Chapter 2

The Sophisticated Mouse: Protecting a Precious Reagent

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Abstract

Definable, genetically and environmentally, the humble mouse has become a reagent with which to probe the human condition. The information thus gained is leading to a greater understanding of interindividual variation in drug responses and disease processes and is forming the basis for personalized medicine. Inbred mice are the tool of choice as each strain is essentially clonal in nature creating a defined, uniform setting where the effects of genetic background and modifications can be evaluated coherently. However, the creation and characterization of novel mouse strains remain expensive and time consuming. Further, the continual maintenance of these valuable animals as live colonies is financially draining and carries continual potential risks, including disastrous loss due to fire, flood, disease, etc. There are also other more insidious disasters including genetic contamination and genetic drift, either of which can go undiscovered until their effects ruin experiments. With this in mind, we strongly recommend that all mouse strains be cryopreserved as a matter of standard mouse management. Cryopreservation is a powerful colony management tool, assuring strains are available upon demand, for example, for regulatory requirements, re-initiation of projects, collaborations, re-evaluation of data etc. However, it is essential that any cryopreservation approach be cost-effective for both strain closure and strain recovery. In this chapter, we describe the variables which can afflict an inbred mouse’s genetic background (and hence phenotype), options to consider for strain archiving, and describe how to economically store and recover strains by sperm cryopreservation.

Key words: Genetically modified mice, inbred mice, sperm cryopreservation, embryo cryopreservation, IVF, in vitro fertilization, genetic drift, genetic contamination.

Abbreviations:
IVF in vitro fertilization

1. Introduction

1.1. The Reagent Grade Mouse

Since the time of alchemy those engaged in research have continually needed to further refine and define their basic experimental reagents, their scientific tools of the trade. The resulting progress
has allowed alchemy to become science and has driven the scientific revolutions of the 20th and 21st centuries. The term “reagent grade” is usually only applied to chemicals where impurities are either vanishingly absent or have been defined. In the field of experimental biomedical and genetic research, the evolution of the mouse as a reagent has been similar to other reagents whose precise definition and refinement has continually improved. Now, with the sequencing of the mouse genome and improvements in genetic monitoring of inbred mouse strains, we believe that the term reagent grade can justifiably be applied to the mouse (1). This particular reagent is, however, one of the most sophisticated scientific tools available. It is a living creature (and as such deserving respect), a highly complex mammal capable of independent survival and is the net result of millions of years of evolution. So as with any reagent, its basic attributes must be understood if it is to be used successfully, including purity and/or quality, and how to maintain its full functionality.

Two things make the reagent grade mouse invaluable as a model organism. First, it is possible to introduce precise genetic modifications into the mouse genome at will. These genetic modifications including gene ablation, addition, and modulation enable the rapid examination of the effects of these modifications in a complete living organism (2, 3). Second, comparisons of the mouse and human genomes reveal that mice and humans share a common ancestor which diverged about 75 million years ago, as such these two species have maintained many similarities in gene function. Comparisons between the two species show 99% shared gene function (4). Combined, these attributes make the mouse a reagent of exquisite subtlety and sophistication, enabling us to understand gene interactions within the complete organism, test their interplay with the environment, and extrapolate these data to human biology. However, “living reagents” require continual maintenance if they are not to be lost or degraded. It is here that mouse management by cryopreservation can be used, providing versatile archiving of these valuable resources.

Lastly, the scientific method is based on the altruistic notion that others can take data and information, repeat it, and build upon it. Without repeatability there is no scientific advancement. As Sir Isaac Newton reportedly said, “If I have seen further it is by standing on the shoulders of Giants.” Treating the mouse as a living reagent will help ensure the creation of a sound foundation on which others can stand upon, build, and hopefully see further.

Mice are a key element in many biological experimental designs, but their origin and quality are often overlooked. The variability introduced by using ill-defined mice, for example, randomly crossed strains, e.g., CD-1, or incomplete inbred
congenics, increases experimental noise within and among experiments. If this is not understood and part of an experimental approach, this genetic variation will lead to a lack of reproducibility and difficulty in interpretation (see Chapter 1 by Festing this volume).

Inbred mice, defined by at least 20 generations of brother-sister matings, are the most defined mammal available for experimental manipulation, with individual mice within each inbred strain being essentially clonal in nature (>99% homozygosed at all loci). This allows precise experimental comparisons within strains, between multiple inbred strains, and between genetically modified versus non-modified mice of the same genetic background (5). However, all life has an innate biochemical, evolutionary capability to change and mutate, generating variation; mice are the selective result of this evolutionary past and their current environment (6). To work with these complex reagents successfully requires an appreciation of this.

Inbred mice are generally maintained as a continually breeding colony requiring precise control of breeding. If done incorrectly their genetic background will change. There are two major sources of genetic change reported in live mouse colonies: (i) fast, disastrous genetic contamination (one breeding cycle) and (ii) insidious genetic drift. Until recently genetic drift was viewed as a slow process, however, recently the phenomena of copy number variation (CNV) has been discovered as a rapid source of genetic variation and hence drift. Although its impact is not fully understood, it is thought to cause very rapid genetic drift (within one generation) via duplication or deletion of one to thousands of kilobases of DNA, potentially containing entire genes. The resulting varying copy number effectively changes gene dosage which can result in phenotypic shifts (7, 8). Appropriate use of cryopreservation can forestall the cumulative adverse effects of genetic drift, including CNV, and allow rapid restoration of strains if genetic contamination, disease, phenotypic shifts, etc. occur (for a review ref. 1). Additionally, cryopreservation increases mouse management options, facilitating more cost-effective colony management.

In regard to mainly custom genetically modified strains, although it is tempting to believe that these strains are safe during active experimental work, all vivariums carry risks including the possibility of disease, breeding cessation, genetic contamination, and other disasters. For example, in June 2001 the tropical storm Allison caused the flooding of vivariums at the Texas Medical Center killing more than 30,000 mice and rats, causing incalculable losses (9). Strain backup is therefore a prudent management step and also facilitates dynamic cost management allowing strains to be closed down and only upon demand, rapidly re-initiated.
1.3. Mouse Archiving – Options

In the management of any resource a key consideration is “Return on Investment,” i.e., in this case, it is of little value to cheaply store/archive mouse strains in a format which makes their recovery prohibitively expensive and/or unpredictable (see Table 2.1 for summary of approaches). While at the same time it is not viable to invest large sums into the archiving of strains if the likelihood of them ever being wanted at a later date is small or totally unknown.

Mouse strains can be archived as embryos (2–8 cell), gametes (sperm, oocytes), or as sources of gametes (spermatogonial stem cells, ovaries), see Table 2.1. When looking at costs to cryopreserve and recover mouse strains, sperm is in general the most logical choice based on the ease of collection and the sheer numbers of sperm available from a single male (∼30 × 10^6 sperm/male). However, until very recently recovery of live born from C57BL/6 sperm, the most commonly used background, was less than 5%, making routine recovery expensive and unpredictable.

Females can provide naturally ∼6–8 oocytes or upon superovulation up to 50 oocytes. However, this is highly strain dependent, for example, C57BL/6J give high numbers, while 129 strains give very few embryos. Complicating this there is also evidence suggesting that embryo quality falls with the high oocytes yields (10, 11).

Thus upon comparing the economics of the two methods, embryos and sperm, it is very apparent that the freezing of embryos is considerably more expensive due to the need for more resources; for example, with a C57BL/6 background, to produce ∼250 two-cell embryos for cryopreservation by IVF requires >15 females. If strains are never recovered or only recovered once or twice, then the bulk of this expense remains forever frozen. In contrast, cryopreserving sperm has a low initial cost as only few animals (1–3 males) and relatively little labor and materials are required (12). It is upon recovery from sperm by in vitro fertilization (IVF) that animals and labor are used, but then only the required number animals per recovery are used as the IVF process can be scaled to produce the desired number of offspring.

A major disadvantage of sperm cryopreservation of a strain is that only half the genome is stored by this approach (i.e., the donor sperm is haploid!). This does not represent a major problem when a genetic modification is on a readily available standard background, as high-quality female animals (oocyte donors) are readily available from reputable providers for most standard inbred mice. Although it should be appreciated that genetic drift still occurs unless the supplier has addressed this issue by restoring the breeder stock from cryopreserved pedigreed embryos every few generations (1).
<table>
<thead>
<tr>
<th>Cryopreservation method/gametes</th>
<th>Cryopreservation Pros</th>
<th>Cons</th>
<th>Recovery Pros</th>
<th>Cons</th>
<th>Cumulative cost/animal to cryopreserve and recover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm cryopreservation</td>
<td>Very simple</td>
<td>Needs only 1–3 carrier males</td>
<td>Only half the genome is preserved</td>
<td>Recovery</td>
<td>Requires IVF to recover strain</td>
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<td></td>
<td></td>
<td>~30 × 10⁶ sperm/male</td>
<td>Needs IVF quality control</td>
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<td>Requires appropriate oocyte donor strain</td>
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<tr>
<td></td>
<td></td>
<td>Inexpensive</td>
<td></td>
<td></td>
<td>Some strains and mutations adversely affect IVF success. Offspring will be heterozygotes</td>
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<tr>
<td>Ovary cryopreservation</td>
<td>Simple</td>
<td>Only half the genome is preserved.</td>
<td>Represents female lineage</td>
<td>Due to potential rejection</td>
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<td></td>
<td>Inexpensive</td>
<td>Needs multiple donor females, i.e., one female has only two ovaries</td>
<td>Needs multiple donor females, i.e., one female has only two ovaries</td>
<td>needs appropriate recipient animals.</td>
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<td>Moderate level of surgical skill to implant.</td>
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<td>Low yield of offspring/ovary</td>
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<td>Off-spring will be heterozygotes</td>
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<td>Can transmit disease</td>
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<td></td>
<td>Not scalable</td>
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<tr>
<td>Two cell embryo cryopreservation (heterozygotes embryos generated via IVF)</td>
<td>Moderately simple</td>
<td>Needs only 1–3 carrier males</td>
<td>Only half the genome is preserved.</td>
<td>Simple recovery into pseudopregnant animals</td>
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<tr>
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<td>Needs IVF to make embryos Needs female (wild type) oocyte donors. Strain dependent 5–50 oocytes/female</td>
<td>Needs IVF to make embryos Needs female (wild type) oocyte donors. Strain dependent 5–50 oocytes/female</td>
<td>Can be used to achieve strain rederivation.</td>
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<td></td>
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<td>Only half the genome is persevered.</td>
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<td>Low yield of offspring</td>
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<td>Offspring will be heterozygotes</td>
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<td></td>
<td>Not scalable (based in initial investment)</td>
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(continued)
Table 2.1 (continued)

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<thead>
<tr>
<th>Cryopreservation method/gametes</th>
<th>Cryopreservation</th>
<th>Recovery</th>
<th>Cumulative cost/animal to cryopreserve and recover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two to eight cell embryo cryopreservation (flushed homozygous embryos)</td>
<td>Simple to implant</td>
<td>Expensive resource to restock</td>
<td>$$$$</td>
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<tr>
<td></td>
<td>Provides “homozygous” storage</td>
<td>Can be used to achieve rederivation of strain</td>
<td>Not scalable</td>
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<td></td>
<td>Needs a large colony of strain to be cryopreserved (expense) to provide embryos</td>
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<tr>
<td>ICSIa</td>
<td>Very simple</td>
<td>None</td>
<td>$$$$</td>
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<tr>
<td></td>
<td>Inexpensive</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Only half the genome is preserved</td>
<td>Requires a high level of resources/skill to recover</td>
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<tr>
<td></td>
<td>None</td>
<td>Requires high level of resources/skill to recover</td>
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<td></td>
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<td>Pathogen transmission could occur</td>
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<td></td>
<td></td>
<td>Offspring will be heterozygotes</td>
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<td></td>
<td></td>
<td>Associated with genetic damage</td>
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<td></td>
<td></td>
<td>Not scalable</td>
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<tr>
<td>ES cellsb</td>
<td>Simple</td>
<td>None</td>
<td>$$$$</td>
</tr>
<tr>
<td></td>
<td>Inexpensive</td>
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<tr>
<td></td>
<td>None</td>
<td>Need to make germline transmitting chimeras</td>
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<tr>
<td></td>
<td></td>
<td>Upon germline transmission only half the ES derived offspring will be heterozygotes</td>
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<td></td>
<td></td>
<td>ES cells carry tissue culture associated genetic damage</td>
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<td></td>
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<td>Not scalable</td>
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</table>

Note: all the above approaches are also subject to strain effects and to possible deleterious effects of gene modification or addition.

aIntracytoplasmic sperm injection, ICSI although not strictly necessary a cryopreservation method – this approach has been used as method to archive and restore mouse strains.

bEmbryonic Stem Cell, ES cell are often generated as part of the process for a targeted genetic modification. Although a strain can be recreated from the original targeted ES line it requires the re-creation of germline chimeras and their successful germline breeding before the strain is recovered. Further, ES cells are known the mutate in culture.
1.4. How Safe Is Gamete Cryopreservation

Any approach for archiving gametes has to provide long-term secure storage. Most methods for archiving mouse strains cryopreserve embryos, sperm, ovaries, etc., in vapor phase or liquid nitrogen. There are many papers discussing the longevity of gametes in cryogenic storage; however, it is generally accepted that once samples pass through the glass transition temperature of water, $\sim -137^\circ C$ all biological activities cease. Although gamma radiation can still cause accumulative damage, simulation studies suggest that this is insignificant over $\sim 2000$ years under normal background radiation levels (13, 14).

Of much greater concern with long-term cryogenic storage is temperature variation, where gametes are exposed to temperature fluctuations above $-137^\circ C$. The most likely causes of temperature variation (increase) is improper handling of the frozen gametes (e.g., while “rummaging” in the liquid nitrogen storage tanks), failure to fill liquid nitrogen tanks, i.e., they run dry, destruction of the storage facility due to fire etc., or the physical failure of the tanks vacuum (15). As such, it is strongly recommended that cryopreserved gametes be stored physically in at least two liquid nitrogen storage tanks and, additionally, that tanks be in two or more separate facilities as one part of a comprehensive approach to repository operation (16).

2. Materials

2.1. Cryopreservation of Mouse Sperm

2.1.1. Cryoprotective Medium

1. Distilled water (Invitrogen, cat # 15230-238)
2. 18% w/v raffinose (Sigma cat # R7630)
3. 3% w/v skim milk (BD Diagnostics cat # 232100).
4. MTG: 447 $\mu$M monothioglycerol (Sigma cat # M6145)

2.1.2. Consumables

1. 0.25 mL French straws (IMV cat # AAA201)
2. Cassettes (Zander Medical Supplies, 145 mm 16980/0601)
3. Styrofoam box internal dimensions $\sim 35$ cm $\times$ $\sim 30$ cm, a Styrofoam float (piece should be approximately $\sim 2$–$3$ cm thick and be cut to cover $\sim 80\%$ of internal area of box)
4. Monoject insulin 1 mL syringe

2.1.3. Mice – Strain to be Cryopreserved

Two to three male mice, preferably 10–16 weeks old (see Note 1)

2.2. In Vitro Fertilization Method

2.2.1. Hormones for Superovulation

1. Pregnant mare serum gonadotropin (PMSG)
2. Human chorionic gonadotropin (hCG)
3. Sterile physiological saline
2.2.2. Mice for Superovulation

Five to ten female mice, 3 weeks or 6–12 weeks of age, depending on the strain (17)

2.2.3. In Vitro Fertilization

1. MVF media: Research vitro Fert (K-RVFE-50) COOKS Mouse In Vitro Fert Fertilization medium (Cook MVF, Australia, see http://www.specialtyvet.net/page/page/6095801.htm) or Human Tubal Fluid media (see 18):

- NaCl (FW 58.44, Sigma S-5886) 5.9375 g
- KCl (FW 74.55, Sigma P-5405) 0.3496 g
- MgSO4 7H2O (FW 246.5, Sigma M-7774) 0.0493 g
- KH2PO4 (FW136.09, Sigma P5655) 0.0504 g
- CaCl2 2H2O (FW 147, Sigma C-7902) 0.3 g
- NaHCO3 (FW 84.01, Sigma S-5761) 2.1 g
- Glucose (FW180.16, Sigma G-6152) 0.5 g
- Na-pyruvate (FW110.0, Sigma P-4562) 0.0365 g
- Na-lactate 60% syrup (FW 112.1, Sigma L-7900) 3.42 mL
- Penicillin-G (FW 372.5, Sigma P-4687) 0.075 g
- Streptomycin sulfate (FW 1457.4, Sigma S-1277) 0.05 g
- Phenol red (5%) (Sigma P-0290) 0.20 mL
- BSA (Equitech-Bio BAC62-0050) 4.0 g

Fill with water-cell culture grade (Sigma 59900C)

Weigh each component and dissolve in high quality water (cell culture grade, double glass distilled or reverse osmosis, and filtration, i.e., 18 MΩ) in a 1 L volumetric flask, but withhold the BSA for addition later. Bring the volume up to 1 L. Measure the osmolarity (290 ± 5).

Bubble gas (5% CO2, 5% O2, 90% N2) through the medium for ~5 min, add the BSA to the media, mix gently to avoid frothing.

Filter through a 0.2 μm filter into sterile bottles.

Gas the medium with a mix of 5% CO2, 5% O2, 90% N2 to displace air above the medium, cap tightly, and store at 4°C for not more than 2 weeks. Repeat gassing after every use to maintain a pH of 7.2–7.4.

2. Mixed gas (5% O2, 5% CO2, balanced with N2)
3. One large 60 × 100 mm Falcon Petri dish for every three females (BD Biosciences)
4. One small 35 × 10 Falcon Petri dish for each male (BD Biosciences)
4. Embryo-tested mineral oil
5. Phosphate Buffered Saline(PBS)
3. Methods

It is critical that all media are carefully prepared and have the correct pH and temperature, as well as batch-tested reagents. Where possible we suggest buying reagents readymade. Further, it is helpful to pay attention to details and efficient laboratory setup, e.g., small incubators, heated stages, or other devices that ensure proper temperature and pH stability.

3.1. Sperm Cryopreservation

3.1.1. Preparation of Cryoprotective Media (CPM)

1. Place ∼80 mL of bottled distilled water in a beaker.
2. Heat for ∼40 sec in microwave to ∼60–80°C (do not boil).
3. Place beaker on heated stir plate, add 18 g of raffinose, and heat and stir till solution clears (see Note 2).
4. Add 3 g of skim milk to the raffinose mixture and heat and stir until dissolved (see Note 3).
5. Transfer solution to volumetric flask and bring to 100 mL with bottled distilled water.
6. Add MTG now or after thawing (see Note 4).
7. Mix well and divide the solution into two 50 mL centrifuge tubes.
8. Centrifuge at 13,000 × g for 15 min at room temperature (∼22°C).
9. Filter through a 0.22 μm cellulose filter (a prefilter may help the flow).
10. Verify that the osmolarity is in the range of 470–490 mOsm.
11. Aliquot 10 mL of filtered cryoprotective media into labeled 15 mL conical tubes.
12. Cap and store at −20°C until ready for use (see Note 5).

3.1.2. Sperm Cryopreservation Setup

1. Thaw and warm CPM in 37.5°C water bath (see Note 6). While the media is warming.
2. Label and mark straws and affix to a 1 mL monoject syringe.
3. Fill Styrofoam box to a depth 6–9 cm of liquid nitrogen.
4. Place Styrofoam float into Styrofoam box.
5. Replace Styrofoam box lid to slow the evaporation of liquid nitrogen.
6. Place the lid from Petri dish on the warming tray and lean the bottom of a Petri dish against it so that one side of the
Petri dish is elevated. This arrangement forces the CPM to collect on one side, making it easier to fill the straws.

7. If monothioglycerol was not added prior to freezing, add it now, to a final concentration of 477 μM. Add 1 mL of CPM to the dish for each male from which sperm will be collected.

3.1.3. Sperm collection

1. Euthanize the males (1–3) and remove the cauda epididymides and vas deferentia, carefully removing the testicular artery to avoid contaminating the sperm with blood.

2. Release sperm into the CPM by making several cuts through the epididymides and vas deferentia using a beveled hypodermic needle while holding the tissues with a pair of forceps.

3. Remove tissue from the CPM after 10 min.

3.1.4. Sperm cryopreservation

1. Aspirate a 4.5 cm column of CPM into a French straw followed by a 2 cm column of air.

2. Aspirate a 0.5 cm column of sperm into the French straw then aspirate additional air until the column of CPM without sperm contacts the PVA powder in the cotton plug.

3. Seal the end of the French straw with a brief pulse from an instantaneous heat sealer.

4. Repeat this process until the desired number of straws has been filled (we suggest minimum of 20/strain).

5. Place five straws into one cassette. Repeat until four cassettes have been filled.

6. Place the cassettes in the liquid nitrogen-filled box on the float (i.e., in vapor phase) so that they are not touching.

7. Put the lid on the box for 10–30 min.

8. Plunge the cassettes into the liquid nitrogen.

9. After at least 10 min in liquid nitrogen the cassettes can be removed and rapidly placed into storage in liquid nitrogen (see Note 7).

3.2. In Vitro Fertilization (IVF) with Frozen Sperm

Once a strain is frozen as sperm we strongly recommend that 1–2 straws be used to assess the quality of the sperm post-thaw. Although various devices exist to measure sperm motility etc., the only relevant test for sperm function is an actual IVF. Additionally, fertilization rates vary widely among commonly available inbred strains; also the introduction of mutations and genetic modifications into a strain can have indirect and unanticipated effects on the quantity and quality of oocytes and sperm produced, as well their performance during IVF (12, 17).

IVF can be difficult to establish – the quality of the reagents is crucial, also during the IVF process it is essential for repro-
ducible success to maintain media pH and temperature and that all practical steps are taken to keep precise control of the culture conditions.

3.2.1. Superovulation

1. Inject females with PMSG 44–48 h prior to injection with hCG (see Note 8).
2. Inject females with hCG 13 h prior to oocyte collection (see Note 8).

3.2.2. IVF Setup

1. Prepare oocyte collection dish by adding 2 mL of PBS to a 25 mm × 10 mm dish and keep at 37.5°C in air.
2. Prepare IVF dish by placing a 250 μL drop of MVF medium (see Note 9) in the center of a 60 mm Petri dish. Place four additional 150 μL drops of MVF medium around the 250 μL drop.
3. Carefully add sufficient oil to cover the media and place in an incubator or sealed chamber filled with mixed gas (5% O₂, 5% CO₂, 90% N₂) at least 1 h prior to IVF (see Note 10).

3.2.3. Thawing Sperm

1. Place the straw in a 37.5°C (clean) water bath.
2. Rapidly swirl the straw in the water until all ice has melted (about 30 sec).
3. Dry the straw with a paper towel.
4. Cut off the sealed end of the straw opposite the cotton plug. Using a metal rod, expel the sperm from the straw into the 250 μL IVF drop.
5. Allow sperm to incubate at 37°C for 1 h prior to adding oocytes.

3.2.4. Oocyte Collection and IVF

1. Euthanize 2–5 superovulated females approximately 13 h post-hCG (see Note 11).
2. Remove the ovary, oviduct, and a small portion of the uterine horn and place in the dish containing PBS from one female at a time (see Note 12).
3. Repeat for all females.
4. Working under low magnification, identify the ampulla. Cumulus enclosed oocytes should be easily visible within the ampulla of the oviduct. Using a beveled hypodermic needle, open the ampulla to release the cumulus-enclosed oocytes.
5. Repeat until all oocytes have been released.
6. Using a 1 mL pipette (or a wide bore pipette tip) transfer the cumulus enclosed oocytes to the dish containing MVF
medium and thawed sperm (∼10 μL), transferring as little medium as possible.

7. Incubate at 37°C for 4 h under mixed gas.

8. Using a finely drawn glass pipette with a diameter slightly larger than an oocyte. Wash the oocytes through the 150 μL media drops to remove cumulus cells and sperm.

9. Culture overnight at 37°C under mixed gas (see Note 13).

10. Count and evaluate embryos the following morning. Embryos can now be cultured, transferred to a pseudo-pregnant animal, or cryopreserved (see Notes 14 and 15).

4. Notes

1. Variations in sperm quality among individual males within a strain are common.

2. This is a nearly saturated solution, heating the solution makes it easier to get the raffinose into solution.

3. The solution will be opaque after the addition of the skim milk, centrifugation at room temperature (13,000 × g for 15 min) is recommended.

4. Addition of MTG is recommended immediately prior to use. Alternatively, it can be added in advance and the solution stored at −80°C for up to 3 months. Solutions containing MTG should not be stored at 4°C for more than a few days. MTG is viscous and needs to be pipetted carefully. Making an MTG stock solution that is added to the media helps reduce the likelihood of errors. MTG diluted stock solution should be used only on the day it is made.

5. This solution can be stored for at least 6 months at −80°C without MTG or up to 3 months −80°C with MTG.

6. Water baths are a common source of bacterial contamination and also liquid nitrogen is not sterile. Straws should be carefully wiped to remove any moisture from the outside of the straw prior to cutting the end off before dispensing sperm to reduce the risk of contaminating the IVF.

7. It is essential that during the transfer from liquid nitrogen to long-term storage to handle the cassettes rapidly thus preventing any warming.

8. Typical doses are in the range of 2.5–5 i.u. per mouse. Optimal dose varies by strain, age, and weight of the mouse. Extending the oocyte collection window beyond 14 h post-hCG may reduce fertilization rates and compromise embryo quality due to oocyte aging.
9. COOKS Mouse Vitro Fert is similar to Human Tubal Fluid media reported by Quinn (18, 19).

10. Oil can be washed and filtered. Oil should be stored in a dark cool place.

11. The response to superovulation is highly strain dependent (see 17) and some strains appear to be entirely refractory to superovulation.

12. In order to reduce the risk of changes in temperature and pH, oocyte collection should take no more than 5 min from euthanasia to oocyte collection (i.e., practice). Typically cervical dislocation is used to prevent possible exposure to agents that may affect oocyte or embryo quality and to reduce the time from euthanasia to oocyte collection.

13. The use of a low O$_2$ culture environment may not improve fertilization rate, but appears to improve embryo quality (20, 21).

14. The laboratory environment can have a significant effect on the outcome of IVF (22, 23). Materials that release volatile organic compounds (VOC), cleaning/sanitizing agents, such as bleach and floor waxes, should be avoided.

15. Prior to embryo transfer, embryos should be washed following the IETS protocol if the sperm or oocytes were collected from animals with an unknown or unacceptable health status (24).

References


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