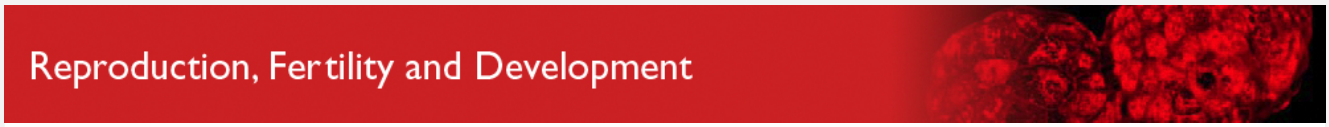

RESEARCH ARTICLE

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21 Investigating the role of serum progesterone, estrogen, and testosterone in cyclic and acyclic black rhinos

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Black rhinos (*Diceros bicornis*) are threatened with extinction due to poaching, making *ex situ* populations valuable genetic reservoirs for species survival. Black rhinos are characterised as a nonseasonal, spontaneously ovulating species with an average oestrous cycle length of 26 days. Although black rhinos have historically reproduced well *ex situ*, progesterone monitoring has revealed periods of acyclicity in a portion of individuals that may disrupt breeding management decisions and impact reproductive output. It is also unknown if ovaries of acyclic animals are active but fail to ovulate or are simply inactive. Oestrogen and testosterone have not routinely been included in female fecal hormone monitoring, but the ability to measure these hormones more precisely in serum could provide additional insight into ovarian dynamics, even in individuals that appear to cycle regularly according to fecal progesterone. Although individuals may display regular progesterone rises, hemorrhagic anovulatory follicle (HAF) formation rather than successful ovulation is known to occur in this species. Our goal is to determine whether serum oestrogen and testosterone can shed new light on rhino ovarian activity. Matched serum (once per week) and fecal (three times per week) samples were collected for 12 weeks from six female black rhinos housed at five institutions. Progesterone was measured in fecal samples using a traditional enzyme immunoassay (EIA, Arbor Assays), and rhinos were categorized as cyclic or acyclic based on these results. Serum samples from cyclic (n = 3; 16–26 years) and acyclic (n = 3; 13–26 years) females then were analysed for progesterone, oestrogen, and testosterone using a competitive assay that assesses multiple analytes concurrently (Luminex-based). The assay was validated for each hormone based on parallelism of serially diluted pooled extracts with the standard curve (R^2 > 0.9). Sample values were positively correlated with those produced with traditional individual EIAs (Pearson R > 0.8; P < 0.1). Average hormone values were significantly lower (P < 0.01; Mann–Whitney test) in acyclic than cyclic individuals for each hormone (117.2 ng/g vs 88.2 ng/g fecal progesterone, 1.51 ng/mL vs 0.44 ng/mL serum progesterone, 35.2 pg/mL vs 16.7 pg/mL serum oestrogen, and 33.0 pg/mL vs 16.1 pg/mL serum testosterone, respectively). Oestrogen and testosterone values fluctuated despite low progesterone in acyclic individuals, suggesting ovarian activity without ovulation. Interestingly, testosterone occasionally spiked in conjunction with both oestrogen and progesterone in cyclic animals. It is possible that cyclic animals are not successfully ovulating but forming HAFs, and testosterone may play a role. Ultrasound data will help elucidate the relationships between these hormonal fluctuations and ovarian activity. These serum hormone data represent the first step in gaining new insight into ovarian dynamics of the North American rhino population.

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