Nonhuman primate models for SARS-CoV-2 research: Cryopreservation as a means to maintain critical models and enhance the genetic diversity of colonies

Nonhuman primate (NHP) models, the most predictive preclinical models for human diseases and treatment outcomes, are in high demand and limited supply. There is a need for improved cryopreservation methods and routine storage of gametes and embryos, which are vital to protecting unique genetic models as well as providing resources for enhancing the genetic diversity of NHP colonies.

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The shortage of NHPs is an ongoing challenge for biomedical research that has been exacerbated by increased demand for these animal models for SARS-CoV-2 research (see previous commentaries in this series on COVID-19 models[1] and infrastructure needs[2]). Cryopreservation methods are needed to safeguard genetically valuable NHP models and maintain primate colonies that are genetically diverse and healthy. Cryopreservation technologies are especially critical during periods of high demand for NHPs because increased pressure on breeding can have negative effects on genetic diversity.

Cryopreservation involves the use of very low temperatures to preserve living cells or tissues so that they remain structurally intact and retain the potential for physiological function, such as fertilization or normal growth.[3] The advancement of fertility preservation approaches depends on cryopreservation and effective recovery of viable biological materials from cryopreserved materials (cryorecovery). Cryopreservation and cryorecovery processes also contribute to the development of new NHP models for human diseases, enable the retention of unique genetic lines, and provide a means to increase the genetic diversity of colonies. In the event of a natural disaster, cryopreservation may be the only way to ensure continued availability of a specific genetic line. Cryopreservation is particularly important for promoting genetic diversity in closed or small colonies without introducing potential pathogens or requiring complicated animal transport and introductions.

Recently, researchers have made stunning advances that rely on the cryopreservation of NHP gametes and embryos, particularly as applied to fertility preservation[4] and genome editing.[5] The benefits and limitations that characterize this area of NHP research vary depending on whether the research goal involves the cryopreservation of sperm, oocytes, or embryos.

Although primate sperm, particularly human sperm, are routinely cryopreserved, methods for NHP sperm cryopreservation remain cumbersome and need further optimization. Standard protocols involve the cryopreservation of NHP sperm in freezing extender solution containing egg yolk, with or without a permeable cryoprotectant, such as glycerol.[6] These and similar methods have been applied to the cryopreservation of sperm recovered from a transgenic rhesus macaque model for Huntington's disease.[7,8] Researchers have used related approaches to cryopreserve sperm from other NHP species that are widely used in biomedical research.[9,10] Less attention has been given to sperm vitrification, an alternative approach involving extremely rapid cooling to produce a glassy state without crystalization.[3] Considering the costs of maintaining primate colonies, the ongoing expansion of unique NHP disease models increases the need for more efficient and effective sperm cryopreservation methods to facilitate the cryobanking and recovery of these critical research resources.

Using a rhesus macaque model, researchers recently demonstrated that immature testicular tissue can be cryopreserved and autologously grafted into the same host, such that the transplanted tissue matures to produce functional sperm.[4] Graft-derived sperm from one of the macaques in this study was used to successfully fertilize oocytes by intracytoplasmic sperm injection. Implantation of one of the resulting embryos into a surrogate mother resulted in the live birth of a healthy monkey. This first in NHP cryopreservation and cryorecovery holds promise for eventual clinical application to preserve the fertility of prepubertal male patients who must undergo potentially gonadotoxic therapies to treat cancer or other conditions. The results also suggest another promising method for long-term storage and recovery of sperm to maintain genetically valuable NHP models.

Given their larger size, oocytes are more sensitive to chilling than sperm, making them highly susceptible to intracellular ice crystal formation.[11] To date, standardized protocols have not been established for cryopreservation and cryorecovery of isolated oocytes from laboratory animals. Notable progress, however, has been achieved with the vitrification of NHP ovarian tissue. Researchers showed that a method for the vitrification of rhesus macaque ovarian cortex tissue preserves both follicular morphology and function following tissue warming and culture.[12] Baboon ovarian tissue also has been successfully vitrified and autografted, resulting in follicle growth and successful ovulation *in vivo*.[13] NHP pregnancies, however, have not yet been achieved using cryopreserved ovarian tissue. The procedures involving the cryopreservation of ovarian tissue also have not been standardized and remain largely experimental.

The cryopreservation of NHP embryos[14] is critical to nascent efforts to create

new NHP models for human disease via CRISPR/Cas9 genome editing, which is typically performed on one-cell zygotes produced by *in vitro* fertilization.[15] A short period of embryo development in culture allows researchers to take a DNA sample by trophectoderm biopsy for genetic profiling without harming the embryo. Subsequent embryo cryopreservation affords researchers the opportunity to validate on-target gene editing from the biopsy and to screen for candidate embryos that are transferred into recipient females to establish pregnancies. In addition, cryopreservation of embryos provides a method for maintaining the genetically engineered line. Achieving the full potential of genome editing research in NHPs[5] will require support for the optimization and standardization of embryo cryopreservation protocols and their integration with other assisted reproduction technologies, as well as support for the expansion of the needed cryopreservation resources.

Although progress has been made toward improving methods for cryopreservation and cryorecovery of NHP gametes and embryos, continued efforts are needed to preserve genetically unique NHP models and to maintain genetically diverse resources. Thus far, cryopreservation efforts have been mainly experimental, focusing on the preservation of NHP genetic models for conditions such as Huntington's disease. Moreover, the use of cryostorage has not been widely promoted for maintaining the genetic diversity of NHP colonies, resulting in limited facilities for the cryostorage of NHP gametes and embryos.

The high research demand for NHPs and their limited availability worldwide amplify the need to ensure that these valuable preclinical models are maintained as both living colonies and cryopreserved resources. A successful response to this challenge will require investments in: methodological studies to compare and optimize the cryorecovery efficiency of existing approaches; the advancement of technical capabilities for cryopreservation; and the development of long-term cryopreservation and cryostorage resources for NHPs. To this end, the Office of Research Infrastructure Programs (ORIP) continues to fund the development of cryopreservation tools and approaches for NHPs and other animal models[16], which will facilitate the biobanking of critical models for COVID-19 and other diseases and the maintenance of genetically diverse and healthy NHP colonies without reliance on uncertain animal importations.❐

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References

1. Chang, M. C., Hild, S. A. & Grieder, F. B. *Lab Anim (NY)* **50**, 113–114 (2021).
2. Hild, S.A. Chang, M.C., Murphy, S.J. and Greider, F.B. *Lab Anim (NY)* (2021) https://doi.org/10.1038/s41684-021-00760-9
3. Pegg, D. E. Principles of cryopreservation. In *Methods in Molecular Biology, Vol. 368: Cryopreservation and Freeze-Drying Protocols, Second Edition*. (eds Day, J. G. & Stacey, G. N.) 39–57 (Humana Press Inc., Totowa, NJ, 2007).
4. Fayomi, A. P. et al. *Science.* **363**, 1314–1319 (2019). Erratum in *Science* **364** (2019).
5. Schmidt, J. K. et al. *Sci. Rep.* **10**, 18457 (2020).
6. Dong, Q., Correa, L. M. & VandeVoort, C. A. *Cryobiology.* **58**, 20–27 (2009).
7. Putkhao, K., Chan, A. W., Agca, Y. & Parnpai, R. *Cloning Transgenes.* **2**, 1000116 (2013).
8. Moran, S. P., Chi, T., Prucha, M. S., Agca, Y. & Chan, A. W. *J. Am. Assoc. Lab. Anim. Sci.* **55**, 520–524 (2016).
9. Oliveira, K. G. et al. *Cryobiology.* **72**, 283–289 (2016).
10. Strelchenko, N. S. et al. *J. Am. Assoc. Lab. Anim. Sci.* **59**, 681–686 (2020).
11. Saragusty, J. & Arav, A. *Reproduction.* **141**, 1–19 (2011).
12. Ting, A. Y. et al. *Hum. Reprod.* **28**, 1267–1279 (2013).
13. Amorim, C. A. et al. *Hum. Reprod.* **28**, 2146–2156 (2013).
14. Curnow, E. & Hayes, E. *Methods Mol. Biol.* **2006**, 321–339 (2019).
15. Kang, Y., Chu, C., Wang, F. & Niu, Y. *Dis. Model Mech.* **12**, dmm039982 (2019).
16. Office of Research Infrastructure Programs. *Strategic Plan 2021–2025*. (National Institutes of Health, Bethesda, MD, 2021, available at https://orip.nih.gov/strategic-plan).