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Commentary

Wild-Derived Inbred Mice No Longer ART-Resistant[1]

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Assisted reproduction technologies (ART) are of key importance for maintenance and storage of valuable mouse models. Unfortunately, these techniques are not equally effective for all mouse strains. Wild-derived inbred mice are descendants of wild mice trapped at different times and locations, and because of their high genetic diversity, they are prized research tools. Such mice offer an unlimited pool of genetic polymorphisms, not available with classical inbred strains, that facilitate a variety of genetic studies, including gene interactions, functional genomics, behavioral genetics, and others. These mice also have high incidence of Robertsonian translocations, which are of interest to cytogeneticists studying the effects of aneuploidy.

Wild-derived strains are particularly difficult to propagate by ART and are primarily maintained as live colonies, which is both cost and space ineffective. Therefore, methods for preservation of these valuable stocks are urgently needed. In the current issue of the *Biology of Reproduction*, Hasegawa et al. [1] successfully address the assisted reproductive technology necessary to propagate wild-derived inbred mice. The authors tested several modifications of the standard ART procedures to adapt these procedures for use with two wild-derived inbred mouse strains: MSM/Ms and JF1/Ms.

The first ART advance that Hasegawa et al. introduced in their study pertains to ovarian stimulation. They observed that females of the MSM/Ms and JF1/Ms strains are poor responders to conventional superovulation induction, which is traditionally induced by injection of equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) in a time- and dose-specific manner. These gonadotropins mimic the activities of the two hormones secreted by the pituitary, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which in normal ovulatory cycles induce egg maturation and ovulation, respectively. Injection of gonadotropins is highly successful and routinely used to achieve superovulation in common mouse strains. Nevertheless, reports of its negative effects on fertilization and development have appeared, and a long half-life of eCG and, perhaps, its LH activity were considered to be a primary reason for these problems [2–4]. In an attempt to increase the oocyte yield in MSM/Ms and JF1/Ms strains, Hasegawa et al. [1] tested the effects of eCG replacement with anti-inhibin serum (AIS). Inhibin is a major hormone that inhibits FSH secretion, and a negative relationship between its concentration and that of FSH in plasma has been documented [5]. Immunization against inhibin also stimulates follicle development and is successful in the induction of multiple ovulations in a variety of species [6–9]. Hasegawa et al. [1], however, are the first to show that AIS administration is highly successful in facilitating follicle progression and oocyte production in wild-derived inbred mice. Whereas eCG/hCG treatment of MSM/Ms and JF1/Ms females yielded few oocytes ($n \sim 5$), replacement of eCG with AIS resulted in ovulation of as many as 25 oocytes. This fivefold increase is a truly spectacular result. The oocytes obtained after AIS injection were of good quality, as evidenced by high fertilization rates during in vitro fertilization (IVF). So, low number of oocytes retrievable from MSM/Ms and JF1/Ms females is no longer an obstacle in generating embryos in vitro. One issue to consider, however, is commercial availability of AIS, which is not immediately apparent. If a goat immunization needs to be done each time that AIS is needed, then at least certain criteria for the quality of AIS should be established so that comparable serum is used across laboratories.

The second ART modification that Hasegawa et al. applied to increase the overall ART efficiency with wild-derived mice was the inclusion of reduced glutathione (GSH) in the fertilization medium. GSH is an antioxidant that protects both sperm and oocytes from oxidative stress [10], and it increases the fertilization rates with frozen-thawed sperm from C57BL/6 mice [11, 12]. The exact mechanism whereby GSH enhances fertilization is not known, but it has been suggested that it may be related to the modifications of zona pellucida [11]. The fertilization rates in the JF1/Ms strain reported by Hasegawa et al. [1] were very high (>85%) with both fresh and frozen sperm, even without GSH. With MSM/Ms mice, however, sperm freezing decreased the fertilization rates, and this was partially rescued by inclusion of GSH. Considering the difficulty in obtaining the oocytes for IVF, any improvement in their fertilizability is important. Towards this end, it might be valuable to use intracytoplasmic sperm injection instead of IVF. This, however, would first require testing the sensitivity of MSM/Ms oocytes to injections, something that has not yet been done.

The third ART improvement reported by Hasegawa et al. pertains to embryo transfer (ET). Using conventional ET, high implantation and live fetus rates (averaging 84% and 47%, respectively) were obtained with the JF1/Ms strain. Under the same conditions, however, MSM/Ms embryos implanted much less efficiently (45%) and yielded essentially no viable offspring. Significant postimplantation loss resulted in many resorptions and dead term fetuses. During normal in vivo development, the establishment and maintenance of pregnancy

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requires cross-talk between the conceptus and maternal environment [13]. Hasegawa et al. [1] speculated that the reason for the fetal loss might be impaired maternal-fetal interaction, such as maternal intolerance to the fetus and/or insufficient trophoblast growth and expansion. They first tried overcoming the fetal loss problem by using females of different strains, including one consomic for MSM Chromosome 17 encoding the major histocompatibility complex. These trials proved ineffective. Next, they tested two other conditions: cyclosporin A (CsA) treatment of recipient females and cotransfer with laboratory mouse embryos. CsA is an immunosuppressant drug widely used in organ transplantation to prevent rejection; it acts by interfering with the activity of T cells and, consequently, reducing the responses of the immune system. CsA injection around the time of implantation has been shown to improve pregnancy outcome and overcome fetal death in mice with high abortion rates [14]. Cotransfer of normal healthy embryos has also been shown to improve pregnancy rates, particularly with nuclear transfer-derived and transgenic embryos [15, 16]. In the hands of Hasegewa et al. [1], CsA and cotransfer treatments, applied individually, allowed the birth of the first few viable ART-derived MSM/Ms fetuses. The combination of both treatments resulted in up to 29% of transferred embryos surviving to term. This is an impressive achievement, considering that with conventional ET, no live pups could be generated.

To summarize, Hasegawa et al. demonstrated that ART could be successfully applied to two wild-derived inbred mouse strains, opening the possibility for preserving these valuable genotypes for distribution and future applications. The development of efficient ART applicable to these mice will undoubtedly expand their use within the scientific community. In addition to its practical aspects, the study raised some interesting scientific questions. What factors render the wild-derived inbred females unresponsive to conventional ovarian stimulation? What are the mechanisms of postimplantation fetal loss in the MSM/Ms strain? Perhaps now that the maintenance of these two strains has become easier, they could become a valuable model for similar ART problems observed in humans.

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