A Genetic Management Toolkit: Hormonal and Behavioural Research towards the Development of Artificial Insemination Technology in the African wild dog (*Lycaon pictus*)

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African wild dogs have a complex pack structure with separate male and female dominance hierarchies in which reproduction is typically exclusive to the alpha male and female. Current efforts to maintain genetic diversity involve translocation of live animals in captivity, a process that involves the combination of male and female single-sex groups to form a new social pack. Due to their complex social structure, such introductions are difficult: regularly leading to aggression and injuries to the animals. Moreover, this may not result in breeding among genetically valuable individuals if they do not become dominant.

To overcome these translocation-associated problems of intra-pack aggression our team has studied a number of facets of basic reproduction as well as stress and behavioural management in African wild dogs with the goal of developing strategies for semen cryopreservation and artificial insemination as a key to the introduction of new genes into existing stable packs (Van den Berghe *et al.* 2012, *Anim Reprod Sci* 133, 1-9). We began by establishing detailed reproductive and adrenal hormone profiles from multiple females in several reproductive states in both captive and free-ranging populations. Faecal samples from captive females in four European institutions and from wild males and females in South Africa were analysed for oestradiol, progestagens, testosterone and glucocorticoids. Captive data demonstrated that female wild dogs have reproductive characteristics similar to other canids, such as spontaneous ovulation and obligate pseudopregnancy. Almost all adult females became pseudopregnant, implying that the mechanism of reproductive suppression in this sex is likely to be behavioural rather than physiological. This was supported by data from free-ranging populations. Adrenal activity in captive and free-ranging animals was measured by faecal cortisol metabolites. Captive females had higher concentrations than free-ranging females. Glucocorticoid concentrations were more variable in captive individuals, whereas in both wild males and females, they were highest during gestation and denning. Adrenal activity in males, but not females, was also affected by age, with the highest glucocorticoid concentrations measured in yearlings.

We have also investigated the use of dog appeasing pheromone (DAP) collars to down-regulate aggression associated with the formation of new packs in captivity. Our initial findings indicate that DAP may reduce baseline stress levels in females and, when applied during regrouping of same-sex individuals, results in a relative reduction in observed levels of aggression.

Building on these findings, work is underway to develop a semen bank and artificial insemination techniques. Although reproductive suppression in females is likely to be behavioural, the effect of dominance on male fertility is still unresolved. We have begun investigating the relationship between hierarchy and sperm quality across the seasons. This will guide our development of a

robust semen collection and freezing protocol that will permit the establishment of a high-quality semen bank. We are also progressing with non-invasive techniques to predict ovulation for timed artificial insemination of females. These techniques must first be established in captivity before our ultimate goal to implement them as tools for genetic meta-population management in semi-captive and free-ranging populations.