Durrant BS. 1989. Coprehensive analysis of cryopreservation techniques for semen of the cheetah.

Keywords: Acinonyx jubatus/artificial insemination/breeding/cheetah/cryopreservation/reproduction technology/semen/zoo

Abstract: The potential of artificial reproductive techniques of enhance captive propagation of endangered species is a topic of much discussion within the zoo community. The most widely applicable artificial reproduction technique is artificial insemination (AI). It's use makes possible the breeding of physically or behaviorally incompatible pairs and brings together animals separated by space or time. The latter, of course, depends upon semen cryopreservation for transport or long-term storage.
If natural reproduction cannot be stimulated, artificial techniques are empolyed in an increasingly aggressive strategy. Hormonal induction of estrus and ovulation followed by natural breeding and gestation is the least invasive technique and has been successful employed at the Center for Reproduction of Endangered Species (CRES).
Preliminary results in following experiment is encouraging: fresh and cryopreserved cheetah sperm will penetrate ova.

COMPREHENSIVE ANALYSIS OF CRYOPRESERVATION TECHNIQUES FOR SEMEN OF THE CHEETAH (*Acinonyx jubatus*)

F I N A L R E P O R T

Barbara S. Durrant, Ph.D.
Reproductive Physiologist

Center for Reproduction of Endangered Species (CRES)

Zoological Society of San Diego
P.O. Box 551
San Diego, CA 92112
(619) 557-3947

INTRODUCTION

The potential of artificial reproductive techniques to enhance captive propagation of endangered species is a topic of much discussion within the zoo community.

The most widely applicable artificial reproduction technique is artificial insemination (AI). It's use makes possible the breeding of physically or behaviorally incompatible pairs and brings together animals separated by space or time. The latter, of course, depends upon semen cryopreservation for transport or long-term storage.

Embryo transfer (ET) and *in vitro* fertilization (IVF) are applicable to specific cases of infertility that cannot be remedied through less invasive protocols. Their use in the propagation of captive species as in the livestock industry is limited (approximately 85% of America's dairy cattle are produced annually by AI whereas 3% are the result of ET).

Even more manipulative procedures such as embryo splitting and inner cell mass transposition will be useful for an extremely small number of individuals, and are unlikely to be of significant value to endangered species propagation efforts.

At the Center for Reproduction of Endangered Species (CRES), a multidisciplinary approach to reproduction comprises basic and applied research in physiology, genetics, behavior, and infectious disease. A strong commitment to the development and implementation of non-invasive research and animal management methods guides experimental design.

After the genetic and somatic health of an individual, pair or group is confirmed, solutions to captive breeding problems are sought through behavioral observation and modification.

If natural reproduction cannot be stimulated, artificial techniques are employed in an increasingly aggressive strategy. Hormonal induction of estrus and ovulation followed by natural breeding and gestation is the least invasive technique and has been successfully employed at CRES.

The next step, artificial insemination and semen cryopreservation comprise the foci of CRES' reproductive physiology program.

ANIMALS

The cheetah's increasingly dwindling numbers in the wild and poor captive breeding performance make it a species in need of intensive research efforts.

The Zoological Society of San Diego's cheetah colony is housed in a 12-acre off-exhibit facility at the Wild Animal Park. This breeding facility is managed by CRES with the assistance of an in-house propagation group comprising scientists, veterinarians, and curators. Of the 12.14 cheetahs currently residing at the facility, 10.10 are adults and 9 are proven breeders.

All adult males have been electroejaculated for semen analysis. Three of these (two hand-raised and one mother-reared) have been trained to service an artificial vagina for frequent sample collection without chemical or physical restraint.

METHODS

Experimental semen cryopreservation protocols are designed to compare the effects of various diluent compositions and pHs, packaging methods and freeze rates.

Semen samples are thawed in a 37°C water bath and capacitated *in vitro*. Motility, speed of progression and morphology at thaw are assessed and compared to pre-freeze values. Sperm is then coincubated with denuded superovulated hamster ova. Ova are subsequently fixed, stained and examined for evidence of sperm penetration (swollen sperm heads or male pronuclei within the egg cytoplasm). Frozen human sperm and fresh cheetah sperm serve as controls.

RESULTS

Both fresh and cryopreserved cheetah sperm will penetrate hamster ova. Three swollen sperm heads are visible in the attached photomicrograph of frozen cheetah sperm on the hamster ova assay. Associated sperm (A) are attached to the vitilline membrane but have not penetrated the ovum. Of the assays performed during this grant period, 22% of fresh and 15% of frozen sperm samples have penetrated hamster ova.

These preliminary results are most encouraging. However, at this time, insufficient data have been gathered to indicate which cryopreservation protocol results in optimal post-thaw viability.

FUTURE STUDIES

The failure of some fresh and frozen sperm samples to achieve penetration

may be the result of incomplete capacitation or less-than-optimal coincubation conditions. Maximum capacitation will be attempted through a series of experiments comparing media, temperature, and duration of sperm culture prior to coincubation with ova. Coincubation conditions will be optimized through variation of media, temperature, and duration.

BUDGET

Nixon Griffis funds were utilized in the manner described in the project proposal. All monies have been spent except "publication costs." When sufficient data have been analyzed, they will be published in an appropriate peer-reviewed journal and the NGFZR will be acknowledged for its support of the project.