IEF Research Project Grant Report

a. Project Title: Investigating a Low Tech Method of Cryopreserving Elephant Sperm

b. Final report

c. Principle Investigator: Laura H Graham, PhD; University of Guelph, Guelph, Ontario, Canada 519-824-4120, ext 56516; endolaura@yahoo.com
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d. Project Starting Date: January 2013

e. Project Completion Date: January 2014

2. Overall and specific conservation needs this project addressed

The population of Asian elephants in captivity in North America is currently not self –sustaining (Wiese 2000). There are numerous reasons for this, not the least of which is the lack of bull elephants in captivity. As a result, many zoos are now attempting artificial insemination (AI) to increase reproduction and maximize the genetic diversity of the captive population. Cryopreservation of semen for use with artificial insemination has the potential to be a valuable tool in the management of captive elephants. Cryopreserved semen has the advantage that it can be collected at any time and frozen indefinitely until needed, overcoming the need to collect semen shortly before it is needed and the risk of quality inconsistency that is found with chilled semen.

3. **Project Objectives**

Hypotheses

Our goal is to develop a method of Asian elephant semen cryopreservation that can be employed successfully and cost-effectively in a zoo situation.

Objective: Our objective is to build on previous studies and test the following two hypotheses.

Hypothesis 1: If previous success in the cryopreservation of Asian elephant semen (Saragusty et al., 2009) was due to controlling ice crystal formation during the cryopreservation process then manual seeding of extended semen will result in superior post-thaw sperm quality compared to samples that are not seeded.

Hypothesis 2: If some of the loss in post-thaw semen quality in Asian elephant sperm is due to large ice crystal formation during the thawing process then thawing the sperm at high temperatures will result in superior post-thaw quality compared to samples that are thawed at 37C.

In addition to assessing the effect of seeding and different thaw temperatures on post-thaw semen quality we also assessed the effect of very fast freeze rates in a dry shipper on post-thaw semen quality as well as the affect of storage at 4C prior to cryopreservation on post-thaw semen quality.

4. Specific Actions

A total of 29 semen collection attempts were made on four Asian elephant bulls. Of those 29 attempts, 14 ejaculates met our quality criteria to be included in the published study. The ejaculates were treated as described in the grant proposal with additional treatments of fast freeze rates (1cm and dry shipper) and storage at 4C for 24 hours prior to cryopreservation.

5. Changes from the original proposal

We only included 3 bulls in the data that is to be published as the fourth bull (St Louis) did not provide good samples and was logistically difficult to collect. To compensate for that we increased the number of collection attempts on the other three bulls.

6. The following is the abstract for the scientific article to be submitted for publication.

The specific objectives of the present study were to investigate the effects of manual seeding, differing freeze and thaw rates as well as storage for 24 hrs at 4°C prior to cryopreservation on post-thaw sperm quality in Asian elephants. Extended semen was cooled in an equitainer to 4°C, frozen in liquid nitrogen vapour at various rates with and without manual seeding or in a dry shipper and thawed at 37, 50 and 75°C. There was a significant effect of freeze rate on post-thaw motility ($P<0.0001$) and acrosomal integrity ($P<0.005$). The faster freeze rates in the dry shipper and at 1cm or 2 cm above liquid nitrogen consistently provided better cryopreservation than slower freezing rates. Thaw temperature had no effect on post-thaw semen quality but there was an interaction between freeze and thaw rates with higher thaw rates resulting in superior post-thaw semen quality in straws frozen at fast rates. Storage of samples prior to freezing had a detrimental effect on post-thaw semen quality. In summary, our results indicate cooling extended semen in an equitainer and cryopreserving it by placing straws directly in a dry shipper is a simple technique for effectively cryopreserving Asian elephant semen in the field or zoo.

7. Yes our project was successful. From the scientific article to be submitted for publication:

To our knowledge this was the first study to assess different freeze rates, including the use of a dry shipper, different thaw temperatures and seeding on post-thaw quality of cryopreserved Asian elephant semen. Our results indicate that faster freeze rates and higher thaw temperatures can significantly improve post-thaw semen quality in Asian elephants. Our overall goal was to develop a method of Asian elephant semen cryopreservation that can be employed successfully and cost-effectively in a zoo or field situation. The use of an equitainer to cool semen, a dry shipper to cryopreserve semen and thawing at temperatures of at least 50°C results in acceptable post-thaw semen quality as high as any previously reported in Asian elephants using expensive and technically challenging techniques. This is a breakthrough in the cryopreservation of Asian elephant semen, employing simple and inexpensive techniques and equipment that can be used in zoos and in the field alike.

8. Summary of Progress and Results Achieved

This was the first study to assess different freeze rates, including the use of a dry shipper, different thaw temperatures and manual seeding on post-thaw quality of cryopreserved Asian elephant semen. Extended semen was cooled in an equitainer to 4°C, frozen in liquid nitrogen vapour at various rates with and without manual seeding or in a dry shipper and thawed at 37, 50 and 75°C. There was a significant

effect of freeze rate on post-thaw motility (P<0.0001) and acrosomal integrity (P<0.005). The faster freeze rates in the dry shipper and at 1cm or 2 cm above liquid nitrogen consistently provided better cryopreservation than slower freezing rates. Thaw temperature had no effect on post-thaw semen quality but there was an interaction between freeze and thaw rates with higher thaw rates resulting in superior post-thaw semen quality in straws frozen at fast rates. Storage of samples prior to freezing had a detrimental effect on post-thaw semen quality. In summary, our results indicate that faster freeze rates and higher thaw temperatures can significantly improve post-thaw semen quality in Asian elephants. Our overall goal was to develop a method of Asian elephant semen cryopreservation that can be employed successfully and cost-effectively in a zoo or field situation. The use of an equitainer to cool semen, a dry shipper to cryopreserve semen and thawing at temperatures of at least 50°C results in acceptable post-thaw semen quality as high as any previously reported in Asian elephants using expensive and technically challenging techniques. This is a breakthrough in the cryopreservation of Asian elephant semen, employing simple and inexpensive techniques and equipment that can be used in zoos and in the field alike.

9. Organizations

Dept of Animal and Poultry Science, University of Guelph – employer of PI Dr. Laura Graham

African Lion Safari, Cambridge, Ontario – employer of Charles Gray and Sebastian Mitchell both of whom were instrumental in the collection of ejaculates from all the bulls. In addition, one bull used in the study was housed at the African Lion Safari.

Columbus Zoo – one of the bulls used in the study was housed at Columbus Zoo

Rosamond Gifford Zoo – one of the bulls in the study was housed at this zoo

Cincinnati Zoo – all processing of the samples from the bulls housed in the US was performed at the laboratories of CREW.

10.

BUDGET ITEM	Cost	Total
Liquid nitrogen	$750	$750
Chemicals	$998	$998
Disposable Laboratory Supplies	$1000	$1000
Dry shipper	$800	$800
Travel (mileage) African Lion Safari	$27 x 10	$270
Travel (mileage) St Louis Zoo	$640 x 1	$640
Travel (mileage) Columbus Zoo	$350 x 10	$3500
Travel (mileage) Rosamond Gifford Park	$216 x 8	$1728
Cincinnati Zoo	$405 x 2	$810
Travel Lodgings/food	$120 * 3	$360
TOTAL		$10856

11. see attached

12. Paper will be submitted for publication in Reproduction, fertility and development in 2014.