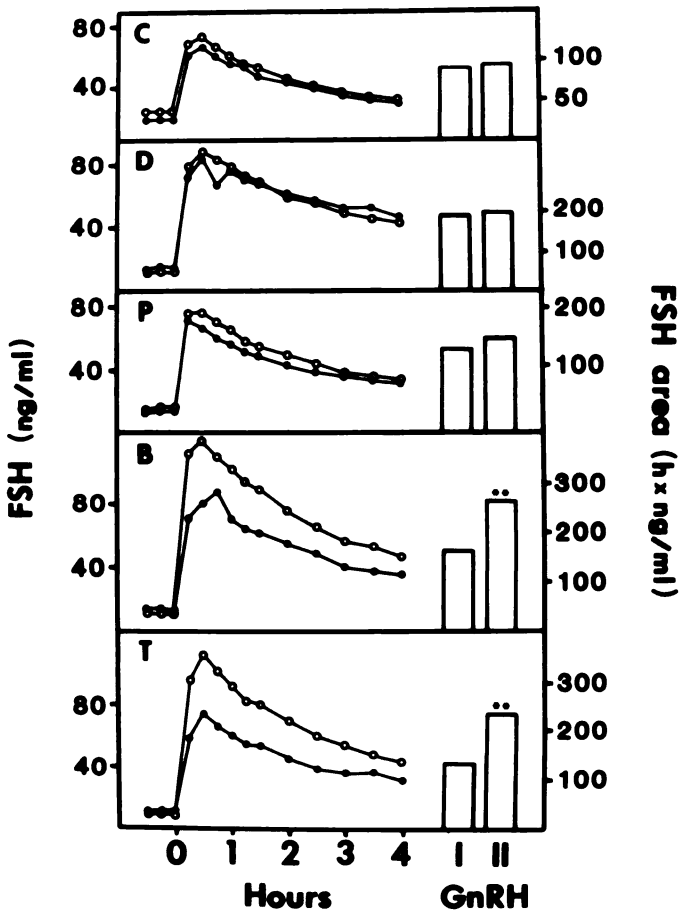

FIG. 3. Mean concentrations of FSH in plasma of anestrous mares in response to an injection of GnRH before (*solid circles*) and after (*open circles*) treatment with vehicle (*C*), DHT (*D*), P, DHT+P (*B*), or T daily for 14 days. GnRH was administered at Time 0 at 1.0 μg/kg of body weight. Bar graphs indicate the average areas under the curves for the pretreatment (*I*) and post-treatment (*II*) injections of GnRH. Random variation among groups before treatments were initiated (within GnRH I) was accounted for by the orthogonal contrasts used after the analysis of variance. *Asterisks* indicate significant difference between pre- and post-treatment responses within a group ($p<0.01$). Pooled SEM from the analyses of variance was 7.9 ng/ml for FSH concentration and 20.3 units for areas under the curve.

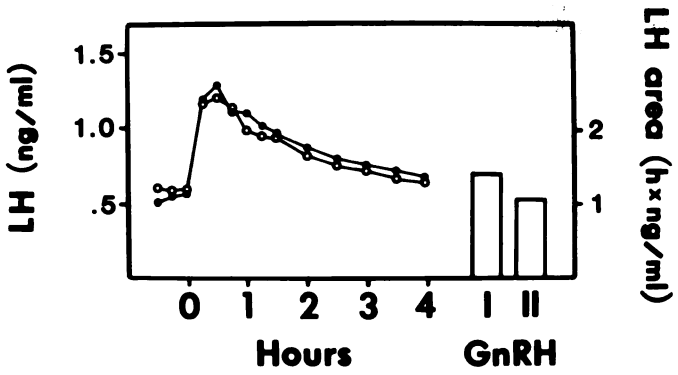

FIG. 4. Mean concentrations of LH in plasma of anestrous mares in response to an injection of GnRH before (*solid circles*) and after (*open circles*) treatment with vehicle or steroid. Bar graphs indicate the areas under the curves for the pretreatment (*I*) and post-treatment (*II*) injections of GnRH. There was no effect ($p>0.10$) of treatment or any treatment X time interaction in the analysis of variance for either characteristic, so the pooled data are presented. Pooled SEM from the anlayses of variance were 0.10 ng/ml for LH concentrations and 0.38 units for areas under the curve.

lar treatment in winter did not (Garcia and Ginther, 1978).

Although neither DHT nor P treatment of anestrous mares affected FSH secretion after exogenous GnRH, both steroids given simultaneously increased the amount of FSH secreted after the post-treatment GnRH injection to a degree similar to that of T. The failure of DHT alone to affect FSH secretion in the present experiment is in contrast to the results of Thompson et al. (1983c), who found that DHT treatment of intact cyclic mares increased FSH secretion in a manner similar to TP. However, other work in our laboratory (Garza et al., 1985) indicated that DHT treatment of long-term ovariectomized pony mares had no effect on FSH secretion. Thus, anestrous and ovariectomized mares are similar in their lack of response to DHT treatment. The intact cyclic mares that responded to DHT treatment in the experiment of Thompson et al. (1983c) developed normal corpora lutea during treatment (as assessed by plasma P concentrations) and therefore received a steroidal exposure similar to the DHT+P-treated mares in the present experiment. Thus, the presence of P appears to be necessary for the DHT-induced stimulation of FSH after exogenous GnRH.

Garza et al. (1985) hypothesized that some metabolite of T (or T itself) was needed in ovariectomized mares for them to respond to pure androgenic stimulation (DHT treatment) because ovariectomized mares do respond to TP alone with increased FSH production and secretion after exogenous GnRH (Reville-Moroz et al., 1984; Thompson et al., 1984b). Based on this hypothesis, Garza (1985) treated ovariectomized pony mares with DHT, estradiol, or the combination of both steroids and found that there was an estradiol effect on FSH secretion but there was no interaction with DHT. That experiment indicated that the factor(s) present in the intact mare that enabled her to respond to DHT alone was not estrogen. Although the present results indicate that P may be partially responsible for the cyclic mare's response to DHT, it does not explain why ovariectomized mares are able to respond to TP but not DHT alone. That is, in most species, T can be metabolized to 5α-reduced androgens, to estrogens, and to hydroxylated forms of T and these metabolites, but cannot be converted back to progestogens (Oh and Tamaoki, 1970; Ganjam and Kenney, 1975; Selmanoff et al., 1977; Callard et al., 1978). Therefore, the TP effect in ovariectomized mares (and the T effect in anestrous mares) would not likely be due to an interaction with progestogens. It is possible that the TP effect observed in cyclic mares is an androgenic effect dependent upon the presence of P while the T (or TP) effect observed in ovariectomized and anestrous mares is actually an estrogenic effect similar to that observed by Garza (1985) in ovariectomized mares.

Although previous researchers reported that P or Altrenogest treatment of anestrous mares increased FSH secretion (Evans and Irvine, 1979; Squires et al., 1983; Thompson et al., 1984a), we did not observe any effect of P on daily FSH secretion or on FSH secretion after exogenous GnRH. The duration of treatment in the present experiment was similar to those in the previous experiments, whereas the dosage was approximately half of that reported by Evans and Irvine (1979). However, the subcutaneous injection of P in vegetable shortening in the present experiment resulted in P plasma concentrations of 1.5–2.0 ng/ml, which were similar to those reported by Evans and Irvine (1979). Thus, other factor(s) may be responsible for the difference in results between the present and previous experiments. It is difficult to compare P treatment with the oral administration of Altrenogest because: 1) the concentration of Altrenogest circulating in the blood after feeding is unknown, 2) the progestogen activity of Altrenogest relative to P in the mare is unknown, and 3) the degrees of estrogenic and androgenic activities of Altrenogest in the mare are unknown.

Based on highly purified equine standards, approximately 94 times more FSH than LH was secreted after administration of GnRH to these anestrous mares. For comparison, the ratio of FSH:LH secreted

in normal cyclic mares (Thompson et al., 1983c) and ovariectomized mares in summer (Reville-Moroz et al., 1984) averages approximately 2—3:1 in our laboratory. These results are in good agreement with those of Hart et al. (1984), who found that LH concentration in the pituitary was reduced in anestrous mares by approximately 85% relative to mares in the summer; concentrations of FSH in the pituitaries of those mares were not influenced by season. This effect of season appears to be expressed in average plasma concentrations of the two gonadotropins as well. In the present experiment, concentrations of FSH and LH in daily plasma samples averaged 12.7 and 0.56 ng/ml, respectively. If LH production and secretion are reduced in anestrous mares due to a reduced hypothalamic GnRH content (and hence a reduced input to the pituitary; Hart et al., 1984), then FSH production and secretion must be relatively unaffected by endogenous GnRH in these mares. Because FSH is secreted in response to exogenous GnRH in horses of both sexes and of various reproductive states, we must conclude that the FSH-producing gonadotropes are responsive to GnRH. Therefore, the difference between LH and FSH secretion during anestrus may be influenced more by a difference in sensitivity to endogenous GnRH (i.e., the low GnRH input from the hypothalamus that results in the low LH production and secretion in winter may be adequate to maintain normal FSH production and/or secretion).

Qualitative as well as quantitative changes in FSH have been reported for steroid-treated, gonadectomized rats (reviewed by Bogdanove et al., 1975). Similar qualitative changes have been described for pituitary FSH in pre- and peripubertal male rats (Chappel and Ramaley, 1985). In the present experiment, the disappearance constants and half-times of FSH concentrations (after peak levels had been reached following administration of GnRH) were similar for all groups of mares. Thus, we conclude that no major qualitative differences in FSH were induced by T or DHT+P treatment in these mares.

In conclusion, T treatment of intact anestrous mares in winter increased the FSH response to exogenous GnRH in a manner similar to TP treatment of intact cyclic mares and ovariectomized mares in summer. Treatment with DHT or P had no effect on gonadotropin secretion in these anestrous mares, whereas treatment with the combination of DHT and P increased the FSH response to exogenous GnRH similar to T treatment. Thus, the presence of P may account for the response of intact cyclic mares to DHT alone with increased FSH secretion after exogenous GnRH, whereas ovariectomized and anestrous mares do not respond.

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