

## **Cheetah Conservation Fund (CCF) Announces Breakthrough in Cheetah Reproductive Research**

*Otjiwarongo, Namibia* - The first ever *in vitro* cheetah embryos that have developed to the blastocyst stage have been produced at the **Cheetah Conservation Fund (CCF)** in collaboration with the **Smithsonian Institution** and the **University of California, Davis, USA**.

The oocytes (eggs) were recovered from some of CCF's captive cheetahs, and inseminated with sperm *in vitro* (in the lab). During this procedure new culture systems were implemented to optimize embryo development. Embryos were assessed and the **first ever cheetah embryos to reach the blastocyst stage** was frozen for storage in the Cheetah Conservation Fund's Genome Resource Bank, a reservoir of frozen genetic and biological materials. The oocytes (eggs) used to produce these historical embryos were collected from Nestlé and Hershey --two of CCF's resident non-releasable cheetahs, of which four blastocysts from Nestlé were frozen. What makes this even more exciting is that the sperm used were collected over a year ago from a CCF's resident male, Cruise, and had been frozen and stored in the Genome Resource Bank. This proves that the techniques researched at CCF to freeze and thaw cheetah sperm are viable.

Last year another major breakthrough was made when the timing of aspiration (collection) of oocytes was established at 28 to 30 hours post hormone treatment. Building on what was discovered last year, scientists were able to take this research to the next level with the help of the captive cheetahs living at CCF, as a large enough sample size was needed in order to test different incubation conditions needed by embryos to develop successfully. All these methods have been established using domestic cats as models, but need to be adapted for each wild carnivore species.

While *in vitro* fertilisation is relatively routine in many other species, including humans, carnivores have proven to be a challenge when it comes to assisted reproduction and pioneering methodologies need to be developed. In order to successfully grow cheetah embryos *in vitro*, the correct temperature, CO<sub>2</sub> and growth mediums needed to be established that are very specific to cheetah embryos. Dr. Pierre Comizzoli, a veterinary reproductive physiologist from the Smithsonian Institution's National Zoo has been studying *in vitro* processes in antelope and carnivores for over 14 years. Dr. Comizzoli said, "This groundbreaking research has been made possible by the long-term collaboration between the Smithsonian and CCF, which has proven invaluable for the conservation of this unique species."

Dr. Adrienne Crosier, a reproductive physiologist from the Smithsonian Institution's National Zoo, and team leader said, "Typically, cheetahs reproduce poorly in captivity and the efficiency of reproduction for female cheetahs drops after ~8 years of age. This research, initiated in December 2005, is the first of its kind on any of the non-domestic carnivore species and is evaluating the physiology of the influence of age on cheetah reproduction. Understanding how oocyte quality and uterine morphology and anatomy are affected by age are vital to improving assisted reproductive techniques for the cheetah." Dr. Autumn Davidson, veterinarian and reproductive specialist and Tom Baker, a specialist in ultra-sound technology, both from the University of California, Davis, USA, used ultrasound technology to investigate the morphology of female cheetah reproductive tracts.

This study includes 33 cheetahs from seven facilities in two countries. Information gathered will conclude a long-term project investigating reproductive health in older female cheetahs and documenting reproductive senescence in these felids. Dr. Laurie Marker, CCF's Executive Director, said, "This breakthrough in cheetah reproductive research has far-reaching implications for the conservation of cheetah and demonstrates the benefits of an integrated approach of both captive and wild cheetah conservation programmes to ensure the survival of the species."