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The obligate need for accuracy in reporting preclinical studies relevant to clinical trials: autologous germline mitochondrial supplementation for assisted human reproduction as a case study

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***Abstract*:** A now large body of work has solidified the central role that mitochondria play in oocyte development, fertilization, and embryogenesis. From these studies, a new technology termed autologous germline mitochondrial energy transfer was developed for improving pregnancy success rates in assisted reproduction. Unlike prior clinical studies that relied on the use of donor, or nonautologous, mitochondria for microinjection into eggs of women with a history of repeated *in vitro* fertilization failure to enhance pregnancy success, autologous germline mitochondrial energy transfer uses autologous mitochondria collected from oogonial stem cells of the same woman undergoing the fertility treatment. Initial trials of autologous germline mitochondrial energy transfer during - *in vitro* fertilization at three different sites with a total of 104 patients indicated a benefit of the procedure for improving pregnancy success rates, with the birth of children conceived through the inclusion of autologous germline mitochondrial energy transfer during *in vitro* fertilization. However, a fourth clinical study, consisting of 57 patients, failed to show a benefit of autologous germline mitochondrial energy transfer–*in vitro* fertilization *versus in vitro* fertilization alone for improving cumulative live birth rates. Complicating this area of work further, a recent mouse study, which claimed to test the long-term safety of autologous mitochondrial supplementation during *in vitro* fertilization, raised concerns over the use of the procedure for reproduction. However, autologous mitochondria were not actually used for preclinical testing in this mouse study. The unwarranted fears that this new study's erroneous conclusions could cause in women who have become pregnant through the use of autologous germline mitochondrial energy transfer during-*in vitro* fertilization highlight the critical need for accurate reporting of preclinical work that has immediate bearing on human clinical studies.

Keywords: AUGMENT, *in vitro* fertilization, IVF, mitochondria, mitochondrial supplementation, oocyte, oogonial stem cells, ovary, three-parent baby

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Introduction: mitochondria and *in vitro* fertilization outcomes

Experimental and clinical observations have collectively underscored the central importance of mitochondrial function to oocyte maturation, fertilization success, and preimplantation embryonic development.[1–14] In turn, the prospects of improving human *in vitro* fertilization (IVF) success rates by supplementing oocytes with additional mitochondria through microinjection during intracytoplasmic sperm injection (ICSI) were first reported in the late 1990s using subject-mismatched (nonautologous) mitochondria collected from donor eggs or trinucleate embryos.[15–20]

Although initial clinical studies of nonautologous cytoplasmic or ooplasmic transfer in women with a history of repeated IVF failure showed highly promising outcomes,[15–20] the procedure was quickly halted by the United States Food and Drug Administration (FDA) because of the transfer of foreign genetic material (namely, donor mitochondrial DNA or mtDNA) into human eggs during the process.[21] Indeed, mitochondrial genomes derived from both the natural mother and the oocyte donor have been identified in children conceived through the use of nonautologous ooplasmic transfer during assisted reproduction.[22,23]

The ruling of the FDA, and subsequent preclinical mouse studies showing that offspring carrying heteroplasmic mitochondrial genomes can develop a number of abnormalities during adult life,[24,25] prompted a re-thinking of the ooplasmic transfer procedure to possibly achieve the clinical benefit for assisted reproduction reported earlier[15–20] without the downside of using nonautologous (subject-mismatched) mitochondria. As efforts in this area continued, additional studies were published with animal models confirming the benefits of mitochondrial supplementation in eggs to IVF success rates.[26,27] On the heels of this growing body of work, a new technology termed autologous germline mitochondrial energy transfer (AUGMENT) was then developed using autologous mitochondria isolated from oocyte precursor or oogonial stem cells (OSCs) of the same individual undergoing the conventional IVF protocol.[28,29] Preliminary results from approved clinical studies of AUGMENT-IVF at three different sites with 104 total patients enrolled yielded positive early indications of the procedure for improving pregnancy success rates across a total of 369 IVF cycles.[30,31] The benefits of AUGMENT-IVF reported from these trials were consistent with similar positive outcomes demonstrated in animal studies[26,27] as well as with outcomes of prior clinical studies using donor (nonautologous) mitochondria.[15–20] Importantly, AUGMENT-IVF appeared to achieve these outcomes while circumventing the issue of introducing nonautologous germline mitochondria into human eggs at the time of fertilization.[32]

However, a fourth trial of AUGMENT-IVF reported 4 years later with 57 enrolled subjects failed to show a clinical benefit of the procedure for enhancing cumulative live birth rates *versus* those obtained with IVF alone.[33] Although the basis of this discordance in results generated to date across the four published AUGMENT-IVF trials remains unclear, it has been speculated that the relatively small numbers of patients enrolled in the various studies, differences in patient cohorts and/or protocols employed, and intercycle variability of IVF procedures are all potential explanations.[34] While these possibilities seem reasonable, we would offer yet another explanation for the discordance in AUGMENT-IVF outcomes reported by Fakih and colleagues[30] compared with those of Labarta and colleagues[33] several years later. Our explanation is rooted in the technical approaches required for the successful purification of OSCs from human ovarian cortical tissue as the source for mitochondria that are subsequently transferred into eggs at the time of ICSI.

As designed, isolation of OSCs from ovarian tissue for AUGMENT-IVF requires the use of fluorescence-activated cell sorting (FACS) coupled to antibodies that recognize an externalized domain of the C-terminus of DEAD-box helicase 4 (DDX4) exposed on the surface of OSCs in adult ovaries.[35] To prepare the cells for FACS, ovarian tissue must be properly dispersed using mechanical and enzymatic approaches in a manner that achieves adequate tissue dissociation for release of the OSCs as single cells while simultaneously maintaining cellular viability.[36] Indeed, application of excessive mechanical force, use of plastic pipette tips for tissue dispersion and/or enzymatic overdigestion (either temporally or through the use of a collagenase source with a very high level of activity per unit of enzyme) will significantly compromise the integrity of the cells obtained. Moreover, the cell fractions are then subjected to flow cytometry, which can also be a primary source of extensive cellular damage or death if the nozzle pressures are not properly calibrated for viable cell collection. We have experienced, and experimentally resolved, all of these issues firsthand in our development of the FACS-based procedure to purify OSCs from human ovaries,[35] and we have emphasized the extreme precautions that must be taken to minimize cellular damage through each step of the protocol.[36] Given all of this, and the fact improper gating strategies and/or failure to include key controls during FACS can generate cell preparations contaminated with nontarget cells and debris,[37] we believe a critical data set missing from all studies of autologous mitochondrial transfer reported to

date is clear documentation of the identity and viability of the sorted cells used to isolate the mitochondria for injection.

Why is this so important? It is quite likely that mitochondria isolated from OSCs which are contaminated with unwanted cell types and debris, or, more importantly, which are damaged or dead, would behave quite differently following injection into eggs than mitochondria isolated from pure preparations of viable OSCs. Moreover, of all of the possibilities offered to explain the discordance in AUGMENT-IVF study outcomes reported thus far (see above), this possibility seems to best explain how one study reported a significant improvement in blastocyst quality,[30] while another reported the opposite,[33] when AUGMENT was included. Specifically, for 25 of their 59 enrolled subjects, Fakih and colleagues[30] utilized a procedure termed Matched Best Embryo Selection and Transfer (MBEST). The approach enabled a direct comparison of outcomes from ICSI alone *versus* ICSI plus AUGMENT in the same pool of eggs retrieved from a single IVF cycle in each individual subject. In other words, a patient's own eggs from a given cycle served as the control (non-AUGMENT) group; once that, patient's pool of retrieved eggs were randomly allocated as ICSI alone or ICSI-AUGMENT for parallel study under otherwise identical conditions. Holding all other parameters for embryo culture constant, the ICSI-AUGMENT group showed a substantial increase in embryo transfer rates (14/25) compared to the ICSI alone group (2/25). This was attributed specifically to marked improvements in the criteria used for blastocyst quality assessment and selection, as recorded by embryologists blinded to the treatment groups. In turn, a 32% pregnancy rate was achieved in the ICSI-AUGMENT group, which is striking since this same cohort of patients historically exhibited a 0% pregnancy rate per initiated cycle.[30] In contrast, Labarta and colleagues[33] subsequently reported that the inclusion of AUGMENT in a study of 57 subjects undergoing IVF not only failed to improve cumulative live birth rates but also negatively affected the quality of the embryos produced following fertilization. Putting pregnancy rates aside as an endpoint, it is perplexing how two studies could produce completely opposite outcomes in terms of embryo quality if both employed the exact same protocols. The most logical explanation is that there were critical differences in the approaches used, and this could very well be reflective of differences in the purity or integrity of the OSC preparations obtained by FACS for subsequent mitochondrial isolation and injection at the two clinical sites.

In closing this section, the published clinical studies of AUGMENT-IVF reported to date from four different sites,[30–33] when considered with results of prior studies of mitochondrial transfer in eggs using animal models,[26,27] as well as nonautologous mitochondrial transfer in humans,[15–20] collectively weigh in favor of a benefit of boosting eggs, at least eggs compromised by advanced maternal aging, with additional mitochondria during IVF on subsequent reproductive success.[32] However, questions remain about the potential for AUGMENT-IVF to enhance pregnancy success rates in women seeking fertility care.[33] Additional preclinical studies that replicate the procedure as closely as possible, as well as randomized controlled human clinical trials involving much larger cohorts of patients, will be needed to address this further. Moving forward, we feel it will also be imperative, based on the discussions presented above regarding the complexity of the technical protocols involved in obtaining OSCs from ovarian tissue for mitochondrial isolation and injection, that any future studies of mitochondrial transfer employing OSCs, or even other cell types, provide extensive evidence of the purity and viability of the cells sorted and used for the procedure as a part of the study outcomes.

Syngeneic is not the same as autologous

Recently, St. John and colleagues[38] claimed to characterize the long-term safety of autologous germline mitochondrial supplementation as an assisted reproductive technology, using mice as a model system. However, after careful evaluation of the methodological approaches described, the results obtained, and the conclusions drawn by St. John and colleagues, we were surprised by the repetitive misrepresentation of what was actually performed experimentally and, more importantly, by the unfounded fears that this study's erroneous conclusions could raise in women who have already participated in clinical studies of true autologous germline mitochondrial supplementation during IVF. At the heart of the problem is a repeated misuse of the term "autologous" by St. John and colleagues.[38]

In biology and medicine, autologous refers to some type of biological material – whether it is an organ, tissue, cell, or even an intracellular component in the body of, or sourced from, a single individual. In transplantation biology, under which mitochondrial supplementation falls, autologous transplantation refers specifically to a biological material being transferred from one part of the body into the same or different part of the body of the same individual. The key defining feature of the term autologous, or autotransplantation, traces back to the prefix "auto-," which means "of the self" or "of the same organism." In other words, the recipient and the donor are the same individual. If, however, that same material is collected from one individual for transfer into another individual or into material from another individual, the procedure is termed allotransplantation (transfer of material between individuals of the same species), syngeneic transplantation (transfer of material between two genetically identical individuals of the same species, namely, monozygotic or identical twins), or xenotransplantation (transfer of material between individuals of two different species). In all cases, the distinguishing feature of nonautologous transplantation, irrespective of the type (allo-, syngeneic, or xeno-), is the involvement of more than one individual. Simply put, the recipient and the donor are not the same individual.

The authors recognize how important this difference in terminology is to clinical outcomes. Specifically, they state,

> [A]autologous mitochondrial transfer has been proposed as an assisted reproductive technology to ensure sufficient copies of mtDNA are present to support post-fertilisation developmental events in embryos until post-gastrulation when mtDNA replication is initiated. Autologous transfer would not compromise the genetic identity of the offspring as is the case when third party or 'donor' mitochondria are used in an approach commonly referred to as '3-parent IVF'. Furthermore, the use of '3-parent IVF' in both mouse models and humans has been associated with a number of disorders that have resulted in this approach being banned by regulators in many countries.[38]

In fact, the authors emphasize this issue as the driving force for the design, experimental execution, and interpretation of their study.

However, in reading the publication details, the authors actually performed syngeneic mitochondrial supplementation, in which OSC mitochondria and oocytes for IVF were collected independently from two different, but genetically inbred (genetically "identical"), groups of female mice. This approach, which is clearly nonautologous by definition, was then unfortunately misrepresented throughout their published study as autologous mitochondrial supplementation. This is not a question of semantics, and it is not a minor issue given that true autologous mitochondrial supplementation has already been performed with women seeking fertility assistance in approved clinical studies.[30–32] In fact, concerns raised about nonautologous mitochondrial supplementation from both animal experiments and human studies are exactly why autologous germline mitochondrial supplementation was developed in the first place. Specifically, AUGMENT-IVF removes the use of donor mitochondria in human assisted reproduction since the OSC mitochondria and the eggs for IVF are both derived from the same individual.[32]

Why is the autologous nature of mitochondrial supplementation during human IVF so critical to consider? Aside from potential societal and ethical issues surrounding children conceived through nonautologous mitochondrial transfer during IVF having "three genetic parents" (namely, the biological mother, the biological father, and the mitochondrial donor who provides yet another source of genetic material in the form of mtDNA),[39,40] and the largely unknown long-term consequences, if any, of children conceived through nonautologous mitochondrial transfer during IVF possessing mitochondria from two entirely different maternal sources,[22,23] the reasoning is straightforward. From biological, medical, and regulatory standpoints, microinjection of mitochondria collected from OSCs of the same woman undergoing AUGMENT during IVF to enhance pregnancy success rates increases the levels of a natural product that is already in eggs, in the form of maternal germline mitochondria.

To this end, genetic tracing studies in mice have shown that oocytes newly formed from OSCs in ovaries during adult life are used directly for the generation of offspring in natural mating trials.[41] Hence, the progressive differentiation of OSCs into oocytes that complete maturation to form metaphase-II eggs for fertilization is a physiological

cell lineage continuum such that mitochondria present in eggs initially derive from OSCs as their source. The only exception would be those oocytes formed in ovaries during the embryonic period from primordial germ cells (PGCs). While it is not feasible to perform autologous mitochondrial supplementation during human IVF using embryonic PGCs from that same individual as the source of the mitochondria, the same principles of a physiological cell lineage continuum in a single individual would still apply if it were feasible. In any case, syngeneic transfer of mitochondria from OSCs of one inbred strain of mice to eggs of other mice of the same inbred strain circumvents, for the most part (but not entirely), issues surrounding genetic (mtDNA) identity mismatch in the eggs used for fertilization. However, this approach is still, without question, nonautologous in nature. Hence, the syngeneic mitochondrial supplementation performed by St. John and colleagues[38] in their recent mouse study, which could theoretically be performed in humans with female identical twins, still introduces biological material into recipient eggs that is from another individual. This single conceptual difference, which is indisputable, underscores why syngeneic and autologous are not, and cannot be used as, interchangeable terms, especially when applied to discussions of mitochondrial supplementation for assisted human reproduction.

Even in the case of autologous germline mitochondrial transfer, however, studies are still needed to determine the impact, if any, of introducing different "types" of autologous germline mitochondria into eggs on post-fertilization development. Although OSCs and eggs represent a cell lineage continuum,[41] mitochondria are known to change in appearance and function as female germ cells develop from premeiotic progenitor cells (e.g. oogonia) into oocytes and then mature eggs.[42–45] Until recently, the isolation, much less testing, of specific types or subpopulations of mitochondria, which differ in size, ultrastructure, protein expression or membrane polarity, from the same biological sample was not feasible. However, the development of a nanoscale flow cytometry platform, termed fluorescence-activated mitochondrial sorting or FAMS, has provided a means to study, for the first time, the characteristic properties and functions of mitochondrial subpopulations segregated on the basis of various parameters.[46] In addition, FAMS allows for quantitative evaluation of the absence or presence of, or changes in numbers of, different types of mitochondria in a given cell lineage during the course of differentiation (e.g. OSCs to eggs) as well as in a single cell type, such as oocytes, across chronological age. Introduction of these and other technologies into mitochondrial transfer experiments should allow a more complete understanding of how the injection of germline mitochondria, or even specific types of germline mitochondria, into eggs affects both embryonic (short-term) and offspring (long-term) development.

If syngeneic is not autologous, then why repeatedly represent it as such?

Unintended mistakes can, and do, happen in scientific publications; however, a repetitive error cannot be a simple mistake. Keeping in mind that syngeneic is not in any way equivalent to autologous, to follow are statements taken from the publication of St. John and colleagues,[38] with bold formatting added for emphasis. Each statement is inaccurate based on the approaches employed by these authors in their study, since in no case were autologous mitochondria collected or used.

- '*We have supplemented oocytes with* ***autologous*** *populations of mitochondria to generate founders*.'
- '*These data highlight the need for caution when using* ***autologous*** *mitochondrial supplementation to treat female factor infertility*.'
- '*As oocytes for assisted reproduction are normally collected following hormonal stimulation or less frequently through in vitro maturation and the mtDNA content of an oocyte cannot be directly assessed prior to treatment, we have supplemented superovulated mouse oocytes at the time of fertilization with* ***autologous*** *populations of mitochondria to generate founders.*'
- '*We found that* ***autologous*** *supplementation enhanced fertility but led to structural defects in cardiac tissue suggesting that the regulation of chromosomal gene expression that is established during oogenesis is perturbed by this process.*'
- '*While there has been much concern about the health and well-being of humans and mice generated from third party mitochondrial supplementation, it appears that there is also cause for concern regarding* ***autologous*** *mitochondrial supplementation.*'

- '*Therefore, a degree of caution is required by regulators before granting licenses or permission to exploit this technology clinically.*' [Note: '*this technology*', in the context used by St. John and colleagues, refers specifically to autologous germline mitochondrial supplementation].
- '*In all, we show that* ***autologous*** *mitochondrial supplementation can enhance reproductive function in a small cohort of founder mice by increasing litter size and the ovarian reserve in subsequent generations. However, this is at the expense of weight gain and the structure of heart tissue.*'

Testing autologous mitochondrial supplementation during IVF

To truly perform autologous mitochondrial supplementation during IVF in mice, one would have to assign each female mouse as a single '*n*', from which superovulated oocytes and egg precursor cells or OSCs (the latter used for subsequent mitochondrial collection) would be isolated in parallel. To meet the definition of autologous, the two cell types from each mouse would then need to be used together, without any other source of material from any other mouse. In other words, samples could not be collected from, or pooled across, different female mice. This latter approach involving only a single individual as both donor and recipient is, in fact, the standard operating protocol in human clinical trials of AUGMENT-IVF, as reported.[30–33] There are two possible ways, and only two possible ways, of performing true autologous mitochondrial supplementation in mice for the purpose of modeling clinical studies of AUGMENT-IVF in women.

Under experimental approach 1, one would need to collect superovulated cumulus-oocyte complexes (COCs) from the fallopian tubes (for use in IVF) and simultaneously collect the ovaries for isolation of OSCs by cell sorting from each female mouse as a single and sole source of both materials. Once the OSCs were isolated, mitochondria would then need to be isolated from the purified OSCs for injection into the oocytes during ICSI. This was not done, as per the methods overview provided by St. John and colleagues.[38] In fact, this is not even technically feasible since it takes, at minimum, 4–6 hours to prepare ovarian tissue and sort OSCs using the antibody-based protocol cited by these authors,[35,36,38] and then at least another hour to isolate mitochondria from the sorted OSCs using the differential centrifugation protocol specified by the authors.[38] After this extended time sitting *in vitro*, the isolated oocytes would be difficult, if not impossible, to successfully fertilize. Even if the above were feasible, past studies have consistently shown that the yield of OSCs per adult mouse ovary is extremely low (around 200 or so cells),[35,36] and that the efficiency of the sorting procedure to isolate OSCs drops considerably (and often does not work well) when less than 4–6 pooled ovaries are used as starting material for a single sort.[35,36] Even if 400 or so OSCs could be obtained from the residual superovulated ovary tissues of one mouse (200 cells per ovary $\times$ 2 ovaries), the mitochondrial isolation procedure used by the authors (differential centrifugation) requires a threshold amount of biological sample input to work,[46] which would not be met using 400 cells (much less 400 cells containing 10–20 mitochondria per cell, as OSCs are primitive female germline stem cells similar in size and mitochondrial numbers to embryonic PGCs). For these reasons, this potential approach is not technically feasible.

Under experimental approach 2, for each female mouse, one ovary would have to be surgically removed to isolate OSCs, and subsequently mitochondria, prior to the superovulation protocol to isolate COCs for IVF once the mouse recovers from the surgical procedure. Once again, there cannot be pooling of samples across mice, and the mitochondria isolated earlier have to be stabilized, stored, and then matched specifically to the mouse from which COCs are harvested for IVF. While this would solve the temporal disconnect between having oocytes ready for IVF *versus* the time it would take to have OSC mitochondria prepared for injection into those oocytes during IVF, use of a single mouse ovary to isolate OSCs is technically challenging, if not impossible, using the antibody-based protocol cited by these authors.[35,36,38] Even if one could isolate 200 or so OSCs from a single starting mouse ovary, the yield of cells is far too low for differential centrifugation to be useful for mitochondrial isolation,[46] unless of course samples were pooled from multiple mice to increase the amount of biological sample input for the protocol to work as intended and presented. However, doing this would remove the autologous nature of the experiment. For these reasons, this potential approach is also not technically feasible.

Autologous *versus* syngeneic aside, other key differences exist

As more minor but very important points, St. John and colleagues[38] emphasize in their discussion that their data are from '*a mouse model that* ***has normal reproductive function*** *and is* ***not hindered by poor gamete quality***' (bold formatting by the authors for emphasis) and that their studies were performed with '*superovulated oocytes*'. While these points may seem innocuous on face value, these details are highly relevant to drawing any relevance of their work to clinical studies in which true autologous mitochondrial supplementation has been performed with women since, in the latter group, the women were seeking IVF because of poor gamete quality and their oocytes were not superovulated for retrieval from the fallopian tubes.[30–32] Hence, the approaches taken in the study by St. John and colleagues, compared with those taken in clinical studies of autologous mitochondrial supplementation referred to by St. John and colleagues, are quite different in terms of the character of the oocytes studied. In other words, mitochondrial 'over-boosting' of an oocyte that is otherwise already competent and replete with the mitochondria it needs to successfully generate an embryo and offspring (namely, the oocytes collected by St. John and colleagues were from very young mice) could, in itself, cause major issues with subsequent development. Equally concerning is the fact that the ages of the female mice subjected to superovulation to obtain COCs for the study by St. John and colleagues[38] (4–6 weeks postpartum) chronologically correspond to obtaining eggs from young human females on the cusp of puberty. For these reasons alone, we feel it is scientifically inappropriate for St. John and colleagues to draw sweeping conclusions from their work with prepubertal mice about the use of mitochondrial supplementation during human IVF involving women at advanced reproductive ages with poor egg and embryo quality.[30–32]

Summary and recommendations

In closing, we contacted the journal in which the St. John and colleagues publication appeared in an effort to bring to the editors' attention the technical flaws in the approaches used to achieve the stated objectives, the repeated misrepresentation of what was actually performed in the work, the scientifically unsupported conclusions and, most importantly, the unwarranted fears this study's erroneous conclusions could cause in women who have already conceived children through true autologous mitochondrial supplementation during IVF. Without timely acknowledgment from the journal editors that our concerns were being considered, we contacted the senior author of the study for additional clarification, and again received no response. Hence, we elected to submit our assessment of this publication from St. John and colleagues as a 'case study' for future work in the area of reproductive medicine. Although we are unsure of where all of this will go in the future, what is clear at present is that the authors did not actually perform autologous mitochondrial supplementation despite their repeated statements otherwise.[38] Hence, this mouse study has no direct relevance to assessing the potential merits or risks of AUGMENT-IVF as performed in approved clinical studies.[30–33] Instead, it simply reinforces results from prior studies with mice that the mixing of maternal mitochondrial genomes, such as that resulting from the presence of 'foreign' or nonautologous mitochondria introduced into eggs at the time of IVF, has negative consequences on the subsequent development of the resultant offspring during adulthood.[24,25]

This critical limitation of the study from St. John[47] must be reconciled, as the senior author of this study has subsequently used the misrepresentation of syngeneic mitochondrial transfer as autologous mitochondrial transfer to once again raise unfounded concerns over AUGMENT-IVF in human clinical trials. Specifically, in a recent review St. John[47] stated,

> A recent study has demonstrated that autologous mitochondrial supplementation using egg precursor cell mitochondria can result in the transgenerational transmission of a heart defect. Whilst this study [reference 38 herein] was conducted in a mouse model, further investigation is required in a large animal model prior to progressing clinical trials. That having been said, a few pregnancies and live births that have been generated using this technology. Nevertheless, the use of this technology in a clinical setting should be halted until the procedure has been fully validated in a large animal model with a similar embryo and pathophysiology to that of humans.

We feel the repetitive drawing of direct parallels between nonautologous syngeneic mitochondrial supplementation during IVF using prepubertal mice and true autologous mitochondrial transfer during IVF as conducted in approved human clinical studies is inappropriate and misleading.

These problems are compounded by the fact that St. John and colleagues repeatedly refer to human clinical studies of autologous mitochondrial supplementation during IVF – even emphasizing that children have been born through this procedure, and yet the authors elected to not cite any published studies describing the preliminary clinical experience with the procedure they refer to[30,31] or a detailed review of autologous mitochondrial transfer in human assisted reproduction that places these clinical studies into a deeper historical context.[32] Considering the rapid progress being made in the development of new potential human assisted reproductive technologies,[48,49] this recent publication with mice,[38] along with related misstatements made in a subsequent review from St. John[47] that references this same work, should collectively serve as a cautionary red flag to ensure that experimental procedures and outcomes of future preclinical studies are reported as accurately as possible. In addition, these types of experimentally unfounded conclusions should not be propagated further into unsupported regulatory or medical recommendations. In sum, studies of eggs from prepubertal mice that were collected from the fallopian tubes after superovulation and then subjected to nonautologous mitochondrial injection during IVF are simply not comparable to studies of eggs from women of advanced maternal age with a history of poor egg/embryo quality that were collected by transvaginal aspiration of ovarian follicles and then subjected to autologous germline mitochondrial supplementation during IVF.

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Conflict of interest statement

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: J.L.T. declares interest in intellectual property described in U.S. Patent 7,195,775, U.S. Patent 7,850,984, U.S. Patent 7,955,846, U.S. Patent 8,642,329, U.S. Patent 8,647,869, U.S. Patent 8,652,840, U.S. Patent 9,150,830, U.S. Patent 9,267,111, U.S. Patent 9,845,482, and U.S. Patent 10,525,086. D.C.W. declares interest in intellectual property described in U.S. Patent 8,642,329, U.S. Patent 8,647,869, U.S. Patent 9,150,830, and U.S. Patent 10,525,086.

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