Current state of marmoset population in Japan and exchange of the marmoset genetic resources

Erika Sasaki
Central Institute for Experimental Animals
### History of Marmoset Research in Japan

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>Investigation of 12 kinds of small primates toward developing non-human primate models.</td>
</tr>
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<td>2008</td>
<td>Strategic Research Program for Brain Sciences</td>
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<td>Development of transgenic marmoset</td>
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<td>2010</td>
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<tr>
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<td>New marmoset facility, Tonomachi</td>
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<td>2012</td>
<td>Japan Society for Marmoset Research</td>
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<tr>
<td>2014</td>
<td>Brain/MINDS project</td>
</tr>
<tr>
<td>2016</td>
<td>Knockout marmoset by genome editing</td>
</tr>
<tr>
<td>2018</td>
<td>Marmoset lab manual</td>
</tr>
<tr>
<td>2020</td>
<td>Transfer breeding colony to CLEA Japan Inc.</td>
</tr>
<tr>
<td>2021</td>
<td>New marmoset facility, Tonomachi</td>
</tr>
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<td>2022</td>
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Image of the Marmoset demands and supplies in Japan

The numbers are not exact

Strategic Research Program for Brain Sciences (SRPB)

Over stock for 4 years, $500,000 loss every year

Brain/MINDS project

Available supplies
Demand
Number of supplies

The numbers are not exact

Courtesy of Mr. Fukasawa, CLEA Japan, Inc.
Transportation of marmosets

In Japan, marmoset is prohibited to import from France. This animal transportation difficulties make less genetic diversity in individual colonies.
Shipping embryos (or sperm) using dry shippers

“dry shippers” are designed to safely transport a variety of materials at cryogenic temperatures.

Don’t forget to get Export / Import approves for CITES before shipping !!
**Sperm Cryopreservation**

Centrifugation: 400Xg for 3min

Sperm swim-up

Suspend in 1mM dbcAMP+BO

IVF

<table>
<thead>
<tr>
<th></th>
<th>dbcAMP treat</th>
<th>Number of trals</th>
<th>Number of oocytes</th>
<th>Number of fertilized embryos</th>
<th>Fertilization rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flesh</td>
<td>-</td>
<td>11</td>
<td>72</td>
<td>34</td>
<td>47.2 %</td>
</tr>
<tr>
<td>Cryopreserved</td>
<td>-</td>
<td>3</td>
<td>10</td>
<td>0</td>
<td>0 %</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>7</td>
<td>36</td>
<td>8</td>
<td>22.2 %</td>
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</tbody>
</table>

Intracytoplasmic sperm injection (ICSI) is needed to obtain fertilized embryos

Courtesy of Prof. Sotomaru
ICSI requires special equipment and technics

Micro manipulator

Furthermore, ovarian stimulations, surgical ovum pickup procedures are also required

In vitro manipulated embryos are low efficiencies of cryopreservation….
Blood or urine sample collection on Day -1, 1, 9, 11, 13

Paired marmosets

Natural fertilized embryo collection

Ovarian cycles controls using PGF2α

Progesterone (ng/ml)

Days

Embryo Collection (P.O. 4-10)

PGF2a (Day 0)

PGF2a (Day 21)

Ovulation
Nonsurgical embryo collection

Thomson et al. J Med Primatology 1994
Comparison of successful rates among the embryo collection methods

<table>
<thead>
<tr>
<th></th>
<th>Number of times of collection</th>
<th>Times of successful collection</th>
<th>Number of collected embryos</th>
<th>Number of Abnormal embryos</th>
<th>Rates of successful collection</th>
<th>Average number of collected embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical</td>
<td>52</td>
<td>22</td>
<td>44</td>
<td>0</td>
<td>42.3%</td>
<td>0.85</td>
</tr>
<tr>
<td>Percutaneous</td>
<td>52</td>
<td>28</td>
<td>58</td>
<td>3</td>
<td>53.8%</td>
<td>1.1</td>
</tr>
<tr>
<td>Transvaginal</td>
<td>52</td>
<td>33</td>
<td>64</td>
<td>3</td>
<td>63.5%</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Vitrification of marmoset embryos and viability after thawing

![Graph showing viability of marmoset embryos after slow freeze and vitrification with different treatments.]

- **DMSO**
- **DAP213**
- **PEPeS**
- **Fresh**

Viability (%): 20, 40, 80, 120

- a, b: p<0.01

Viability after slow freeze and vitrification.
Nonsurgical embryo transfer
Artificial insemination in marmoset

Semen collection

50μl TYH + semen

Developed AI method without anesthesia

<table>
<thead>
<tr>
<th>Fertilization</th>
<th>Number of Embryo collection trial</th>
<th>Number of unfertilized embryo</th>
<th>Number of Fertilized embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural mating</td>
<td>13</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>AI</td>
<td>10</td>
<td>4</td>
<td>8</td>
</tr>
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</table>

Currently, only fresh semen can be applicable for this method
Acknowledgements

Central Institute for Experimental Animal

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