
EARLY PREGNANCY DETECTION IN RHINOCEROS SPECIES USING SERUM GLYCOPROTEINS

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Abstract

There are currently five rhinoceros species still surviving. The Javan (*Rhinoceros sondaicus*), Sumatran (*Dicerorhinus sumatrensis*), Indian (*Rhinoceros unicornis*), black (*Diceros bicornis*), and white (*Ceratotherium simum*) are each critically endangered and all but the Javan has captive populations in North American zoological institutions. Habitat destruction and poaching for the rhino's horn have led to the decreased numbers in the wild; therefore, captive populations need to be self-sustaining with successful reproduction programs. All of the rhino species are difficult to breed in captivity with early embryonic death, stillbirths, and uterine leiomyomas as part of the problems. In fact, fewer than 10% of sexually mature animals have successfully reproduced and hence are not represented in the current captive population. Therefore, an early detection pregnancy test would allow animal care staff to determine first if a rhino has conceived. The test can then be followed up with additional tests to see if she maintains the pregnancy through the first month as well as confirming the pregnancy at 5 mo with the progesterone spike characteristic of rhino pregnancies.

ECF or Early Conception Factor is a protein that is released when an egg is fertilized by a sperm cell that will help with pregnancy detection and diagnosis of conception problems. A lateral flow assay test has been developed for use in horses to detect the ECF protein by Concepto Diagnostics Corporation™. The test can detect the ECF glycoprotein factor in horse serum between 3 and 30 days after breeding. The ECF kits test mare serum using specific monoclonal-polyclonal antibodies with colloidal gold as the indicator. We hypothesize that because rhinoceros are part of the equid family the Concepto Horse ECF™ Test will help detect early pregnancy in captive Indian, black, white, and Sumatran rhinoceros species.

Using a cycling, rhinoceros female with recent breeding activity for the test, we collected fresh blood from her using the saphenous vein of the front leg. The blood was then centrifuged for 10 min at 3000 rpms to separate the serum. The serum was then pipetted out and one drop was placed on a paper towel and the second drop placed in the circular test window on the ECF test kit. The serum was then followed a few seconds later with two drops of assay buffer. The kit was then laid flat, undisturbed at ambient room 25°C for 2 hr. After 2 hr, the kit was read under fluorescent light to detect all lines. A red line was present in the "C" or control region of the indicator window meaning the test had been set up correctly. If the rhino is pregnant a second

red line, which can be faint will form in the “T” or test region, which was seen on the suspected pregnant rhino female. This procedure was then tested on a cycling, non-breeding, white rhinoceros female as a control for the test and she was negative for ECF on the test kit. To test the efficacy of the test on frozen serum the samples collected from each rhino, (both the pregnant female and the control female), were frozen for five days, thawed to room temperature, and vortexed vigorously for 5 min. The protein can stick to the sides of the serum sample tubes causing a false negative result in frozen samples so sufficient vortexing is necessary. The results produced using frozen serum were consistent with those from the fresh samples.

Using test kits, on further breedings and other rhinoceros species, determination will be made as to the accuracy of the test, the approximate testing window to detect ECF protein in the different rhino sp., and the viability of the test on fresh versus frozen serum. We will then confirm any positive or pregnant females by collecting serum progesterone levels, which should peak at 5 mo in pregnant rhinoceros. The ECF test is a revolutionary new way for animal care managers to confirm early pregnancy as well as early embryonic death and could lead to further conclusions that elucidate the cause of captive breeding problems in this difficult to breed equid group. Likewise, once the test is validated for rhino sp. the test can be tried on other endangered equid species.