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Studies of male reproduction in captive African wild dogs (*Lycaon pictus*)

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Abstract

Implementation of assisted breeding in the captive African wild dog is restricted by a current lack of knowledge on their reproductive physiology and the apparent difficulty of effectively manipulating the complex social dynamic of the pack in order to conduct reproductive procedures. In this study, we describe protocols for the safe and repeated capture and restraint of the African wild dog ($n = 7$) as well as techniques for assessment of male reproductive function, semen collection and preservation. In a serendipitous finding, captive African wild dogs appeared to display significant seasonal change in male reproduction. Testicular volume and tone, spermatorrhea and the ability to collect semen by electroejaculation all increased significantly during late summer and then subsequently declined by early spring. While there were no detectable seasonal changes in testosterone concentration in the population as whole, the alpha-dominant male in both years of the study, had a highly elevated testosterone concentration compared to subordinate males. Semen collection by electroejaculation during the late summer was most effective in peri-pubertal males (15 months) when all seven electroejaculates were of adequate quality for cryopreservation. In the second breeding season (27 months), there were numerous changes in the pack hierarchy and electroejaculation was not as successful (3/7). The characteristics of electroejaculated semen collected in the breeding season are described for seven animals including the first descriptions and incidence of sperm abnormalities in the species. Semen ($n = 7$) was frozen using a Tris–citrate fructose buffer and final egg yolk and glycerol concentration of 20% and 4%, respectively. Sperm were loaded into 0.25 mL straws, frozen in liquid nitrogen vapor and then thawed at 37 °C. Initial post-thaw survival of spermatozoa was encouraging (% motile: $31.8 \pm 5.8\%$; rate: 2.8 ± 0.3; % intact plasma membranes: $33.4 \pm 5.3\%$ and the

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% of damaged acrosomes: 4.4 ± 1.5%) but following 2 h incubation at 37 °C, post-thaw survival declined markedly.

Keywords: African wild dog; *Lycaon pictus*; Semen collection; Semen preservation; Electroejaculation; Seasonal male reproduction; Testosterone; Alpha-male suppression; Testis

1. Introduction

The African wild dog or painted hunting dog is a "wolf-like" carnivore unrelated to the domestic dog. They represent a separate evolutionary line unique to Africa going back 15 million years. Painted dogs once occurred in large populations in ten of thousands across Africa but numbers in the wild have declined dramatically over the last thirty years due to habitat loss, predation, viral disease and persecution by man (Woodroffe and Ginsberg, 1997b). In 1997, only 3000–5000 dogs were estimated to be remaining in the wild and many of these were in small fragmented or genetically non-viable populations (Fanshawe et al., 1997). The African wild dog is currently considered by the world conservation union (IUCN) as one of most endangered canids in the world (McNutt et al., 2004).

Captive breeding of African wild dogs has been successfully established in a number of institutions both in and outside of Africa, resulting in what currently appear to be self-sustaining populations (Frantzen et al., 2001). There are approximately 300 wild dogs in captivity in 55 zoos, as listed on ISIS (International Species Information System) and as many as 200 animals occur in zoos or private collections, mostly in South Africa (McNutt et al., 2004). Nevertheless, these populations still need to be managed carefully to avoid inbreeding depression, genetic drift and stochastic events. As of December 2005, there were a total of 38 males and 37 female African wild dogs located across five Australasian zoos (Johnson et al., 2005), with the most abundant population based at Western Plains Zoo, Dubbo in central New South Wales. The effective genetic and reproductive management of this small population involves the regular movement of individual animals between institutions, a situation that is complicated in *Lycaon pictus* by its requirement for functioning in complex social groups.

Most African wild dog packs contain four to eight adults, a breeding pair with 2–6 yearlings and 5–11 pups (Woodroffe and Ginsberg, 1997a). The adaptive value to parents of incorporating post-pubertal young into the pack is that they can contribute to the parental care of their younger siblings (Courchamp and Macdonald, 2001). To overcome the managerial limitations of this complex social system, we propose the development of assisted breeding technologies such as artificial insemination, which would allow the movement of preserved sperm cells rather than live animals. This would result in improved animal welfare outcomes and reduce the cost and dangers of whole animal shipment and integration of an unrelated animal into an established pack. To date, there has been only one study that has examined assisted breeding technology in African wild dogs (Hermes et al., 2001) in which they developed protocols for the sonographical characterisation of the testicles and prostate and attempted to collect and cryopreserve semen from two males, one of which was subsequently found to have testicular pathology.

There are a number of limitations to the implementation of assisted breeding technology in African wild dog and these particularly concern the separation and re-introduction of individual males from the pack in order to conduct repeated anaesthesia and semen collection procedures. Animals that are separated from the pack should be drafted off and re-introduced to the pack with great care, in order to reduce subsequent aggressive encounters or rejection of pack members on