Serum thyroxine concentrations and pregnancy rates 15 to 16 days after ovulation in broodmares

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EQUINE

Objective—To determine whether serum thyroxine (T_4) concentration was associated with pregnancy rates 15 to 16 days after ovulation in mares and to determine whether thyroid hormone supplementation would enhance fertility in mares.

Design—Cohort study.

Animals—329 clinically normal broodmares.

Procedure—Mares were examined 15 to 16 days after ovulation to determine whether they were pregnant; blood samples for determination of serum T_4 concentration were collected at the same time. Sixty mares were receiving thyroid hormone supplementation prior to the study because of low serum T_4 concentration (< 16 µg/dl) prior to breeding.

Results—Serum T_4 concentration ranged from 4.5 to 53.9 mg/dl. Forty (12%) mares had low (< 16 µg/dl) concentrations, 283 (86%) had normal concentrations, and 6 (2%) had high (> 45 µg/dl) concentrations. Two hundred thirty-one mares were pregnant 15 to 16 days after ovulation. A significant association between serum T_4 concentration (low, normal, or high) and pregnancy (yes or no) was not detected, and logistic regression analysis indicated that serum T_4 concentration was not significantly related to pregnancy. Of the 269 mares not receiving thyroid hormone supplementation, 187 were pregnant, and of the 60 mares receiving thyroid supplementation, 44 were pregnant. There was no significant relationship between thyroid hormone supplementation and pregnancy status.

Conclusions and Clinical Relevance—Results suggest that serum T_4 concentration in mares is not significantly associated with pregnancy 15 to 16 days after ovulation. Results also suggest that supplementation of mares that only have low T_4 concentrations is not indicated or likely to be beneficial. (*J Am Vet Med Assoc* 2002;220:64–66)

What role, if any, hypothyroidism plays in fertility of mares is unclear.[1] This is largely because of inadequacies in the diagnostic tests currently available for routine use in private practice.[a] Veterinarians in private practice rely principally on serum **thyroxine (T_4)** concentration and clinical signs to arrive at a presumptive diagnosis of hypothyroidism in horses. Other tests, such as measurement of serum **thyroid stimulating hormone (TSH)** concentration and the thyrotropin stimulation test, are too expensive for routine use, not readily available, or impractical. Additionally, measuring serum **triiodothyronine (T_3)** concentration does not add any information beyond that obtained by measuring serum T_4 concentration alone.[2]

Hypothyroidism is known to cause infertility in women and laboratory animals.[3] For this reason, serum T_4 concentrations are often determined in broodmares, and many are given T_4 supplements because of a perceived thyroid hormone deficiency. However, concerns about the scientific validity of this practice and the cost-to-benefit ratio persist. In 1995, the annual cost of thyroid hormone supplementation in horses was estimated to be $750,000/y.[1] Today, that estimate would possibly exceed 1 million/y. In addition, serum T_4 concentration is routinely measured in broodmares in many breeding programs, adding to the cost of foal production.

To our knowledge, a low serum T_4 concentration has not been shown to affect fertility in mares, and supplementation has not been documented to improve fertility. Previous studies involving adult horses have examined the causes of hypothyroidism,[1] thyroid hormone concentrations,[4] the difficulties in establishing a diagnosis of hypothyroidism,[2] and thyroid hormone concentrations in mares during pregnancy and parturition,[5] but information comparing T_4 concentration and fertility in mares has not been published. The purposes of the study reported here were to determine whether serum T_4 concentration was associated with pregnancy rate 15 to 16 days after ovulation in mares and to determine whether thyroid hormone supplementation would enhance fertility in mares.

Materials and Methods

Three hundred twenty-nine clinically normal broodmares bred on 4 central Kentucky Thoroughbred farms during a single breeding season were used as the study population. Mares ranged from 4 to 24 years old. Mares were examined 15 to 16 days after ovulation and considered to be pregnant if an embryonic vesicle could be detected during transrectal palpation and ultrasonography. A blood sample was collected at the same time and submitted for determination of serum T_4 concentration. For mares that were bred more than once during the season, information was obtained only after the first breeding.

Serum T_4 concentrations were determined with a commercially available fluorescence polarization immunoassay.[b] This assay measures protein-bound and unbound T_4. Sensitivity of the assay was calculated to be 1.05 µg/dl; sensitivity was calculated as the mean plus 2 SD for results of 65 tests run with a calibration solution that had a T_4 concentration of 0 and represents the lowest measurable concentration of T_4 that can be distinguished from 0. Specificity of the assay was determined by studying cross-reactivity with T_3. By mea-

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suring the ratio of the concentrations of T_4 to T_3 that corresponded to the midpoint of the polarization span for the assay's standard curve, cross-reactivity was determined to be < 10%.

A reference range for serum T_4 concentration in adult horses was constructed by measuring serum T_4 concentrations in 100 clinically normal broodmares and comparing values with reference ranges reported by 3 independent commercial laboratories. The lower limit of the reference ranges for the 3 commercial laboratories was roughly equal to the mean concentration minus 1 SD for the 100 clinically normal mares. Therefore, this value was used as the lower reference limit in the present study. The upper limit of the reference ranges for the 3 commercial laboratories was roughly equal to the mean concentration plus 2 SD for the 100 clinically normal mares. Therefore, this value was used as the upper reference limit in the present study. The reference range for serum T_4 concentration in the present study, therefore, was 16 to 45 mg/dl. Values < 16 µg/dl were considered low, and values > 45 mg/dl were considered high.

After pregnancy status and T_4 concentrations were determined, a χ^2 statistical analysis, Fisher exact test, and regressional analysis were used to determine whether T_4 concentrations correlated with pregnancy status 15 to 16 days after ovulation. Values of $P < 0.05$ were considered significant.

Two hundred twelve mares were classified as barren, foaling, or maiden, and statistical analyses were repeated for each subgroup. A mare was classified as barren if it had failed to conceive during the previous breeding season or to carry a foal to term. A mare was classified as foaling if it foaled during the present breeding season. A mare was classified as a maiden if it had never been bred prior to the present breeding season.

One farm routinely measured serum T_4 concentration in all mares prior to breeding and administered a T_4 supplement (0.04 mg/kg [0.02 mg/lb] of body weight, PO, q 24 h) to mares in which serum T_4 concentration was < 16 µg/dl. Thyroxine supplementation had been begun in 60 mares prior to inclusion in the present study; in 59 mares, serum T_4 concentration 15 to 16 days after ovulation was increased, compared with concentration prior to breeding. In the remaining mare, serum T_4 concentration 15 to 16 days after ovulation was decreased, compared with concentration prior to breeding. Pregnancy rate in mares receiving the T_4 supplement at the time of breeding and 15 to 16 days after ovulation was compared with rate in mare not receiving the supplement with the χ^2 test.

Results

Serum T_4 concentration of the 329 mares ranged from 4.5 to 53.9 mg/dl (mean ± SD, 24 ± 7.87 mg/dl; median, 23.8 µg/dl). Forty (12%) mares had low serum T_4 concentrations (< 16 µg/dl), 283 (86%) had normal concentrations (16 to 45 µg/dl), and 6 (2%) had high concentrations (> 45 µg/dl). Two hundred thirty-one mares were determined to be pregnant 15 to 16 days after ovulation. Of these, 31 had low serum T_4 concentrations, 198 had normal concentrations, and 2 had high concentrations. Of the 98 mares that were not pregnant 15 to 16 days after ovulation, 9 had low serum T_4 concentrations, 85 had normal concentrations, and 4 had high concentrations. A χ^2 test for independence indicated that a significant relationship between serum T_4 concentration (low, normal, high) and pregnancy (yes, no) did not exist ($P = 0.282$). Logistic regression analysis of serum T_4 concentration for all 329 mares indicated that serum T_4 concentration was not significantly related to pregnancy status (pregnant vs not pregnant).

Thirty-four mares were classified as barren. Of these, 20 were pregnant 15 to 16 days after ovulation (1 had a low serum T_4 concentration and 19 had normal concentrations), and 14 were not pregnant (13 had normal T_4 concentrations and 1 had a high concentration). A Fisher exact test for independence indicated that there was no significant ($P = 1.0$) relationship between serum T_4 concentration and pregnancy status for this population of barren mares.

One hundred forty-nine mares were classified as foaling. Of these, 107 were pregnant 15 to 16 days after ovulation (17 had low serum T_4 concentrations, 88 had normal concentrations, and 2 had high concentrations), and 42 were not pregnant (6 had low serum T_4 concentrations, 33 had normal concentrations, and 3 had high concentrations). A χ^2 test for independence indicated that there was no significant ($P = 0.808$) relationship between serum T_4 concentration and pregnancy status for this population of foaling mares.

Twenty-nine mares were classified as maiden. Of these, 20 were pregnant 15 to 16 days after ovulation (all 20 had normal serum T_4 concentrations), and 9 were not pregnant (1 had a low serum T_4 concentration and 8 had normal concentrations). A Fisher exact test for independence indicated that there was no significant ($P = 0.310$) relationship between serum T_4 concentration and pregnancy status for this population of maiden mares.

Of the 269 mares not receiving thyroxine supplementation, 187 were pregnant, and 82 were not pregnant. Of the 60 mares receiving supplementation, 44 were pregnant and 16 were not pregnant. A χ^2 test for independence indicated that there was no significant ($P = 0.559$) relationship between thyroid hormone supplementation and pregnancy status.

Of the 6 mares with high serum T_4 concentrations (> 45 µg/dl), 2 were pregnant, and 4 were not pregnant. A Fisher exact test indicated that there was no significant relationship between serum T_4 concentration (high vs normal) and pregnancy status (yes vs no) 15 to 16 days after ovulation.

Discussion

In the present study, we were not able to demonstrate any relationship between serum T_4 concentration in mares and pregnancy status 15 to 16 days after ovulation, regardless of whether data for all mares or for mares grouped on the basis of breeding status (barren, foaling, maiden) were analyzed. The large number of mares included in the study (n = 329) suggests that if an association did exist, we likely would have detected it.

In the present study, a single veterinarian examined all mares to determine pregnancy status and used the same examination protocol in all mares. Only the first breeding of each mare during the breeding season was considered to help eliminate other factors, such as uterine infection, that may have affected the probability of conception with subsequent breedings later in the season.

The extent to which hypothyroidism contributes to infertility in mares is not well understood.[1] However, a published report[1] described 2 mares that conceived after thyroidectomy and carried their foals to term.

Because of the multifactorial nature of conception, it is difficult to assess the effect of any 1 factor.

Studies have shown that serum T_4 concentration alone is not an accurate indication of thyroid function,[2] and many endogenous and exogenous factors can alter serum T_4 concentration, which can lead to a misdiagnosis of hypothyroidism.[6] Therefore, our results do not preclude the possibility of a relationship between hypothyroidism and pregnancy. In humans, measuring serum T_4 and TSH concentrations results in almost 100% accuracy in diagnosing thyroid dysfunction,[2] and research in horses has shown that measurement of TSH concentration in conjunction with thyroid hormone concentrations is useful in the diagnosis of hypothyroidism.[c] However, no assays for measuring TSH concentration in horses are commercially available at this time. More accurate diagnostic methods would enable practitioners to identify horses with true thyroid hormone deficiencies that require supplementation and to minimize supplementation of horses that are normal.

On the basis of results of the present study, we believe that measurement of serum T_4 concentration should not be a routine part of equine breeding programs. Although the true incidence of hypothyroidism in horses is not known, many consider it to be low.[1] Thus, it seems possible that few of the mares receiving thyroid hormone supplementation in the present study could truly have been hypothyroid. In addition, pregnancy rates were not significantly different between mares that were receiving thyroid hormone supplementation and those that were not; therefore, supplementation of these mares did not appear to enhance their fertility. Practitioners who suspect that a mare has hypothyroidism on the basis of clinical signs can use the serum T_4 concentration to aid in arriving at a presumptive diagnosis of hypothyroidism, and mares suspected to have hypothyroidism on the basis of clinical signs and low serum T_4 concentration may benefit from supplementation. However, results of the present study suggest that supplementation of horses with low serum T_4 concentrations that do not have other criteria for hypothyroidism is not likely to be beneficial.

[a]Sojka JE. Thyroid evaluation in horses (abstr), in *Proceedings*. 14th Am Coll Vet Intern Med Forum 1996;546.

[b]AxSYM Total T_4 System, Abbott Laboratories, Abbott Park, Ill.

[c]Breuhaus BA. Thyroid stimulating hormone in euthyroid and hypothyroid horses (abstr), in *Proceedings*. 17th Am Coll Vet Intern Med Forum 1999;243.

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