

ASSISTED REPRODUCTIVE TECHNOLOGIES

In today's competitive research environment, speed and efficiency are critical for evaluating genetically engineered animal models to determine their suitability for research. As novel models are created, an ever-increasing number of them face reproductive challenges that impede the evaluation process. To assist researchers with these growing challenges, Charles River offers an advanced *in vitro* fertilization (IVF) laboratory as well as extensive rescue options for rodent models that may experience reproductive difficulties. IVF is also the primary component of our Rapid Expansion Services.

Rapid Expansion Services

Charles River's Rapid Expansion Services utilize advanced IVF techniques to enhance the production of mouse colonies. By employing this service, Charles River can produce a virtually unlimited number of age-matched, genetically-modified mice in a short period of time (12-15 weeks). This service can provide cohorts of animals, ready for study, in significantly less time and at greatly reduced cost compared with standard mating. We are currently offering rapid expansion for the most commonly used mouse strains and continually develop methods for application with less frequently used background strains. Animal requirements for rapid expansion are two proven breeder males, preferably less than 10 months of age (older animals may be acceptable depending on strain) and wild type females purchased from a commercial vendor. For cases where greater than 150-200 animals are desired, additional males may be required.

Rescue Services

Charles River has extensive experience and capabilities for rescuing valuable, genetically-modified rodent models. Whether the model has fertility issues due to an adverse phenotype or has simply stopped mating due to age or illness, we have a variety of rescue options available. In some extreme cases, it may be possible to rescue a line even if the last animal has recently died.

Ovarian Transplantation

Ovarian transplantation offers researchers a means of rescuing valuable female mice that are either not capable of carrying a litter to term or are unable to breed due to age or poor body condition. This procedure also provides a tool in the maintenance of difficult lines in which the female is unable to or unwilling to nurse her pups, thus necessitating the need for cross fostering and maintenance of a foster colony. Neonatal or adult ovaries can be transplanted to histocompatible or immunodeficient recipients. The technique is similar with freshly collected or previously cryopreserved ovaries. Furthermore, donor ovaries can be split in half, or quartered, resulting in several potential germ line carriers. Briefly, ovaries of the recipient female are removed by making a small slit in the ovarian bursal membrane. The stalk between the ovary and oviduct is cut and the

In vitro fertilization (IVF) is the process by which mature oocytes are fertilized in a Petri dish with capacitated sperm. Sperm is collected from the vasa deferentia and caudal epididymides from genetically-modified males and evaluated with a computer assisted sperm analyzer. A comprehensive report on the morphology, concentration, motility, and progressive movement of each sample is produced to determine the suitability of each male prior to use. Oocytes are collected from superovulated wild type or genetically-modified females at a specific time post-injection. Following capacitation, an aliquot of sperm is added to each fertilization dish containing groups of oocytes. The fertilized oocytes are incubated overnight and, the next day, two-cell embryos are harvested for transfer or cryopreserved for long-term storage. IVF can also help rescue a line in which a physical impairment prevents males from mating and, with some strains, to recover a line from previously cryopreserved mouse sperm.

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ovary is removed. The donor ovary is inserted into the opening in the bursa where it is physically held in place. Within a few weeks, the blood supply to the ovary will be re-established and the ovary should begin to function normally.

Assisted IVF

In addition to standard IVF techniques, Charles River offers laser-assisted IVF services for mouse lines with low quality sperm. A main criteria for successful IVF is the use of sperm with acceptable motility, concentration, and forward movement. If one or more of these parameters is suboptimal, the ability to fertilize oocytes can be severely limited. In these cases, an XYClone™ laser is used to create 2-3 holes in the zona pellucida of each oocyte providing a means for less motile or less progressive sperm to penetrate the oocyte and induce fertilization. This procedure has greatly enhanced the fertilization efficiency of various genetically-modified lines and is a vital tool in the rescue of valuable rodent strains.

Intracytoplasmic Sperm Injection (ICSI)

For genetically-modified mouse lines with extremely low sperm concentration, Charles River offers ICSI. This is the process by which a single spermatozoan is mechanically inserted into a donor oocyte in order to induce fertilization. This technique, requiring the use of a micromanipulator and originally described in the hamster (Uehara and Yanagimachi, 1976), is routinely used today with humans in cases of severe male factor infertility. If the sperm quality is insufficient for standard IVF or laser-assisted IVF, offspring can still be generated utilizing this technique. For mouse ICSI, the sperm tail is removed prior to injection and only the sperm head is introduced into the oocyte. Sperm used for ICSI can be prepared from previously frozen, lyophilized, or completely immotile samples. This technology provides the ultimate rescue of indispensable rodent lines.

Prior to initiating any rescue procedure, we perform a comprehensive fertility assessment on each strain. This aids in identifying the cause of poor reproductive performance and helps determine the best route for overcoming infertility. Reproductive records (when available) are evaluated for past performance, and the history of the line is considered. In addition, we offer extensive phenotyping services to further characterize the model and help explain reproductive challenges. Males and females can be evaluated individually and/or as a reproductive pair.

The male-specific fertility assessment consists of mating the males to synchronized or superovulated females in order to assess plug-to-pregnancy ratio and ability to produce fertilized embryos. Sperm can be collected and evaluated for concentration, as well as rates of motility, forward movement, and normal morphology.

The female-specific fertility assessment includes estrous synchronization with exogenous hormones (eCG and hCG), mating to a fertile male, and the ability to produce fertilized embryos and/or carry a litter to term.