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**Final report
Report period 2005**

**Cryopreservation of Elephant Semen using
Directional Freezing**

We apologize for the long delay in handing in a final report to the IEF on our received grant. There is little excuse, but with the frustrating results we generated, we were rather embarrassed with the poor outcome of our funded project and reluctant with its summary.

In the course of this project we collected semen for the purpose of cryopreservation using directional freezing from 6 elephant bulls. 20 semen samples were collected from four Asian elephants (Munich, Whipsnade, Perris CA, Hannover) and three African elephants (Colchester, West Midlands). Out of 24 semen samples only four samples were suitable for freezing. The general quality of sperm samples across donors was unexpectedly pure and even if good on one day pure on the next (table1).

A total of only four samples were frozen - thawed, three of which by directional freezing technique in large volume and one over nitrogen vapour in 0.5 ml straws. The samples were extended and frozen using a TES / TRIS buffer media containing 16% egg yolk and 6% DMSO as cryoprotectant (Hermes et al., 2005). All frozen samples were from Asian elephants - three from a bull in Hannover (Calvin) and one from a bull in Whipsnade (Emmet).

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Thawing results

Post thaw motility:

Upon thawing motility ranged between 0 and 6% for all samples. The samples were incubated at 37°C for about 20 min and then evaluated again. For Calvin there was no change in the sample frozen in a straw and very little improvement for the sample frozen in tubes. Emmet sample's motility went up after incubation from 5% to 35% total motility.

Post thaw sperm morphology:

- Calvin
In the two samples frozen by directional freezing 59 % of the cells had normal morphology and 34% had damaged acrosome.
In the sample frozen in a straw 48% had normal morphology and 37% had damaged acrosome.
- Emmet
Sample frozen by directional freezing had 54% normal morphology, 23% damaged acrosome.

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This (very) limited post thaw morphology data seemed to indicate that directional freezing indeed is less damaging to the sperm than conventional freezing in straws. Post thaw motility results 0 – 6 % and one maximum of 35 % post thaw motility were well below acceptable values. Published post thaw elephant semen values were not met or surpassed in this study (Thongtip et al., 2005).

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In conclusion the value of consistently good semen samples is crucial for any further progress in elephant semen preservation, cryopreservation or even semen sexing research. Once a consistently good semen donor is identified research should be concentrated on this male to establish working protocols even if the scientific value of n=1 is limited. In the present study we were not able to test our hypothesis in depth and were not able to draw conclusions to our satisfaction. From horses and rhinos (Saragusty et al., 2007, Hermes publication in press) we learnt that directional freezing is a superior freezing technology. Only with directional freezing as a standard freezing technology and a reliable very good semen source the composition of freezing media and thawing protocols can be broadly investigated. Further improving frozen-thawed sperm results might summit at post thaw semen quality suitable for use in AI programs.

With the use of directional freezing the following aspects should be considered as guideline for further investigations:

- Varying cryoprotectants at different concentrations
- cell membrane protection at different concentrations (skimmed milk, egg yolk)
- Testing different buffer solutions as base to the cryoprotectant media.
- Different thawing temperatures

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Sincerely,

Robert Hermes
Reproduction management

Table 1. Semen collection results in Asian and African elephants

Asian Elephants

→ mean ± SD

name	volume (ml)	fractions	<u>motility</u> (%)	concentration (x 10⁶/ml)
Tusko	2.9 ± 3.0	1.5 ± 0.7	66.1 ± 28,1	197,5 ± 17.7
Gaijendra	9.8 ± 7.6	2.7 ± 1.5	0.9 ± 1.7	965.5 ± 632.9
Emmet	35.5 ± 43.7	3.6 ± 0.9	52.3 ± 15.4	51.8 ± 52.3
Calvin	38,8 ± 1.8	1,5 ± 0.7	86,75 ± 9.5	1167,5 ± 1226.8

African Elephants

→ mean ± SD

name	volume (ml)	fractions	motility (%)	concentration (x 10⁶/ml)
Jack	108.0 ± 32.2	5 ± 0	7.5 ± 10.6	350.0 ± 28.3
Tembo	14	2	32.5	65.0
Tooth	21.0 ± 7.1	3.8 ± 1.0	25.5 ± 30.6	382.5 ± 46.3