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## THE QUEST FOR A RELIABLE BIOMARKER OF IRON OVERLOAD DISORDER IN THE RHINOCEROS

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### Abstract

It is well documented that zoo-managed black rhinoceroses (*Diceros bicornis*) and Sumatran rhinoceroses (*Dicerorhinus sumatrensis*) store excessive iron as hemosiderosis in body organ tissue, especially that of the liver;<sup>3</sup> however, the etiology of iron storage and the extent of its impact on rhinoceros health are obscure and likely complex. Recent research has revealed serum ferritin concentration is unreliable as a measure of iron overload disorder (IOD) severity in the rhinoceros.<sup>4,5</sup> Therefore, new diagnostic biomarkers are being pursued.

Hyaluronic acid (HA) is rapidly filtered by the liver of healthy individuals, and serum concentrations increase when the liver is compromised.<sup>2</sup> Two HA assays (Echelon Biosciences Inc., 675 Arapeen Dr., Suite 302 Salt Lake City, UT, 84108 USA [product number K-1200] and Corgenix, 11575 Main Street Suite 400, Broomfield, CO, 80020 USA [product number 029-001]) were employed for testing Sumatran rhinoceros serum samples ( $n = 71$ ). Although concentrations differed between assays, results were highly correlated ( $r = 0.92$ ;  $P < 0.01$ ). Hyaluronic acid concentrations were elevated in symptomatic individuals sick with IOD (Echelon assay values, mean  $\pm$  SD;  $1,084 \pm 359$  ng HA/ml) compared to those in both the same individuals when asymptomatic ( $278 \pm 174$  ng HA/ml) and another sick Sumatran rhinoceros ( $157 \pm 40$  ng HA/ml) with high serum ferritin concentrations ( $4,927 \pm 1,019$  ng/ml) that died of thyroid cancer with only mild hemosiderosis. In contrast, HA concentrations were not markedly elevated in samples from any of the eight outwardly healthy black rhinoceroses tested ( $180 \pm 60$  ng HA/ml; range, 119-280 ng HA/ml) despite the wide range in serum ferritin concentrations among individuals (140-127,891 ng/ml).

Under iron overload conditions, serum can contain non-transferrin bound iron, a fraction of which may be labile plasma iron (LPI). LPI is especially damaging because it is redox active, capable of permeating into organs and catalyzing the formation of reactive oxygen species (ROS) in tissues.<sup>1</sup> An LPI assay (Aferrix Ltd., Kiryat Atidim, POB 58129, Tel-Aviv 61581, Israel, FeROS™ LPI kit, product reference: TSL902 V.12) that relies on a selective iron chelator to quantify iron-mediated ROS generation was used to test rhino serum. Among Sumatran rhinoceros samples ( $n = 19$  samples from seven rhinoceroses), only those from symptomatic individuals sick with IOD tested positive for LPI; however, data for black rhinoceroses ( $n = 16$  samples from six rhinoceroses) were inconsistent. Black rhinoceroses that were LPI-positive were not always clinically ill, nor did they die within 3 yr of sampling. Interestingly, even in some LPI-negative black rhinoceros samples, ROS activity was substantial, suggesting another redox active agent (other than iron) may be present in the serum. In contrast, the ROS reaction was minimal in all Sumatran rhinoceros serum samples, even those with positive LPI values. Follow-up analyses are underway to identify the cause of the excessive ROS activity observed in black rhinoceros serum.

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These preliminary data suggest that 1) HA may be useful in diagnosing end-stage liver failure in rhinoceroses, 2) LPI may be an accurate indicator of end-stage IOD in Sumatran rhinoceroses, 3) test results differ between black rhinoceroses and Sumatran rhinoceroses, and 4) serum from some black rhinoceroses appears highly redox active even in the presence of an iron-specific chelator. Overall, results offer insight into alternative biomarkers that may prove useful for diagnosing rhinoceros IOD or liver failure. Furthermore, inter-specific variability in the results suggests that iron dysfunction may differ between the Sumatran rhinoceros and the black rhinoceros.

**Key words:** Black rhinoceros, hyaluronic acid, iron overload, labile plasma iron, reactive oxygen species, Sumatran rhinoceros

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