POLICY

Disruptive reproductive technologies

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In vitro gametogenesis raises new possibilities for reproductive and regenerative medicine as well as vexing policy challenges.

In vitro fertilization (IVF) represents a transformative technological innovation. Recognized by a Nobel Prize in Physiology and Medicine to Robert Edwards in 2010, IVF cured some forms of infertility and gave rise to numerous therapeutic and diagnostic breakthroughs. Nevertheless, this remarkable feat may one day be supplanted by in vitro gametogenesis (IVG)—the generation of eggs and sperm from pluripotent stem cells in a culture dish. Currently feasible in mice (*1*, *2*), IVG is poised for future success in humans and promises new possibilities for the fields of reproductive and regenerative medicine. At the same time, IVG raises vexing ethical and social policy challenges in need of redress. Here, we describe the state of relevant IVG science and discuss the promise, challenges, and regulatory and ethical implications of IVG.

STATE OF THE SCIENCE

Differentiation of the mammalian germ cell lineage in vivo requires timely signaling along a proscribed multistep fate specification pathway (*1*, *2*). The extragonadal segment of this developmental course—the induction of primordial germ cells (PGCs)—has been successfully recapitulated in vitro using cultured mouse embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), but early reports failed to achieve functional gametogenesis (*3*). In recent pioneering studies from Hayashi and colleagues, in vitro–derived primordial germ-like cells have been transplanted into the seminiferous tubules of germ cell–ablated mice (yielding functional sperm) or into reconstituted ovaries that comprise in vitro–generated PGCs and embryonic gonadal somatic cells transplanted under the ovarian bursa of mice (which restored oogenesis) (*4*, *5*). In vitro–fertilized and implantable embryos followed, resulting in fertile offspring (*4*, *5*). In remarkable recent work, Zhou and colleagues produced sperm-like cells (*1*), whereas Hikabe and colleagues produced oocytes capable of supporting fertilization and parentage from murine ESCs entirely in vitro (*2*). These observations suggest that the molecular cues that drive the specification of mouse PGCs in vivo are operational ex vivo. Optimization of IVG will require that the critically timed cross-talk between the somatic gonadal cells and their germ cell counterparts be thoroughly elucidated and replicated in vitro.

Attempts at IVG in human and nonhuman primates have met with limited success. Recent studies, however, have largely replicated the historical sequence noted in the mouse model, including the in vitro specification of human PGCs from naïve germ cell–competent ESCs and iPSCs (*6*, *7*). These findings suggest that experimental refinements likely will permit derivation of functional eggs and sperm from human iPSCs in the not too distant future.

Viewed in terms of its scientific impact, IVG stands to overcome the limited availability of embryonic germ cells for scientific investigation. Embryonic germ cells have been and remain intractable to study because of the limited number of founder PGCs and the practical, ethical, and political constraints associated with procuring early-stage human embryos for research. IVG raises the prospect of an all but inexhaustible supply of germ cell elements for investigation (*8*). Studies of gametogenesis in null gene mutants and the signaling networks responsible for gamete fate specifications stand to illuminate interactions between germ and somatic cells (*9*).

TRANSLATIONAL POSSIBILITIES

IVG promises to transform the fields of reproductive and regenerative medicine in several ways. First, patient-specific iPSC-derived gametes will permit the characterization of germline disease at the cellular and molecular levels (*10*). Second, fully functional autologous gametes of iPSC origin might substitute for lost or impaired germ cell function, for example, in the case of cancer survivors. Incurable with present-day know-how, chemotherapy-induced germ cell failure might be addressed with iPSC-derived gametes, either in vitro (IVF) or in vivo (insemination or transplantation). Similarly, heritable germ cell dysfunction might be amenable to substitution with genome-edited iPSC-derived gametes. Third, IVG might enable the prevention of mitochondrial diseases via patient-specific iPSC-derived oocytes selected for their low burden of mutant mitochondrial DNA that could yield disease-free progeny. Fourth, IVG could facilitate the derivation of patient-specific stem cell lines through somatic cell nuclear transfer (SCNT), which is presently rate-limited by the scarcity of human oocytes. iPSC-derived oocytes could potentially fill this void by sidestepping the ethical and statutory barriers associated with the procurement of human oocytes.

The availability of fully functional gametes of iPSC origin also may transform the current IVF paradigm by eliminating the need for stimulating the ovary and retrieving eggs and, in so doing, phase out the occasional morbidity and mortality of ovarian hyperstimulation. Similarly, IVG could obviate the need for donor eggs to overcome intractable female infertility. Much would depend on whether IVG could ever become more affordable and thus enhance access to advanced infertility therapy. Some in the bioethics, legal, and public press have speculated that in the future, IVG may also serve female same-sex partners who seek to have a child who shares both partners' genetic heritage, or possibly enable single women to conceive offspring of a single parentage (*11*).

The above possibilities notwithstanding, the challenges facing IVG are multiple. Terminal gametogenesis—the in vitro conversion of stem cell–derived PGCs into fully functional gametes—would have to be accomplished with high fidelity if the promise

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of IVG is to be realized. In particular, meiosis, the hallmark of gametogenesis, would have to be meticulously replicated and carefully validated for the absence of genetic or epigenetic aberrations. This concern assumes special significance in light of the observation that meiotic progression and phenotypic oocyte differentiation are dissociable (*12*). It follows that nothing short of rigorous affirmation of the defining attributes of meiosis and copious preclinical evidence of safety will do to inspire the confidence to proceed to clinical application (*13*). Optimizing of terminal gametogenesis requires breakthroughs in the derivation of developmentally matched somatic gonadal cells, which are indispensable for PGC maturation. Greater attention must be paid to simulating the gonadal "niche" microenvironment, which plays a role in optimizing oocyte differentiation (*14*).

One additional cloud on the IVG horizon is the lingering concern about iPSCs as the starting material for regenerative medicine. Whether human iPSCs have a propensity for genetic and epigenetic aberrations is unresolved. Also, limitations persist regarding the relative stochastic and protracted inability of human iPSCs to reprogram to pluripotency or to erase "residual DNA methylation patterns typical of parental somatic cells" as compared with SCNT-derived counterparts (*15*). With doubts remaining on the relative safety of iPSCs as the substrate for cell replacement therapies, the practice of IVG may well depend on further advances in reprogramming technology and on reassuring results from future clinical studies.

REGULATION AND ETHICS

Any clinical use of IVG raises regulatory and ethical questions that require resolution. Some of these questions are similar to those faced by other cutting-edge reproductive technologies (for example, mitochondrial replacement therapy and gene editing) covered by, among other things, the 2008 consensus statement of the Hinxton Group (*16*). Others are posed for the first time by IVG.

All clinical applications of IVG will be the subject of regulation by the U.S. Food and Drug Administration, and safety concerns are bound to loom large in the eyes of regulators. In this context, IVG-derived eggs and sperm will likely be categorized as a "cellular and gene therapy product" to be regulated under Section 351 of the *Public Health Service Act* and certain sections of the *Federal Food, Drug, and Cosmetic Act* (*17*). Under this regulatory scheme, extensive preclinical safety trials in mammalian species will be required, likely including nonhuman primates. Successful conclusion of the latter could lead to the conduct of phased clinical trials pursuant to an Investigational New Drug application (*17*). The prospect of first-in-human embryo generation and transfer will require careful long-term monitoring of the ensuing progeny, as IVF demanded on its introduction.

Second, refining the science of IVG to the point of clinical use will involve the generation and likely destruction of large numbers of embryos from stem cell–derived gametes. This practice raises religious and secular objections to embryo creation and destruction in the research process. In the United States, for example, the creation (and destruction) of IVG-derived human embryos solely for the purpose of research will be ineligible for public funding as a result of the *Dickey-Wicker Amendment*, an appropriations rider that prohibits the use of federal funding for research wherein embryos are created for research purposes or destroyed (*18*). IVG might also inflame age-old concerns about "commodification" of human reproduction. On the one hand, IVG may supplant human donor egg markets, which some argue raises the risk of exploitation and thus dampens one kind of concern in the commodification debate. On the other hand, IVG may raise the specter of "embryo farming" on a scale currently unimagined, which might exacerbate concerns about the devaluation of human life (*19*). At the very least, large-scale efforts at commercialization will certainly be subject to the same federal and state oversight currently applied to sperm and egg banks (*20*).

Third, IVG may exacerbate concerns regarding human enhancement. Preimplantation genetic diagnosis already allows individuals to select among multiple embryos for implantation, but IVF generates a finite number of embryos from which to select, especially given the physical burdens of harvesting eggs and the risks of ovarian hyperstimulation syndrome. IVG could, depending on its ultimate financial cost, greatly increase the number of embryos from which to select, thus exacerbating concerns about parents selecting for their "ideal" future child. This worry would be exacerbated if IVF were combined with facile genome editing such as CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9), enabling not only selection but also alteration (*21*). Of course, successfully using the technology in this way would require far deeper knowledge of human genetics than we currently have. Nevertheless, even at this stage, it is worth contemplating the ethical issues raised by such a possible future, which would directly force regulators to make difficult decisions on drawing the line between alterations that end harmful conditions versus eugenics (*22*).

Fourth, IVG increases the risk of the "unauthorized" use of biomaterials, absent explicit consent. In the most extreme case, imagine an individual using someone else's sloughed skin cells to derive gametes for reproductive purposes. Should the law criminalize such an action? If it takes place, should the law consider the source of the skin cells to be a legal parent to the child, or should it distinguish an individual's genetic and legal parentage? Thus far, courts have had little experience with nonconsensual parenthood (*23*). In cases of stolen sperm or statutory rape of men, the courts have been largely unwilling to free the wronged father of unwanted legal parenthood. Instead,

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IVG: Possibility and policy. Cryopreservation of frozen sperm straws and embryos.

courts confronting these situations have largely reasoned that the right to have a legal parent and child support attendant to it belongs to the child, and the child's rights cannot be forfeited by either parent's misdeed (*23*). IVG further ups the stakes by raising the possibility of the unauthorized use of human cellular debris subject to daily involuntary shedding. In this regard, it remains uncertain whether the law should and will protect the "right not to be a genetic parent" (*23*). In the short term, insistence on well-documented consent from a would-be genetic parent may well constitute the only, if imperfect, means of deterrence. If or when such deterrence fails, the courts may well have to decide the legal parentage of children conceived consequent to unauthorized IVG, as well as potential tort claims (for example, claims of civil liability) by the aggrieved parties (*23*).

Last, IVG's most disruptive impact might be on our very conception of parentage. Artificial insemination, IVF, and surrogacy have forced us to unbundle our conceptions of genetic, gestational, and legal parentage and recognize that an individual might play one but not another role. Those in the legal and bioethics literature have suggested that IVG may in the future confront us with a still more complex conceptual future, where the number of genetic parents is no longer limited to two. The scientific literature has not yet proven feasibility but, for the purpose of thinking through the implications, consider the speculative possibility of so-called multiplex parenting, where one gamete is derived from two individuals and combined with the gamete of a third individual (*24*). Would we view each of those three as equal genetic parents, or do we give greater rights and duties to the parent who contributed more genetic material? Should the relationship of the three break down, how should the state adjudicate legal parentage? To what extent should the law respect contractual agreements or other indicia of intent, as it has been called to do by some courts considering surrogacy arrangements? The situation becomes still more complex if other reproductive technologies, such as surrogacy, are combined with IVG.

CONCLUSIONS

In the near future, the impact of IVG likely will be limited to enhancing the science of germ cell biology. Given the stringent safety imperative, clinical applications are less likely to be pursued any time soon. Still, with science and medicine hurtling forward at breakneck speed, the rapid transformation of reproductive and regenerative medicine may surprise us. Before the inevitable, society will be well advised to strike and maintain a vigorous public conversation on the ethical challenges of IVG. In this regard, the United States might do well to borrow a page from the U.K., where an exemplary process focused on safety, ethics, and public consultation has recently led to the state-sanctioned conduct of clinical studies of mitochondrial replacement therapy (*25*). This latter example might well be viewed as a model for defining ethically defensible and publicly acceptable pursuits of novel biomedical technologies in the best interest of humankind (*25*). Nothing less will do as humanity contemplates the replacement of gametes of gonadal origin by their stem cell–derived counterparts.

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