Shipping Sperm Frozen Samples in Dry Ice

Marcello Raspa, DVM PhD
Consiglio Nazionale delle Ricerche-EMMA, Monterotondo (Italy)
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°C
General considerations

✓ 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)

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

Animal Rights Conundrum

Does Getting Lab Animals Off Planes Really Help Them?

By Laura Höflinger

Protests from animal rights organizations have prompted major airlines to stop transporting monkeys and other animals slated for use in laboratory experiments. But is this really helping or hurting the animals?

The animal rights organization PETA is one of the leaders of the lawsuit, which is being heard in a federal court. The airlines have been under pressure from animal rights groups, who have been bringing cases to the court.

Before long, the Göttingen animal center hopes to be able to transport the animals using its own methods. The company plans to be able to transport the animals in a more humane way, which will be easier on the animals and the workers.

The company is also considering a new method of transporting the animals. In this method, the animals will be flown to a new facility, which will then be used to house the animals until they are needed for experiments.

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 transport animals is Air France. The airline has defended its stance on its website, saying that the company is convinced of the benefits of transporting lab animals.

"We cannot understand how some airlines are unwilling to transport laboratory animals even though they continue to take pets, animals, reptiles and animals used in agriculture," says Sigrid Thomsen, director at Wannin, who is responsible for international operations at the animal research facility in Göttingen.

"We're concerned about the well-being of the animals, and we're concerned about the ethics of how the animals are being transported. It's a huge problem when a minority uses undemocratic and opaque means to determine what should be allowed regardless of what the laws say or what people want," says Thomsen.

"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 Thomsen.

"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 Thomsen.

For more information, please visit the website of the German Primate Center (DPZ) in Göttingen, about the influence of animal rights activists.

The DPZ supplies German universities and research institutions with monkeys from its own breeding program. All of the DPZ animals are fed on a special diet and are given regular veterinary check-ups.

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 unloading them from the cargo hold.

"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 Thomsen.

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."
From the origins...

Polge, Smith and Parkes; Nature 1949

Polge; Nature 1951

Polge & Rowson; Nature 1952

www.emmanet.org
...up to the present day!


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

- 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 bkgds) 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)
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;
Wild-type mouse sperm:

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

Mutant mouse sperm (B6N, B6J bkgs):

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

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

2x4 B6N and 2x4 B6J frozen at -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

- 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**

<table>
<thead>
<tr>
<th>Time</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 1 REF</td>
<td>N.S.</td>
</tr>
<tr>
<td>2 vs 2 REF</td>
<td>N.S.</td>
</tr>
<tr>
<td>3 vs 3 REF</td>
<td>N.S.</td>
</tr>
<tr>
<td>4 vs 4 REF</td>
<td>N.S.</td>
</tr>
<tr>
<td>7 vs 7 REF</td>
<td>N.S.</td>
</tr>
<tr>
<td>14 vs 14 REF</td>
<td>N.S.</td>
</tr>
<tr>
<td>30 vs 30 REF</td>
<td>N.S.</td>
</tr>
<tr>
<td>90 vs 90 REF</td>
<td>N.S.</td>
</tr>
<tr>
<td>180 vs 180 REF</td>
<td>N.S.</td>
</tr>
<tr>
<td>360 vs 360 REF</td>
<td>N.S.</td>
</tr>
<tr>
<td>450 vs 450 REF</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

**Dry-ice storage (days) vs Refrozen**

- 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)

**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)**

www.emmanet.org
Results and Conclusions

- **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 bkg;

- 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...

1. DRY-ICE BOX
2. LN2 DRY SHIPPER

<table>
<thead>
<tr>
<th></th>
<th>TIME</th>
<th>COSTS</th>
<th>ADVANTAGES</th>
<th>BIOSAFETY/S SECURITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU</td>
<td>1. 24/48H</td>
<td>1. 250/950 Euro</td>
<td>1. Costs (equip/transport)</td>
<td>To be tested</td>
</tr>
<tr>
<td></td>
<td>2. 24/48H</td>
<td>2. 350/960 Euro</td>
<td>2. Safety</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>1. 48/72H</td>
<td>1. 1250/1400 Euro</td>
<td>1. Costs</td>
<td>To be tested</td>
</tr>
<tr>
<td></td>
<td>2. 48/72H</td>
<td>2. 2250/2500 Euro</td>
<td>2. Safety</td>
<td></td>
</tr>
</tbody>
</table>

Shipping Companies: e.g. World Courier, Gabella, Marken, PHSE, FEDEX, TNT, DHL, UPS, etc.
Acknowledgements

- Marcello Raspa
- Ferdinando Scavizzi
- Rafaele Matteoni
- Renata Paoletti
- Francesco Donati
- Martin Fray
- Mo Guan
- Amanda Pickard
- Susan Marschall
- Stefanie Dunst
- Bernhard Rey

EMMA Project Office:
- Michael Hagn
- Sabine Fessele

www.emmanet.org
EMMA
Mutant mice for the scientific community

Thanks for your attention!!!