Shipping Sperm Frozen Samples in Dry Ice

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Arabic: علم الحياة المتجمد, Bulgarian: Криобиология, German: Kryobiologie, Persian: سرد زیست شناسی, French: Cryobiologie, Korean: 저온생물학, Indonesian: Kriobiologi, Icelandic: Lághitalíffræði, Italian: Criobiologia, Hebrew: קריוביולוגיה, Japanese: 低温生物学, Portuguese: Criobiologia, Russian: Криобиология, Tagalog: Kriobiyolohiya, Turkish: Kriyobiyoloji, Ukrainian: Кріобіологія.

EMMAService WP5 Technology Development

•*Task 4 - Transportation of unfrozen embryos;*

INFRAFRONTIER-I3 PROJECT *WP5 Technology Development and Implementation*

•*Task 1 - Optimising the procedure for transporting of both, frozen/thawed sperm and unfrozen sperm in the absence of liquid nitrogen;*

•*Cryopreservation and transport of Mouse Sperm at -79℃*

General considerations

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- ✓ Impact of transport on animal welfare;
- ✓ Compliance of national laws to European directive 2010/63/EU
- ✓ Need to reduce live animal transport (IATA);
- ✓ LN2 dry shipper transport (IATA);
- ✓ Distribution of thousands of new mouse lines from large-scale targeted mutation programs;
- ✓ Operator's safety;
- ✓ Risk of pathogen transmission (contamination of infrastructures, etc.);
- ✓ Economic impact - Logistics;

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND COUNCIL on the protection of animals used for scientific purposes (2007/526/EC)

Guidance on the transport of laboratory animals. Report of the Transport Working Group established by the LASA. Laboratory Animals (2005) 39, 1–39

Lufthansa stopped transporting lab animals, in response to pressure from animal rights groups!

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SPIEGEL ONLINE
04/26/2012 02:03 PM

Animal Rights Conundrum

Does Getting Lab Animals Off Planes Really Help Them?

By Laura Höflinger

Protests from animal rights organizations have prompted many airlines to stop transporting monkeys and other animals slated for use in laboratory experiments. But could this actually be causing more harm than help?

The animal rights organization PETA considered the contents of the Lufthansa jet's cargo hold scandalous. Twenty-four hours later, Lufthansa agreed.

Images of beagles -- with their floppy ears and trusting eyes staring out from behind the bars of their crates -- appeared on the PETA website. Some 50 animals were being flown to Scotland, the group claimed, to the Charles River Laboratories, where they were to be used in experiments.

Before long, the German airline was flooded with hundreds of angry emails. On Facebook, posters berated Lufthansa for contributing to the torture and killing of animals. The company gave in and announced that it would not transport any more dogs or cats being used in experiments.

In the battle over animal research, many activists are directing their attacks at the weakest link in the chain: the transporters. Many airlines have now refused to carry lab animals -- and especially primates, which stir the most controversy.

For years, British Airways has refused to allow lab monkeys into its cargo holds. In the US, no major airlines transport lab animals anymore. And, in Europe, the only international carrier to still accept them onboard is Air France. The airline has defended its stance on its website, saying that the company is convinced of the benefits of animal experiments for biomedical research. Even so, in March, the airline reportedly refused to transport 60 monkeys from Mauritius to the US.

A Battle between Image and Safety

"We cannot understand how some airlines are unwilling to transport laboratory animals even though they continue to take pets, zoo animals, racehorses and animals used in agriculture," says Siegfried Throm, director of research, development and innovation at Germany's Association of Research-Based Pharmaceutical Companies (vfa).

Still, for the airlines, it is time-consuming and costly to transport some animals. With monkeys, for example, airline employees have to wear protective clothing when taking them on and off planes, and the cargo hold has to be disinfected after the flight. Above all, though, the airlines are worried about getting a bad image.

"It is a huge problem when a minority uses undemocratic and opaque methods to determine what should be allowed regardless of what the laws say or what people want," says Stefan Treue, director of the German Primate Center (DPZ) in Göttingen, about the influence of animal rights activists.

The DPZ supplies German universities and research institutes with primates from its own breeding program. Of the 1,400 animals at the facility, between 40 and 80 are sent to scientific institutions each year. As a result, the DPZ claims that German academic researchers hardly have to import any primates.

In 2010, experiments were carried out on 2,789 primates in Germany, or fewer than in previous years. Most of them are used in industrial research, in tests aimed at determining whether substances are poisonous before they are tested on humans.

A Lucrative Business

A large number of the laboratory monkeys used in Western labs originate from China, Vietnam and Mauritius. They come from those countries for the same reason many clothes and toys are produced there: the price. A Rhesus monkey raised in Germany at the DPZ costs more than €5,000 ($6,600) and is subsidized by taxpayer money. The real costs are at least twice that much. A monkey from Africa or Asia, on the other hand, costs between €1,000 and €2,000, including transport.

It's a lucrative business. In China alone, there are about 40 breeding facilities, which supply roughly 70 percent of the laboratory primates in the US. Last year, the US imported 18,044 of them.

Most of the primates are flown to the US on Chinese airlines, making the journey in wooden crates in the cargo hold. A US animal rights organization recently tracked the odyssey of 100 macaque monkeys flown from Indonesia to the Philippines and, from there, to San Francisco. Once there, they were transported to Louisiana by truck. The entire journey lasted 56 hours.

Still, even shorter flights and quarantines put the animals under stress. During the trip, the animals can develop lung infections, suffer from dehydration or show behavioral changes. At the end of a trip, many are found dead in their crates.

Other Targets of Protest

Given the protests and shrinking number of airlines willing to transport animals, pharmaceutical companies and universities are starting to have more of their laboratory animals transported via overland routes. But since these journeys require more time, could it be that the protests are actually increasing the number of strains on lab animals?

Irmela Ruhdel of the German Animal Welfare Federation disagrees, saying: "The more difficult it is for scientists to get animals, the more they will think about using alternative methods."

Activists are also using protests in an effort to block different ways of transporting animals, and with some success. In January, the last ferry company stopped shipping lab mice from continental Europe to Britain.

Translated from the German by Mary Beth Warner

URL:

http://www.spiegel.de/international/world/0,1518,829779,00.html

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Animals

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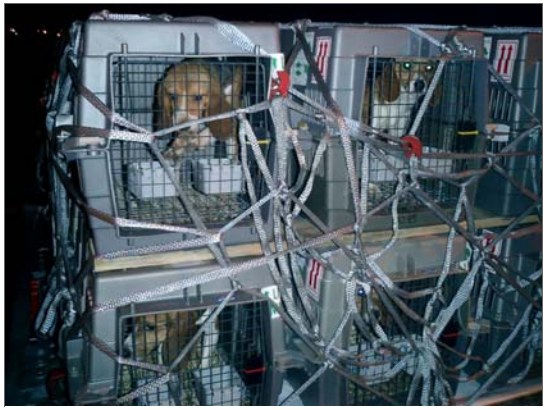

PETA

Lufthansa stopped transporting lab animals, such as these beagles, in response to pressure from animal rights groups.

From the origins…

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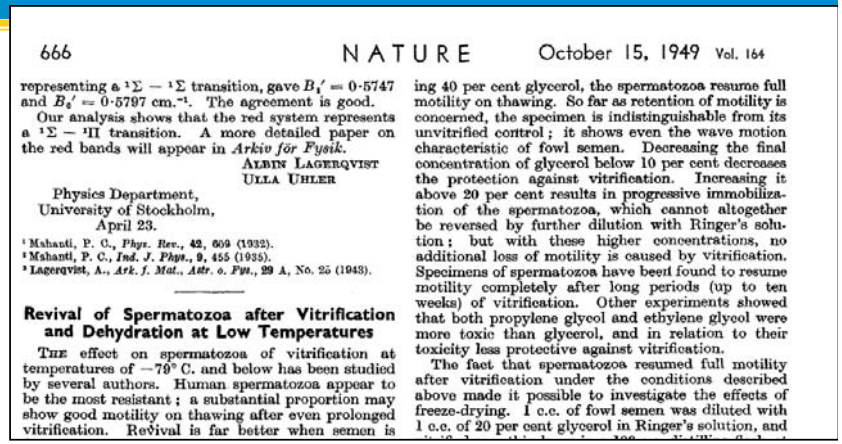
Polge, Smith and Parkes; Nature 1949



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representing a $^1\Sigma - {}^1\Sigma$ transition, gave $B_1' = 0{\cdot}5747$ and $B_2' = 0{\cdot}5797$ cm.$^{-1}$. The agreement is good.

Our analysis shows that the red system represents a $^1\Sigma - {}^1\Pi$ transition. A more detailed paper on the red bands will appear in *Arkiv för Fysik*.

ALBIN LAGERQVIST
ULLA UHLER

Physics Department,
University of Stockholm,
April 23.

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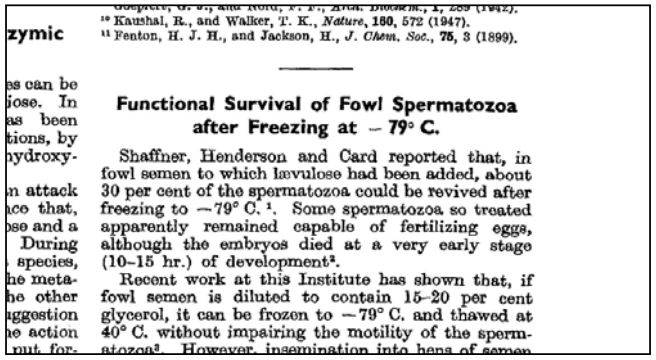
Revival of Spermatozoa after Vitrification and Dehydration at Low Temperatures

THE effect on spermatozoa of vitrification at temperatures of −79° C. and below has been studied by several authors. Human spermatozoa appear to be the most resistant; a substantial proportion may show good motility on thawing after even prolonged vitrification. Revival is far better when semen is ing 40 per cent glycerol, the spermatozoa resume full motility on thawing. So far as retention of motility is concerned, the specimen is indistinguishable from its unvitrified control; it shows even the wave motion characteristic of fowl semen. Decreasing the final concentration of glycerol below 10 per cent decreases the protection against vitrification. Increasing it above 20 per cent results in progressive immobilization of the spermatozoa, which cannot altogether be reversed by further dilution with Ringer's solution; but with these higher concentrations, no additional loss of motility is caused by vitrification. Specimens of spermatozoa have been found to resume motility completely after long periods (up to ten weeks) of vitrification. Other experiments showed that both propylene glycol and ethylene glycol were more toxic than glycerol, and in relation to their toxicity less protective against vitrification.

The fact that spermatozoa resumed full motility after vitrification under the conditions described above made it possible to investigate the effects of freeze-drying. 1 c.c. of fowl semen was diluted with 1 c.c. of 20 per cent glycerol in Ringer's solution, and



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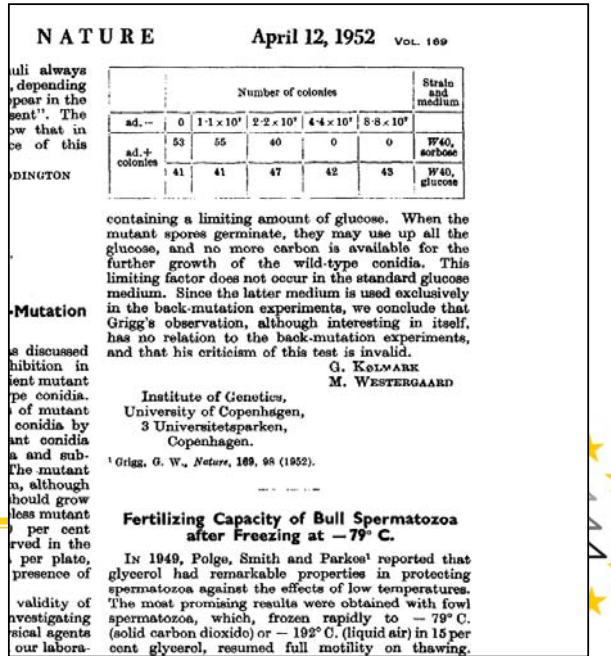
Functional Survival of Fowl Spermatozoa after Freezing at −79° C.

Shaffner, Henderson and Card reported that, in fowl semen to which lævulose had been added, about 30 per cent of the spermatozoa could be revived after freezing to −79° C.[1]. Some spermatozoa so treated apparently remained capable of fertilizing eggs, although the embryos died at a very early stage (10–15 hr.) of development[2].

Recent work at this Institute has shown that, if fowl semen is diluted to contain 15–20 per cent glycerol, it can be frozen to −79° C. and thawed at 40° C. without impairing the motility of the sperm-atozoa[3]. However, insemination into hens of semen

Polge; Nature 1951



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	Number of colonies					Strain and medium
ad.−	0	$1{\cdot}1\times10^5$	$2{\cdot}2\times10^5$	$4{\cdot}4\times10^5$	$8{\cdot}8\times10^5$	
ad.+ colonies	53	55	40	0	0	W40, sorbose
	41	41	47	42	43	W40, glucose

containing a limiting amount of glucose. When the mutant spores germinate, they may use up all the glucose, and no more carbon is available for the further growth of the wild-type conidia. This limiting factor does not occur in the standard glucose medium. Since the latter medium is used exclusively in the back-mutation experiments, we conclude that Grigg's observation, although interesting in itself, has no relation to the back-mutation experiments, and that his criticism of this test is invalid.

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[1] Grigg, G. W., *Nature*, **169**, 98 (1952).

Mutation

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Fertilizing Capacity of Bull Spermatozoa after Freezing at −79° C.

IN 1949, Polge, Smith and Parkes[1] reported that glycerol had remarkable properties in protecting spermatozoa against the effects of low temperatures. The most promising results were obtained with fowl spermatozoa, which, frozen rapidly to −79° C. (solid carbon dioxide) or −192° C. (liquid air) in 15 per cent glycerol, resumed full motility on thawing.

Polge & Rowson; Nature 1952

...up to the present day!

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- Okamoto M. et al. Cryopreservation and transport of mouse spermatozoa at -79 C. Exp. Anim. 50 (1), 83-86, 2001.
- Jin B. et al. Equilibrium vitrification of mouse embryos. Biology of Reproduction. 82, 444-450, 2010.
- Seki S. & Mazur P. Stability of mouse oocytes at -80 C: the role of the recrystallization of intracellular ice. Reproduction APR; 141 (4): 407-415, 2011.

In this scenario we should effectively promote the exchange of frozen mouse germplasm when transferring mutant stocks between Institutions!

EMMA-CNR proposed study: two steps

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- PILOT MONO-CENTER STUDY: Mid-term stability of mouse sperm frozen at -79 °C and long-term storage by re-freezing at -196 °C, from hybrid, inbred and mutant lines;

- MULTICENTER STUDY: Distribution of frozen sperm samples (most common bkgs) cryopreserved at -196°C, shipped to three EMMA Partners at -79°C (dry-ice) and long-term storage by re-freezing at -196 °C;

EMMA Centers involved: CNR (IT), MRC (UK), HMGU (DE), CNB (SP)

Purposes

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- ✓ To verify the suitability of frozen sperm samples from most common bkgs, held at -79°C (including long-term storage);
- ✓ To investigate the effects of transportation at -79°C (dry-ice) on the viability of frozen sperm samples (mutant lines) distributed among EMMA Centers, in the perspective of world-wide distribution with significantly lower costs;
- ✓ To investigate the effect of re-freezing in LN2 (-196°C) on the viability of frozen sperm samples, following shipping at -79°C;

List of planned experiments

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Wild-type mouse sperm:

B6D2F1, B6J and B6N: frozen at - 196 °C ⇒ -79 °C ⇒ 196 °C
Timing: 1-450 days (Re-frozen samples)

Mutant mouse sperm (B6N, B6J bkgs):

6 B6N and 6 B6J: frozen at - 196 °C ⇒ -79 °C ⇒ -196 °C;
Timing: 0-30 days (Re-frozen samples)

1 B6N and 1 B6J frozen at - 196 °C ⇒ dry-ice ⇒ -196 °C;
Timing: 0-3 days (Re-frozen) (in-house study)

2x4 B6N and 2x4 B6J frozen at - 196 °C ⇒ dry-ice ⇒ - 196°C;
Timing: 0-3,5 days (Re-frozen)

QC: Fertilization and culture rates (2-cell and blast. stages);
ED (fertility and offspring rates);

Analysis of the embryo viability: embryo culture and mutant genotype rates

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- ✓ Culture rate: 95% (blastocyst stage);
- ✓ Offspring rate:
  - B6J: 48% (CNR) vs. 30% (CNB/MRC/HMGU)
  - B6N: 48% of pups rate (CNR) vs. 27% (CNB/MRC/HMGU)
- ✓ Genotype screening: 54.1% mutant pups;

Statistical analysis

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Dry-ice storage (days) vs Refrozen N.S.
Wt bkgs vs. corresp. mutants N.S.
All Wt bkgs vs. all mutants N.S.(p=0,05)
All EMMA Centers vs. Controls (B6N) N.S.
All EMMA Centers vs. Controls (B6J) S.S.(P<0,05)

Time	t-test
1 vs 1 REF	N.S.
2 vs 2 REF	N.S.
3 vs 3 REF	N.S.
4 vs 4 REF	N.S.
7 vs 7 REF	N.S.
14 vs 14 REF	N.S.
30 vs 30 REF	N.S.
90 vs 90 REF	N.S.
180 vs 180 REF	N.S.
360 vs 360 REF	N.S.
450 vs 450 REF	N.S.

Conclusions:

✓The two sperm storage methods, dry ice and refrozen, are comparable between them and against controls;

✓In all 4 EMMA Centers the dry ice and refrozen methods are comparable among mutant strains and WT bkgs, except for B6J strains that are S.S. vs. controls at 1 center (CNR);

❖***SPSS software package (t-test)***

Results and Conclusions

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- Viability of frozen sperm samples stored in dry-ice
- Suitability for their national/international transportation

➢The procedure is applicable to wild-type and mutant mouse strains of most common bkgs;

➢Sperm shipping in dry-ice is possible and applicable (and much less cumbersome than shipping in LN2 vapour containers);

➢*Also re-frozen sperm samples show good viability - they could be utilized after long transfers and when an immediate IVF is not possible;*

➢*Dry-ice in a proper styrofoam box lasts max 72-84 hours;*

LN2 dry-shipper vs. styrofoam box packed with dry-ice...

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1. DRY-ICE BOX
2. LN2 DRY SHIPPER

	TIME	COSTS	ADVANTAGES	BIOSAFETY/SECURITY
EU	1. 24/48H 2. 24/48H	1. 250/950 Euro 2. 350/960 Euro	1. Costs (equip/transport) 2. Safety	To be tested
USA	1. 48/72H 2. 48/72H	1. 1250/1400 Euro 2. 2250/2500 Euro	1. Costs 2. Safety	To be tested

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EMMA
Mutant mice for the scientific community

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