Evaluation of the PATHFAST Chemiluminescent Enzyme Immunoassay for Measuring Progesterone in Whole Blood and Serum of Mares

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Evaluation of a new chemiluminescent enzyme immunoassay, the PATHFAST assay system (PATHFAST), for measurement of circulating progesterone in mares was performed. Five mares at the mid-luteal stage were administrated a single i.m. injection of prostaglandin F2α analog (PGF2α; cloprostenol 250 µg/ml), and then blood samples were collected from the jugular vein at 0, 15, 30 and 45 min, at one-hour intervals until 24 and at 48 hr via a catheter in the jugular vein. To monitor the physiological changes in circulating progesterone in mares after induced luteolysis, concentrations of progesterone in whole blood and serum samples were measured by PATHFAST. In addition, concentrations of progesterone in serum samples measured by PATHFAST were compared with those measured by radioimmunoassay (RIA) and enzyme immunoassay (EIA). Using PATHFAST, the serum concentrations of progesterone in mares correlated highly with those of whole blood samples (r=0.9672, n=88). The serum concentrations of progesterone as measured by PATHFAST correlated well with RIA (r=0.9654, n=88) and EIA (r=0.9323, n=112). An abrupt decline in circulating progesterone in whole blood samples was observed within 2 hr (50%), followed by a gradual decline until 48 hr later. The results for progesterone in whole blood samples correlated highly with those in serum samples, and the declining pattern paralleled that of the serum samples. These results demonstrated that PATHFAST is useful in the equine clinic as an accurate diagnostic tool for rapid assay of progesterone within 26 min, using unextracted whole blood.

Key words: enzyme immunoassay, horses, PATHFAST, progesterone, radioimmunoassay

The physiological function of progesterone is to prepare the uterine endometrium for implantation of embryos and maintain uterine conditions for the growing fetus during pregnancy in females. Therefore, measurement of progesterone is useful for detecting luteal function and placental function in cyclic and pregnant mares. There is a need to rapidly measure progesterone in equine clinical sites for early diagnosis of luteal activity during the estrous cycle. It is also important to know the levels of circulating progesterone of estrous mares before covering with stallions to get normal fertilization and growing embryos. The PATHFAST assay system (PATHFAST) was developed as

a small, automated, bench top analyzer that uses a chemiluminescent enzyme immunoassay and can measure protein and steroid hormones in whole blood without extraction [1, 2, 4]. The purpose of this study was to evaluate the validity of PATHFAST for measurement of circulating progesterone in mares using whole blood and serum samples without extraction. In addition, concentrations of progesterone measured by PATHFAST were compared with values measured by radioimmunoassay (RIA) and enzyme immunoassay (EIA).

Materials and Methods

Animals

Five Thoroughbred mares (4–11 years old) in Iburi, Hokkaido, Japan, kept under natural conditions were used for measuring circulating progesterone. All mares were subjected to daily ultrasonographic investigation using a B-mode scanner (ALOKA SSD-620, Hitachi Medical Corporation, Tokyo, Japan), and their ovaries were monitored. After confirming the existence of a corpus luteum, all mares were administrated a single i.m. injection of 250 μg/ml of a synthetic analogue of prostaglandin F2α (F2α, Estrumate, Intervet, Tokyo, Japan). Blood samples were then collected from the jugular vein at 0, 15, 30 and 45 min, at one-hour intervals until 24 and at 48 hr via a catheter in the jugular vein.

Sample preparation and experiments

For the correlation experiments between whole blood and serum, whole blood samples were collected from the jugular vein into commercially supplied plastic tubes with heparin sodium as an anticoagulant. For collection of serum samples, whole blood was drawn from the jugular vein of the same mares into a plain blood collection tube. Serum was separated by centrifugation at 1,700 g for 10 min. Concentrations of progesterone in all samples were measured by PATHFAST. Serum samples were used for comparisons between PATHFAST and RIA or EIA.

Hormone assay. PATHFAST

Concentrations of progesterone in whole blood and serum samples were determined with the PATHFAST analyzer using the PATHFAST reagent kit for progesterone as described previously [1, 2, 4]. In brief, measurement of progesterone by PATHFAST was performed using a single reagent cartridge with 100 μl of whole blood and serum samples without extraction. In the competitive assays for progesterone, an alkaline phosphatase-conjugated antigen was used as the tracer to compete formation of immunocomplexes by progesterone in samples. Following the 5-min immunoreaction, separation of bound and free

hormones was performed using Magtration technology [2]. After the chemiluminescent substrate was added to the immunocomplexes, the amount of chemiluminescence was measured. The assay results were obtained within 26 min. The assay range of PATHFAST was 0.2–40 ng/ml. The intra-assay coefficients of variance were 3.61–10.23% for serum and 7.1–16.7% for whole blood samples.

RIA and EIA

Serum concentrations of progesterone were determined by a double-antibody RIA system using ^{125}I-labeled radioligands as described previously [5]. Serum samples and standard were extracted once with 2 ml ether. The assay results were obtained within 48 hr. The intra- and inter-assay coefficients of variance were 6.3 and 7.2%.

Serum concentrations of progesterone were also determined by double-antibody EIA system without ether extraction as described previously [3]. The assay results were obtained within 48 hr. The intra- and inter-assay coefficients of variance were 6.3 and 10.6%.

Statistics

Pearson's r was calculated to find correlation between variables using the GraphPad Prism 5 software for Windows (GraphPad Software, San Diego, CA, USA).

Results

The correlation of concentrations of progesterone in whole blood and serum samples measured by PATHFAST was examined (Fig. 1). There was excellent positive correlation between whole blood and serum samples (r=0.9672, P<0.0001 n=88).

The patterns of circulating progesterone in whole blood and serum measured by PATHFAST were compared in three mares after the single injection of PGF2α (Fig. 2). Significant correlation of concentrations of progesterone in whole blood and serum samples was observed in all mares. Circulating progesterone in whole blood and serum samples declined rapidly and had declined by about 50% at 2 hr after the PGF2α injection; the levels reached the basal levels in whole blood (mare A, 1.07 ng/ml; mare B, 1.69 ng/ml; mare C, 1.66 ng/ml) and serum (mare A, 0.84 ng/ml; mare B, 1.33 ng/ml; mare C, 1.36 ng/ml) at 24 hr after injection. A further decline in concentrations of progesterone was observed in both whole blood and serum samples at 48 hr after injection.

The concentrations of progesterone in serum samples measured by PATHFAST were compared with those measured by RIA (Fig. 3). The results obtained by PATHFAST showed a significant correlation with those obtained by RIA (r=0.9654, P<0.0001, n=88). The pattern of
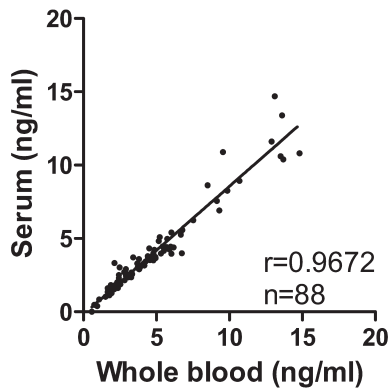
Fig. 1. Correlation plots between progesterone measured by PATHFAST in whole blood and serum samples of mares.
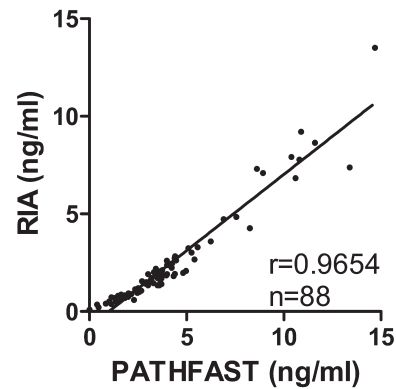

Fig. 3. Correlation plots between progesterone measured by PATHFAST and RIA in serum samples.
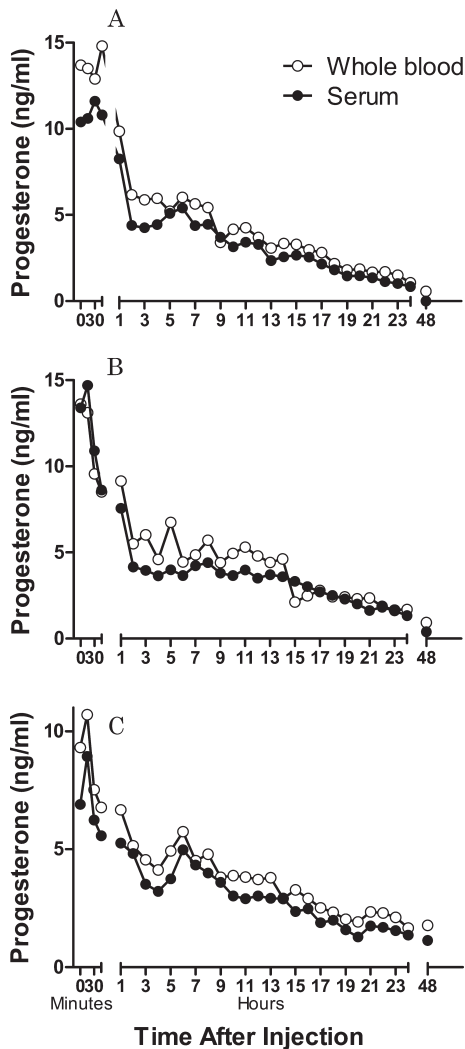

Fig. 2. Changes in circulating progesterone in whole blood (○) and serum samples (●) of three mares (A, B, C) assayed by PATHFAST after administration of a single i.m. injection of PGF2α.

circulating progesterone in serum obtained by PATHFAST showed excellent correlation with that obtained by RIA, although the values measured by PATHFAST were about two times higher than those measured by RIA (Fig. 4).

In addition, the concentrations of progesterone in serum samples measured by PATHFAST were also compared with those measured by EIA (Figs. 5 and 6). A high positive correlation was observed between the concentrations of progesterone measured by PATHFAST and EIA (Fig. 5, r=0.9323, P<0.0001, n=112). The concentrations of progesterone in the serum of the four mares measured by PATHFAST showed a parallel pattern to those measured by EIA (Fig. 6).

Discussion

The present study evaluated the validity of rapid measurement of equine progesterone using a new practical assay system, PATHFAST.

Measurements of progesterone in whole blood and serum samples of mares showed excellent correlation in the present study, indicating that concentrations of progesterone can be measured in whole blood as well as serum samples in mares using PATHFAST. In the experiment in which PATHFAST was compared with RIA and EIA, PATHFAST showed excellent correlation, with correlation coefficients of 0.9654 (n=88) and 0.9323 (n=112). In the present study, the concentrations of progesterone in serum measured by PATHFAST were about two times higher than those measured by RIA. Although the exact reason for this is not clear at the present time, it seems to be related to the different methods of sample preparation, such as extraction of steroid hormones using ether in RIA. In addition, the different cross-reactivities of the anti-progesterone sera
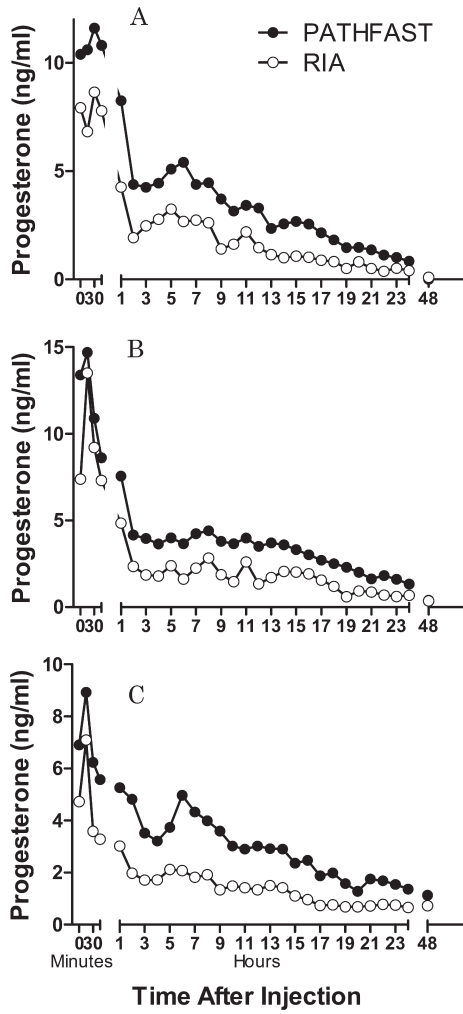
Fig. 4. Changes in circulating progesterone in serum samples of three mares (A, B, C) after administration of a single i.m. injection of PGF2α assayed by PATHFAST (●) and RIA (○).
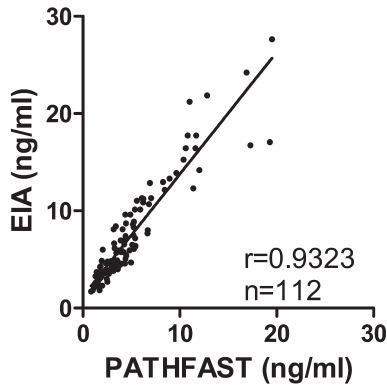

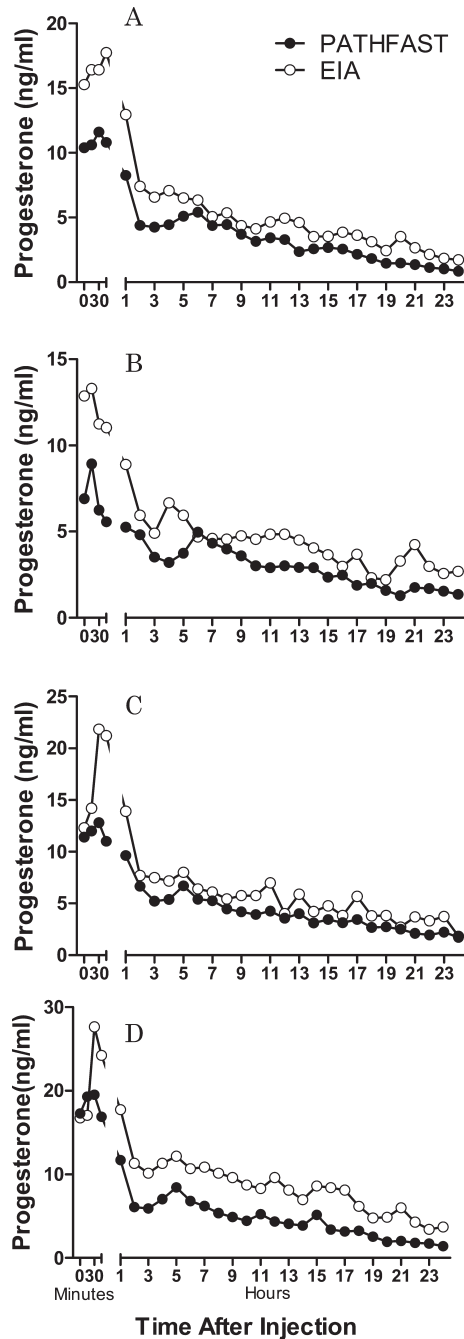**Fig. 5.** Correlation plots between progesterone measured by PATHFAST and EIA in serum samples.

Fig. 6. Changes in circulating progesterone in serum samples of four mares (A, B, C, D) after administration of a single i.m. injection of PGF2α assayed by PATHFAST (●) and EIA (○).

used in PATHFAST and RIA may affect estimation of the concentrations of progesterone. Physiological changes in circulating progesterone during the process of induced luteolysis in mares were examined using PATHFAST. Measurement of progesterone in whole blood and serum

samples showed excellent parallel patterns, indicating that PATHFAST can be used to measure circulating progesterone in mares. The parallelism of the pattern of progesterone levels in serum samples measured by PATHFAST and RIA was confirmed. This is also true for PATHFAST and EIA. These results clearly demonstrated that PATHFAST correctly monitored the physiological changes in circulating progesterone during induced luteolysis in cyclic mares.

In clinical application of progesterone measurement to diagnosis of reproductive condition in the equine clinic, there is a need for a rapid measurement system in addition to a measurement system that uses whole blood samples. In the present study, the concentrations of progesterone in both whole blood and serum samples could be measured by PATHFAST within 26 min without ether extraction. In conclusion, PATHFAST is useful in the equine hospital as an accurate diagnostic tool for rapid assay of progesterone.

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