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ELISA Analysis of Progesterone in Biological Fluids Using Cuvettes and a Densimeter

Dr. Terry Nett, Dr. Patrick McCue and Samantha Hollingshead from Colorado State University have developed a method to measure concentrations of progesterone in biological fluids (urine, serum, saliva) using a cuvette that can be read in a densimeter. The basis of the methodology is that an antibody that binds gamma globulin from the species in which the primary antibody was generated is coated to the cuvettes prior to the analysis. During the analysis, a primary antibody to progesterone is added to the cuvettes followed by a sample of biological fluid containing an unknown amount of progesterone along with a known quantity of progesterone conjugated to an enzyme that will react with a particular substrate to form a colored product. These reagents are incubated together for a fixed time and the reagents that have not bound to an antibody are removed by washing. The substrate that produces the color is added and allowed to react with the enzyme for a fixed time. The reaction is stopped by adding a reagent that inactivates the enzyme. The intensity of the color is quantified in the densimeter. The amount of progesterone in the biological sample is determined by comparing the amount of color in the unknown sample to the amount of color in samples containing known concentrations of progesterone.

Progesterone levels provide information to aid in determining whether a mammalian species that have a corpus luteum as part of their reproductive physiology have ovulated, have healthy luteal tissue, and have a sufficient source of progesterone required to maintain a pregnancy. This is particularly important in the equine family.

This technology allows for a quick, easy, and inexpensive means to determine progesterone levels using equipment available at most veterinary and reproductive facilities. In addition, this technology eliminates many of the costs and time delays of the current means of testing along with rapid results so patients aren't unnecessarily supplemented with expensive progesterone.

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