Assisted same-sex conception: reproduction reimagined

The fast-approaching 50th anniversary of the birth of Louise Joy Brown—the first ever "test-tube baby"—serves as a vivid reminder of the scourge of infertility as well as of the import of the alleviation thereof (1). Thus far, in vitro fertilization (IVF) treatment has focused all but exclusively on the infertility endured by opposite-sex couples. The childlessness experienced by same-sex couples remains to be resolved. Indeed, the notion of bimaternal and bipaternal conceptions remains hypothetical at this time. The above notwithstanding, recent experimental work, the byproduct of several scientific breakthroughs, now suggests that same-sex parenthood could be possibly achieved in the foreseeable future. This special contribution reviews recent scientific developments with the intent of advancing the possibility of same-sex parenthood and the future prospects thereof.

Data derived by the US Census Bureau reveal the total number of same-sex US households will exceed the 1.2 million mark in 2021 (2). The lion's share of the households in question (>0.7 million) comprised same-sex married couples, whose legal status was formally affirmed by the Supreme Court of the US. Notably, ≤20.5% of same-sex households report the presence of children aged <18 years (2). Absent options for genetically related offspring, same-sex couples are presently limited to adoption or donor-assisted conception. It follows that same-sex female couples in pursuit of parenthood are obliged to resort to a sperm donor. Same-sex male couples, in turn, are expected to secure an egg donor as well as a legal contract with a gestational carrier and her partner and spouse, if any. The latter contract, compensated or otherwise, details the rights, obligations, intentions, and expectations of the parties involved with the arrangement in question. Importantly, a legally executed surrogacy agreement assures same-sex male couples of their status as the legal parents of the prospective newborn, whose name may thus be recorded in a US birth certificate.

Currently, the costs incurred in the course of same-sex family building are uncovered largely by commercial insurers, nonprofit health service plans, and health maintenance organizations (3). Statutory redress for this ongoing reality is presently limited to a few states that are home to an enacted same-sex infertility health insurance state mandate (4). The leader of this slow-moving national trend is the state of Maryland which saw the enactment of the first-in-the-nation same-sex infertility health insurance mandate in 2015 (3). As written, Maryland House Bill 838 prohibits "insurers, nonprofit health service plans, and health maintenance organizations from requiring specified conditions of coverage for specified infertility benefits for a patient who is married to an individual of the same sex" (3). Comparable statutes were since enacted by the states of New Jersey (2017), New York (2020), Illinois (2022), Colorado (2023), and Maine (2023) (4).

The prospect of viable bimaternal offspring, heretofore unthinkable, was first broached by Kawahara et al. (5–8) in a series of scientific articles dating back to 2007. Seeking to render the notion of viable bimaternal murine offspring a reality, the investigators set out to construct oocytes in which 2 paternally imprinted-control regions of the oocytic genome were deleted. These previously characterized regions of the genome all but preclude the development of bimaternal offspring (5–8). Specifically, the investigators saw the deletion of the *H19* differentially methylated region as well as of the *Dlk1-Dio3* intergenic germline-derived differentially methylated region in both fully grown oocytes and nongrowing counterparts (5–8). Nuclei of early oocytes isolated from a neonate oocyte bearing the double knockout of the imprinted regions were then transferred into an enucleated, fully grown oocyte and activated for development (5–8). The resultant bimaternal embryos were consistent with the notion that imprinted genes, the subject of regulation by paternal imprinting-control regions, constitute the sole barrier to the successful development of bimaternal offspring (5–8). The investigators made further note of the fact that the bimaternal mice so derived went on to develop into viable and fertile adults at a success rate equivalent to that obtained with IVF-treated normal embryos (5–8).

Further affirmation of the aforementioned conclusions was afforded by Li et al. (9) whose studies entailed the deployment of haploid embryonic stem cells (ESCs) derived from either androgenetic or parthenogenetic (female) embryos (androgenetic haploid ESCs [ahESCs] or parthenogenetic ESCs [phESCs]). In laying out the rationale for the experiments planned, the investigators made note of the fact that ahESCs "can replace gametes to produce offspring" (9). The investigators make further note of the observation that, subject to imprinting modifications, the nuclei of mouse phESC "can efficiently produce viable fertile offspring on intracytoplasmic injection into MII oocytes" (9). The investigators go on to note that the capacity of haploid ESCs to display primordial germ cell-like methylation profiles in the wake of in vitro cultivation was firmly established (10). It is against this backdrop that the combination of a metaphase II oocyte with phESCs replete with 3 deleted imprinted regions gave rise to normally growing bimaternal mice (9, 10). Similarly, the combination of sperm with ahESCs carrying a total of 7 deleted imprinted regions yielded live, full-term bipaternal mice (10).

Further efforts to generate viable bipaternal male and female mice were undertaken by Min Deng et al. (11). Using induced pluripotent stem cells (iPSCs) from an XY mouse (father 1), the investigators undertook to isolate cultured subclones characterized by the spontaneous loss of the Y chromosome to yield XO iPSC counterparts (11). The latter iPSCs were, in turn, deployed by the investigators to generate female chimeras (11). Breeding of the female chimeras in question with a genetically distinct XY mouse (father 2) gave rise to progeny the genetic makeup of which was contributed equally by both fathers (11). Taken together, these observations are in keeping with the notion that functional oocytes can be derived from male somatic cells on reprogramming and spontaneous sex reversal (11).

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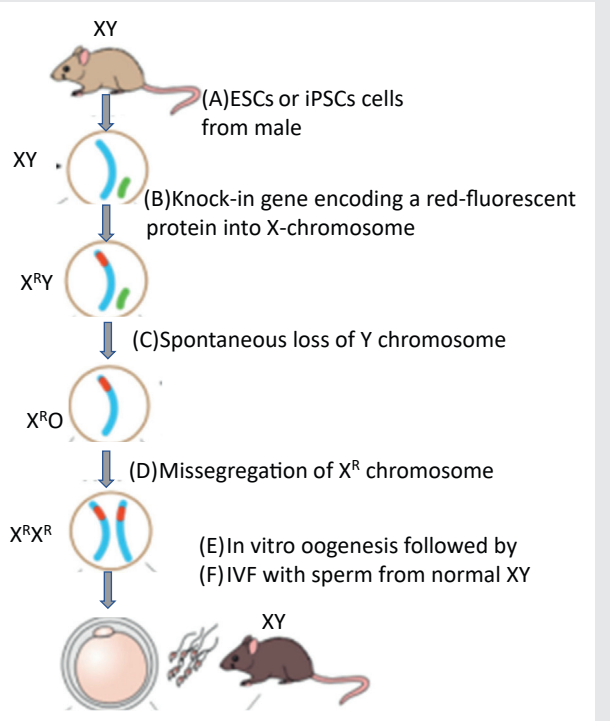
More recently, Murakami et al. (12) reported the in vitro generation of functional oocytes from male mice with an eye toward yielding bipaternal offspring (Figure 1). To this end, the investigators derived XY somatic cells from the tail of a sexually mature male mouse (12). The latter, in turn, were converted to XY iPSCs and placed in culture. With an eye toward enhancing chromosomal missegregation of the cultured XY iPSCs, use was also made of reversine (2-6-cyclohexylaminopurine), a small molecule inhibitor of monopolar spindle 1-like 1 kinase that is capable of inactivating the spindle assembly checkpoint (13). In addition, the investigators inserted a gene encoding DsRed (a brilliantly red fluorescent protein) into the X chromosome using the CRISPR–Cas9 system with an eye toward monitoring the number of X chromosomes in the cultured iPSCs (12). Because the 2 X chromosomes in mouse ESCs are both transcriptionally active, the number of X chromosomes in the cultured iPSCs could be determined by the difference in intensity of the DsRed fluorescent protein (12). It was in the course of the experiment that 6% of the cultured XY iPSCs converted spontaneously to XO iPSC counterparts (12). In time, some of the latter underwent duplication of the X chromosome to yield XX iPSCs (12). It was at that point that the latter were isolated and converted via in vitro gametogenesis into mature oocytes (12). Fertilization of the resultant oocytes, in turn, gave rise to two-cell embryos, which on transfer to pseudopregnant mice, yielded bipaternal offspring (12). As noted by the investigators, several of the aforementioned transitions were marked by low efficiency: circa 30% of the XX iPSCs were successfully converted into oocytes, of which only 40% were successfully fertilized (12). In addition, only 1.1% of embryos transferred to a surrogate "gave rise to pups (7 of 630)" (12). The above notwithstanding, the investigator concluded that "the oocytes from sex-converted iPSCs were functional" (12). It remains to be seen whether the aforementioned process can be duplicated in a human context. When so, it is at least possible that the technology in question could be brought to bear on the treatment of same-sex infertility.

Assuming continued experimental progress, the prospect of bimaternal and bipaternal parenthood in humans could well become a clinical reality. Arriving at this endpoint, however, is bound to prove challenging in a number of ways. First, clinical deployment of the requisite technology in the US can only proceed subject to the approval of its safety and efficacy by the US Food and Drug Administration (FDA). It follows that the cognate technology sponsors will be called on to conduct careful clinical trials, the outcome of which will be thoroughly scrutinized by an FDA public advisory committee. Second, any and all of the preliminary experimental efforts would have to be underwritten by private rather than public (e.g., National Institutes of Health) funds. The latter statutory constraint, one proscribed by the Dickey-Wicker Amendment, precludes the use of federal funds for "the creation of a human embryo or embryos for research purposes" (14). The Amendment goes on to prohibit "research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death" (14). Although not directly germane to the FDA, the Dickey-Wicker Amendment may also give pause to the FDA, whose concerns over its annual Congressional appropriation are ever-present.

FIGURE 1



Schematic of protocol to make oocytes from male mice in vitro (12). (**A**) Use mouse embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs) from a male mouse using convention. (**B**) Knock in a red fluorescent protein into the X chromosome of the male iPSCs using CRISPR-Cas9 technology. This enables the investigators to identify how many X^R chromosomes are present in a cell without damaging the cell. (**C**) At low frequency (approximately 5%), male iPSCs cultured and repeatedly replated will spontaneously lose their Y chromosome. Testing clones using polymerase chain reaction for the Y chromosome yielded the identification of several clones lacking the Y chromosome but retaining the X^R chromosome. (**D**) Male iPSCs lacking the Y chromosome were treated with reversine, a small molecule that enhances chromosome missegregation and serves as an inhibitor of the spindle assembly checkpoint. The endpoint here was a duplication of the X^R chromosome as identified by the inserted red fluorescent protein. (**E**) The now female (X^RX^R) cells from male (XY) cells were used for in vitro gametogenesis. (**F**) The engineered egg was fertilized by sperm from a normal XY male adult, with embryos inserted into a female surrogate host. Pups were born that were healthy and reproductive. IVF = in vitro fertilization.

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Eli Y. Adashi: Conceptualization, Writing – original draft, Writing – review & editing. **Gary M. Wessel:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of interests

E.Y.A. has nothing to disclose. G.M.W. has nothing to disclose.

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