Enzyme Immunoassay for the Measurement of Estrone Sulfate in Cryptorchids, Stallions, and Donkeys

G. F. Carneiro, DVM; I. K. M. Liu, DVM, PhD; J. C. Illera, DVM, PhD; and C. J. Munro, MS

The measurement of estrone sulfate, performed by an enzyme-linked immunosorbent assay, is a useful and reliable diagnostic alternative test for the detection of cryptorchidism in the horse and donkey. Mean values are provided in this report for 48 known cryptorchid horses, 49 geldings, and six stallions ranging from 1 to 10 years of age. These values show significant differences between geldings and cryptorchids (p = 0.0005), cryptorchids and stallions (p = 0.01), geldings and stallions (p = 0.003) and between horses <2 years of age (p = 0.0003). Authors' addresses: Dept. of Population Health and Reproduction, School of Veterinary Medicine, University of California at Davis, Davis, CA 95616 (Carneiro, Liu, and Munro) and Depto. de Fisiologia Animal, Facultad de Veterinaria UCM, 28040 Madrid, Spain (Illera). © 1998 AAEP.

1. Introduction

Cryptorchidism is a condition in which one or both testicles fail to descend into the scrotum, and it is considered to be a prevalent defect in horses.1,5 Although many authors consider this condition to be an inherited abnormality,1,5 convincing evidence is lacking and the exact mode of transmission remains unknown. Bilaterally cryptorchid stallions do not produce viable spermatozoa but often exhibit normal secondary sexual characteristics such as libido, because of testosterone production by the interstitial cells of the retained testes.1,5 Bilateral cryptorchids, or hemicastrates, must be differentiated from geldings who exhibit stallionlike behavior. Thus, the correct laboratory diagnosis of this condition is very important, especially when exploratory abdominal surgery is considered for the removal of retained testes. Several investigators have described hormonal assays for the diagnosis of cryptorchidism in horses.1-5 In particular, isotopic immunoassays (radioimmunoassays, or RIA's) measuring testosterone and estrone sulfate serum levels are considered to be reliable diagnostic aids for the condition.1-5 These methods have disadvantages, including the health hazards associated with handling radioisotopes, the overall expense, and the heavy regulation of radioisotope purchase, use, and disposal. It is also time consuming and harder to use than the enzyme-linked immunosorbent assay (ELISA) method.

The evidence presented here in our experiment suggests that a single-sample plasma level of estrone sulfate, as performed by an ELISA, was reliable, less time consuming, and equally accurate for the detection of the presence of functional testicular tissue in
cryptorchids as the paired-sample hCG stimulation test in horses of all ages. Since the stallion testis produces high amounts of estrogen, it seems reasonable to diagnose the presence of testicular tissue by estrone sulfate measurement alone.

2. Material and Methods

Clinical samples were derived from the clinical endocrinology laboratory at the University of California at Davis. A highly sensitive, reliable, and accurate ELISA method was used to measure the serum estrone sulfate concentration in the male equine. This competitive, solid-phase, microtiter plate ELISA procedure was described and validated by Stabenfeldt et al. Serum estrone sulfate concentrations in 48 known cryptorchid horses (Equus caballus), 49 geldings, and six stallions of various breed and ages varying between 1 and 10 years old were measured. In addition, serum estrone sulfate concentrations in six intact and three castrated donkeys (E. asinus) with ages between 7 months and 8 years were also measured.

3. Results

The mean value for serum estrone sulfate as measured by an ELISA was 0.0714 ± 0.0193 ng/ml for geldings, 49.46 ± 13.13 ng/ml (p = 0.0005) for cryptorchids, and 167.07 ± 31.50 ng/ml (p = 0.003) for stallions (Fig. 1). There was a 95% CI (confidence interval) for geldings and cryptorchids (23, 75.8), a 95% CI for cryptorchids and stallions (−201, −34), and a 95% CI for geldings and stallions (−248, −86). Nine known cryptorchid horses ranging in ages from 12 months to 2 years had an estrone sulfate mean value of 8.95 ± 5.16 ng/ml (p = 0.0003) and a 95% CI (−415, −181). Estrone sulfate levels were detected in a small sample size (nine) in both intact and castrated donkeys, 49.33 ± 42 ng/ml and 0.78 ± 0.58 ng/ml, respectively, with p = 0.3 and a 95% CI (−60, 157). Standard serum testosterone concentrations to confirm the absence or presence of functional testicular tissue as performed by RIA were also measured in these horses to determine correlative values with estrone sulfate concentrations. Mean testosterone values as measured by RIA for the geldings and cryptorchids were 35.48 ± pg/ml and 500.04 ± 64.90 pg/ml, respectively (p < 0.0001; Fig. 2). A significant correlation (p = 0.01) was noted between serum testosterone and serum estrone sulfate concentrations in the cryptorchid horses.

4. Discussion

These results suggest that the enzyme immunoassay method for the detection of estrone sulfate concentrations in the male equine may be a useful and reliable diagnostic aid for the detection of cryptorchidism in the horses of all ages in a single sample. These results also provide preliminary evidence for the presence of serum estrone sulfate concentrations in the male donkey, although levels were lower in male donkeys than in horses. In this study the ELISA was able to detect estrone sulfate in donkeys and horses of all ages. To our knowledge, this is the first report comparing estrone sulfate levels in geldings, stallions, and cryptorchids by an enzyme-linked immunosorbent assay.

References