Development of a Tandem Hormone Assay for the Detection of Pregnancy in the Miniature Mare

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The measurement of serum estrone sulfate (E1S) and equine chorionic gonadotropin (eCG) can be used as an accurate, noninvasive technique for pregnancy detection in the miniature mare. Authors’ address: Depts. of Animal Science (Foristall and Roser) and Population Health and Reproduction (all other authors), School of Veterinary Medicine, University of California at Davis, Davis, CA 95616. © 1998 AAEP.

1. Introduction
Miniature mares have become increasingly popular over the past 10 years as pets, show horses, and breeding stock. Unfortunately, the small size of these animals has generally precluded the use of rectal palpation and ultrasonography. Therefore, many miniature horse owners have turned to hormonal methods for the early detection of pregnancy. To date, it has been assumed that the endocrinology of miniature horses is essentially the same as that of standard size horses, involving estrone sulfate (E1S) and equine chorionic gonadotropin (eCG) as the primary hormonal signals. The source of elevated E1S in maternal circulation after day 70 of gestation is the fetoplacental unit. Thus, E1S is a reliable indicator of fetal viability in the standard size mare.1,2 Equine chorionic gonadotropin, in contrast, is present in maternal circulation between 35 and 130 days of gestation.3,4 Levels of eCG can remain elevated in cases of fetal abortion and may lead to false-positive results if used as the sole determination of pregnancy status. In the miniature mare, the efficacy of an E1S and eCG analysis for pregnancy diagnosis is still unclear. Thus, the main objective of the current research was to validate the use of a tandem assay for E1S and eCG in miniature mares for the diagnosis of pregnancy.

2. Materials and Methods
This study involved two parts. The first was the serial measurement of E1S and eCG in pregnant (n = 14) and nonpregnant (n = 11) miniature mares. (Serum samples were obtained from these animals approximately once a week between the months of May and October, 1995.) The second was the measurement of E1S and eCG on serum samples from miniature mares that were submitted to the Clinical Endocrinology Laboratory at the University of California at Davis for pregnancy diagnosis between August of 1995 and March of 1997. Following a hormonal analysis of the samples, owners were contacted by phone or mail to verify the actual pregnancy status. Responses were received from 76% of the 82 owners or veterinarians contacted. Estrone sulfate in serum was analyzed by a com-
petitive microtiter plate enzyme immunoassay described by Stabenfeldt et al. The ability of each assay to detect pregnancy accurately was determined by calculating the sensitivity (percentage of correctly identified pregnant mares) and specificity (percentage of correctly identified nonpregnant animals) of the assay at various threshold levels for all samples. The sensitivity and specificity of each individual assay was then compared with the tandem assay in order to determine the most effective protocol for a diagnosis of pregnancy in the miniature mare.

3. Results
Although absolute concentrations varied, the overall profiles of secretion for both hormones were similar to those of standard size mares. Levels of E1S consistently rose by day 75, and levels of eCG consistently rose by day 42. Interestingly, some non-pregnant mares had higher E1S levels than pregnant mares, making the tandem assay essential for the diagnosis of pregnancy.

For the tandem assay, the thresholds that resulted in the best sensitivity (90.1%) and specificity (97.5%) for the diagnosis of pregnancy in miniature mares were at E1S > 6.0 ng/ml and eCG > 1000 ng/ml. Further improvements in sensitivity and specificity can be obtained by using a two-sample technique, which involves the measurement of eCG and an increase in E1S over time. Once eCG levels exceeded 300 ng/ml, an increase in E1S over 45–60 days indicated the presence of a viable fetus with 100% sensitivity and specificity.

4. Discussion
The present research provides data that support the use of a noninvasive, tandem hormone assay for pregnancy diagnosis in miniature mares. The use of E1S alone may result in false-positive results because of elevated baseline levels in nonpregnant mares, whereas eCG alone may result in false-positive results because of abortion. The use of the tandem assay improves the accuracy of a hormonal analysis as a tool for pregnancy detection in the miniature mare.

References